Science of the Total Environment 683 (2019) 559–567

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Haematology, blood biochemistry, parasites and pathology of common eider (Somateria mollisima) males during a mortality event in the Baltic

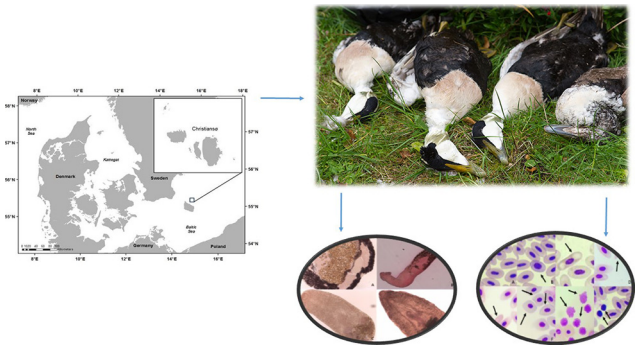
Svend-Erik Garbus [a,c], Jens Peter Christensen [b], Kurt Buchmann [b], Thomas Binger Jessen [c], Peter Lyngs [d], Mona Lykke Jacobsen [a], Grete Garbus [c], Emil Lund [b], Pelle Gorm Garbus [e], Jesper Johannes Madsen [f], Kasper Thorup [f], Christian Sonne [a,*]

[a] Aarhus University, Department of Bioscience, Arctic Research Centre (ARC), Frederiksborgvej 399, PO Box 358, DK-4000 Roskilde, Denmark
[b] University of Copenhagen, Faculty of Health and Medical Sciences, Department of Veterinary and Animal Sciences, DK-1870 Frederiksberg C, Denmark
[c] Dyrlægehuset Randers (Randers Veterinary Hospital), Sallingvej 5, DK-8940 Randers, SV, Denmark
[d] Christiansø Scientific Field Station, Christiansø 97, DK-3760 Gudhjem, Denmark
[e] Aarhus University, Department of Chemistry and iNANO, Center for Materials Crystallography, Langelandsgade 140, DK-8000 Aarhus C, Denmark
[f] University of Copenhagen, Natural History Museum of Denmark, Universitetsparken 15, DK-2100 København Ø, Denmark

HIGHLIGHTS

- From a Baltic mortality event, 39 male eiders were necropsied.
- Twenty-nine eiders had hunger related pathologies.
- All birds were infested with endoparasites.
- Blood biochemistry showed starvation and liver disease.
- These starvation and mortality events should be monitored in the Baltic.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 April 2019
Received in revised form 18 May 2019
Accepted 19 May 2019
Available online 21 May 2019

Editor: Damia Barcelo

Keywords:
Haematology
Biochemistrystry
Endoparasites
Multi-organ failure

ABSTRACT

A mortality event at the Christiansø colony in the Baltic proper killed 115 common eiders (*Somateria mollissima*) in mid-May 2016. To complement previous studies of incubating females, 39 males were necropsied and from a subsample of these a biochemical and haematological profile was obtained. The birds were emaciated and cachexic having a 50% reduction in body mass. Twenty-nine eiders were diagnosed with hydropericardium, 15 had hunger edema, three birds had enteritis and a single air sac infection. All birds were infested with intestinal *Polymorphus minutus* and 32 of these with the intestinal *Trematoda* spp. Microscopic parasitic investigations identified endoparasitic trematodes of the families *Bucephhalidae*, *Echinostomidae*, *Notocotyluridae* and *Levinsiniella*. White blood cell count showed slight heterophilia and lymphopenia while the albumin:globulin ratio (0.28) indicated stress, immune supression and inflammatory reactions supported by a high heterophil:lymphocyte index (13). Declined plasma concentration of glucose, fructosamine, amylase, albumin and protein likewise indicated long-term starvation prior to mortality indicating phase III starvation (catabolism of protein). The dramatic increase in aspartate transaminase, glutamate-dehydrogenase, lactate-dehydrogenase and bile acids indicate

* Corresponding author at: Dipl. ECZM (Wildlife Health), Aarhus University, Faculty of Science and Technology, Department of Bioscience, Frederiksborgvej 399, PO Box 358, DK-4000 Roskilde, Denmark.
E-mail address: cs@bios.au.dk (C. Sonne).

https://doi.org/10.1016/j.scitotenv.2019.05.281

Starvation
Body weight

liver disorders while dehydration, renal, heart and bone disorders was reflected in the increased uric acid, urea, phosphor and potassium values. These findings show that male eiders undergo long-term starvation and multi organ failure similar to that of incubating females previously reported from the same colony. It increases our knowledge of the physiology of starving eiders and add to our understanding of the recurrent mortality events in the colony that seems to be linked to changes in food availability being an important factor together with a warmer climate in a declining Baltic eider population. We recommend future studies to focus on food composition, migration patterns and environmental changes including parasitic infections and global warming.

1. Introduction

Metabolism of fasting or starving wild birds has been studied in geese (Boismenu et al., 1992; Robin et al., 1987), penguins (Robin et al., 1988; Stevens, 1996) and eiders (Garbus et al., 2018a; Hollmén et al., 2001). During avian food deprivation three distinct metabolic stages exits. Stage I is after last food intake where the bird switches its primary energy source from dietary digestion to oxidization of fatty reserves (Stevens, 1996). In stage II, only stored fatty reserves are almost exclusively used for energy and in stage III, proteins serve as primary energy source (Groscolas, 1986; Hollmén et al., 2001; Le Maho et al., 1981). This last stage is often reflected in decreased concentrations of proteins and elevated levels of creatinine and uric acid being the principal nitrogenous wastes of birds (Harr, 2006; Kolmstetter and Ramsay, 2000; Thrall et al., 2006).

Common eiders constitute an important key species in the Baltic ecosystem and their energy restriction is often related to the nutritional quality of blue mussels (*Mytilus edulis*) that constitute the primary food source (Cramp and Simmons, 1977). Food access to blue mussels is therefore an important driver for the population dynamics in the region, and years with fewer and slowed growing mussels may explain the declines over the past decades (Laursen and Møller, 2014). In addition, high prevalence of acanthocephalan parasites has earlier been associated with mortality in this subpopulation of eiders (Buchmann, 2016; Camphuysen et al., 2002; Garbus et al., 2018a).

The colony of eider ducks located in the Southern part of the Baltic Proper at the island Christiansø is the second largest in Denmark (Christensen and Bregnballe, 2011). The local breeding population numbered 1445 and 1500–1750 nesting females in 2007 and 2015, respectively (Lyngs, 2014; Garbus et al., 2018a). The eiders at Christiansø are migrating between wintering grounds at the western part of the Baltic Sea south to the Dutch part of the Wadden Sea (Bønløkke et al., 2006). They return to the summer breeding grounds from late February to early April (Lyngs, 1992).

Mortality events in commen eiders (*Somateria mollisima*) caused by starvation have been reported in the literature (Camphuysen et al., 2002; Garbus et al., 2018a; Madin, 2009). While it is not unusual to find few dead eiders during the breeding season, the colony at Christiansø has experienced an unusually high mortality of 125 and 110 birds found dead during May–June 2007 and 2015, respectively, composing 5–10% of the total colony (Garbus et al., 2018a). In May 2016, a 3rd starvation mortality event was observed in the Christiansø breeding colony where at least 115 birds died. To complement previous studies of incubating females (Garbus et al., 2018c), 39 males were necropsied and a biochemical and haematological profile was obtained from a subsample of these.

In the present study, we hypothesize that the males a) were dying from starvation and multi organ failure, 2) have high loads of parasites and 3) showed general stress and immune suppression. A veterinary standard autopsy protocol was applied to uncover the causes of mortality including biochemistry and haematology based on information in the available scientific literature trying to point out influential factors that add to the starvation and mortality disease events (Garbus et al., 2018a; Harr, 2002; Lumeij, 2008; Sonne et al., 2013; Thrall et al., 2006).

2. Materials and methods

2.1. Study area and field sampling

The investigation was performed on Christiansø, northeast of Bornholm in the Central Baltic Sea (55°19′N 15°11′E; Fig. 1) during the annual incubating eider health assessment (Garbus et al., 2018b). In Mid-May 2016, locals had during the last hatch of ducklings observed several lethargic unresponsive eiders having difficulty standing and holding up their beak. Birds were easily collected with bare hands. Due to the poor state and prognosis of the birds all were euthanised through cervical dislocation followed by pithing with a 16G needle (Kollias, 2017).

Eleven of the dying male eiders (9 adults and 2 subadults) were euthanised and an additional 28 adult males of the total 115 dead eiders found at shore was sampled for autopsy. Blood sampling (12–20 mL) was performed from the euthanized birds with the heart still beating ensuring good sample quality. Blood was transferred to a 4 mL BD Vacutainer® Lithium Heparin tube and a 4 mL BD Vacutainer® EDTA tube. The Lithium Heparin tube was centrifuged at 2500 rpm for 10 min (~838.5G) and supernatant plasma was transferred to a sterile Eppendorf® tube and remained frozen at −20 °C until chemical analysis in May 2016. The body mass was recorded with a Pesola Spring balance with 10 g accuracy.

2.2. Permits, legislation and animal welfare

Wild birds are protected according to Danish law (Wildlife Management and Hunting Act; LBK no. 735 of 14/06/2013) and a permission to handle eiders was therefore granted by the Danish Environmental Protection Agency (NST-304-0008). According to the permit we were allowed to collect and euthanize sick eiders in accordance with dispensations from the general Danish hunting legislation and environmental legislation. In addition, we obtained a laboratory animal permit including blood sampling in the general monitoring program and study of incubating eiders through the Natural History Museum of Denmark, University of Copenhagen (permission no. 2011/561–17).

2.3. Biochemical analysis

The analyses were conducted at IDEXX and included a panel of 28 parameters (Table 1). For the evaluation of hepatic health we included alkaline phosphatase (ALKP; U L^{-1}), (ASAT; U L^{-1}) alanine aminotransferase (ALAT; U L^{-1}), glutamate-dehydrogenase (GLDH; U L^{-1}), lactate-dehydrogenase (LDH; U L^{-1}) and gamma-glutamyltransferase (GGT; U L^{-1}), two hepatic/erythrocyte metabolic products, i.e. total bilirubin (TB; μmol L^{-1}) and bile acids (BA; μmol L^{-1}). To access digestive and pancreatic health we included two digestive enzymes, i.e. amylase (AMY; U L^{-1}) and lipase (LIP; U L^{-1}). Total protein (TP; g L^{-1}), globulin (GLOB; g L^{-1}) and albumin (ALB; g L^{-1}), two carbohydrates, i.e. glucose (GLU; mmol L^{-1}) and fructosamine (FRU; μmol L^{-1}), three muscle and protein metabolic products, i.e. creatinine kinase (CK; U L^{-1}), creatinine (CREA; μmol L^{-1}), urea (UREA; mmol L^{-1}), uric acid (UA; μmol L^{-1}) and cholesterol (CHOL; mmol L^{-1}) was included to assess stage of starvation. One neuro enzyme cholinesterase (CHE; U L^{-1}), one metal iron

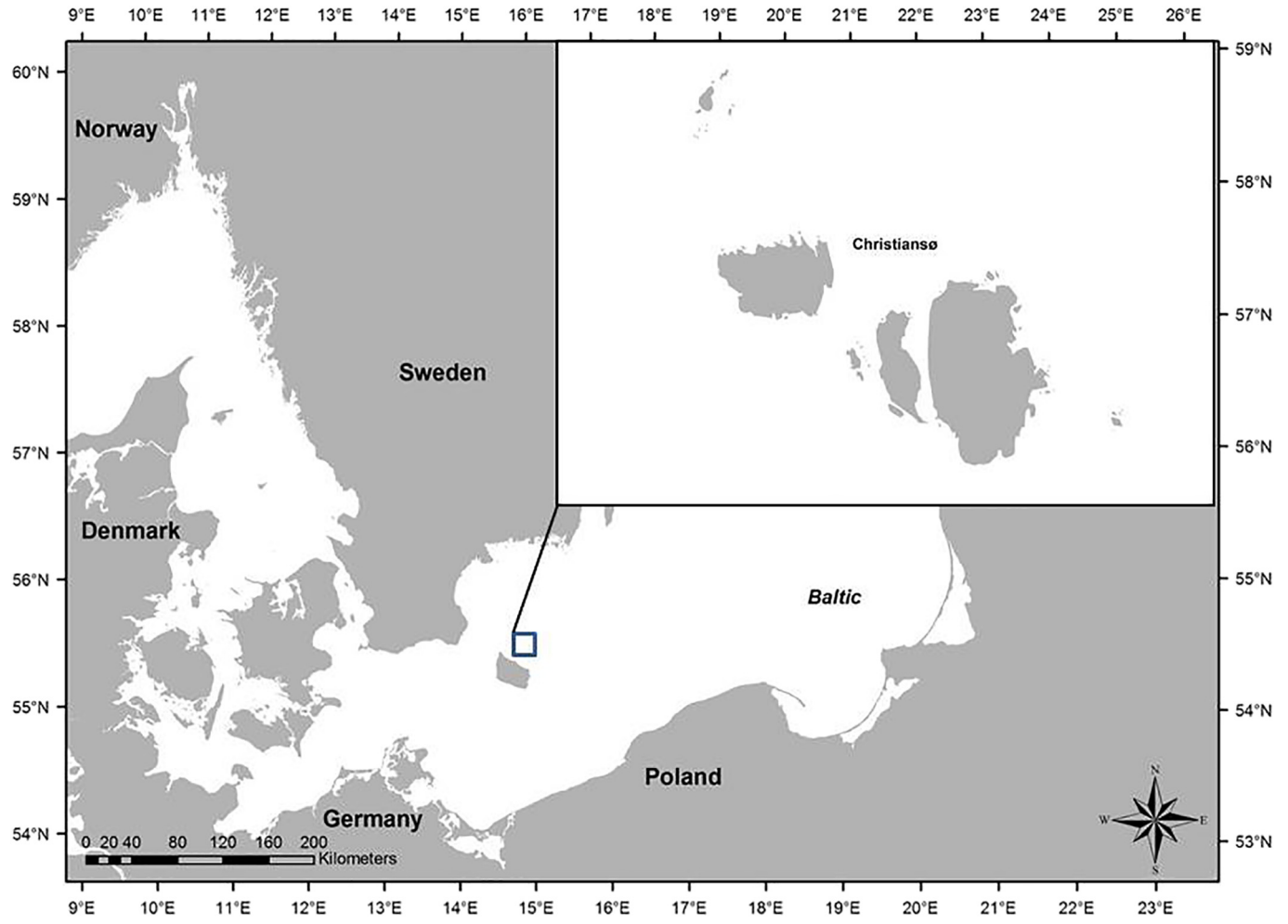


Fig. 1. Study area at Christiansø, Ertholmene in the Central Baltic.

(FE; μmol L^{-1}) and finally six electrolytes, i.e. phosphorus (PHOS; mmol L^{-1}), calcium (Ca; mmol L^{-1}), magnesium (Mg; mmol L^{-1}), sodium (Na; mmol L^{-1}), potassium (K; mmol L^{-1}) and chloride (Cl; mmol L^{-1}). For reference the information on common eiders is sparse. We used the International Species Information System for common eiders (ISIS, 2015), Laursen and Frikke (2008) and own unpublished data on healthy eiders.

2.4. Haematology

Blood from 2 subadult and 9 adult males was kept for haematology in 4 mL BD Vacutainer® EDTA tubes. Blood smears was made from a 5–10 μl droplet that was fixed in methanol after air-drying and finally colored with red-blue Dip Quick® Stain (Hema color). A complete blood count (CBC) including differential WBC count and leukogram was performed at Dyrlægehuset Randers' internal laboratory following Fudge (2000) (Table 2). The relative proportion of 100 white blood cells were examined and counted (heterophils, lymphocytes, monocytes, eosinophils and basophils) using the 100× lens in the first zone of the blood smear (monolayer) following a vertical bottom-top direction a horizontal direction and a top-bottom direction toward zone 2 (multilayer). The leukocytes were counted in 10 independent fields that were finally added and multiplied by 2000 to get the total white blood cell count;

$$\frac{Total\ slide\ leukocyte\ cont}{Number\ of\ high\ power\ fields}\ X\ 2000 = Total\ white\ blood\ cells$$

The counting method described by Fudge (2000) was done at 400× magnification and since we used 1000× magnification our count was corrected by a factor 6.25 using the formulary:

$$\frac{\left(\frac{1}{40}\right)^2}{\frac{1}{100}^2} = 6.25$$

Erythrocytes were evaluated using 1000× magnification by the method explained in Samour (2006). The abbreviations used throughout are WBC (white blood cells), LYM (lymphocytes), MONO (monocytes), HET (heterophils), EOS (eosinophils) and BAS (basophils).

2.5. Parasite investigations

For parasite investigation, a standard faecal sedimentation and flotation test was applied on fresh faecal matter from the ampulla colon using a saturated solution of sodium chloride (Smith, 1993). This is efficient for the identification of a wide variety of different parasites, e.g. nematodes, cestodes and acanthocephalans, but does not determine the intensity of parasites. Acanthocephalans were counted macroscopically and parasites were harvested from the intestines and kept in 70% ethanol prior to morphological analyses and species determination at the Department of Veterinary and Animal Sciences, University of Copenhagen where parasites were cleaned, stained and casted in plastic mounts for microscopy.

Table 1
Summary statistics (median ± SD and range) of body mass and biochemistry of dead male eiders (n = 11) collected at Christiansø in 2016. For clinical interpretation, reference values are shown. ¥: ISIS (2015). §: Laursen and Frikke (2008). €: unpublished data on early incubating eiders Christiansø 2016–2018 (n = 44). ↓: decrease in relation to reference interval. ↑: increase in relation to references interval. Abbreviations are found in materials and methods.

Parameter	Christiansø 2016	References values	Change (↑, ↓) and clinical interpretations
Body mass (g)	1314	2494§	↓: The birds are starving
ALKP (U L^{-1})	419 ± 197 (142–892)	84 ± 59 (32–265)¥	↑: The birds are starving, remodel bones or have liver disorders (cholestatis, biliary obstruction, parasites)
ASAT (U L^{-1})	524 ± 380 (144–1180)	44 ± 38 (16–192)¥	↑: The birds are starving or have liver disorders (cholestatis, biliary obstruction, parasites)
ALAT (U/L)	14.2 ± 8.6 (4–29)	12 ± 9 (4–29)¥	Normal
GLDH (U L^{-1})	21.7 ± 31.1 (3–116)	1 ± 12 (1–45)€	↑: The birds are having liver disorders (cholestatis, biliary obstruction and/or parasites)
LDH (U L^{-1})	825 ± 646.5 (238–2572)	235 ± 87 (116–463)¥	↑: The birds are having liver disorders (cholestatis, biliary obstruction and/or parasites)
GGT (U L^{-1})	3.6 ± 1.3 (2–6)	3 ± 2 (1–6)¥	Normal
Amy (U L^{-1})	408 ± 212 (408–1202)	553 ± 101 (436–692)¥	↓: The birds are starving
LIP (U L^{-1})	28.36 ± 10.3 (13–49)	5 ± 1.2 (5–10)€	↑: The birds are starving
ALB (g L^{-1})	3.5 ± 1.3 (1–6)	24 ± 9 (14–42)¥	↓: The birds are starving
GLOB (g L^{-1})	12.5 ± 2.8 (6–16)	29 ± 7 (20–40)¥	↓: The birds are starving
ALB:GLOB	0.28	1.2 ¥	↓: The birds are starving. Stress. Immunusupression.
TP (g L^{-1})	15.8 ± 3.7 (8–21)	54 ± 12 (27–81)¥	↓: The birds are starving
BA (μmol L^{-1})	17.6 ± 27 (1.9–98.9)	9 ± 7.2 (1.9–37.7)€	↑: The birds are having liver disorders (cholestatis, biliary obstruction and/or parasites)
TB (μmol L^{-1})	3.9 ± 2 (2–8.7)	3 ± 3 (0–9)¥	Normal
GLU (mmol L^{-1})	3.4 ± 1.9 (0.8–7.8)	13.2 ± 2.4 (7.8–21.2)¥	↓: The birds are starving
FRU (μmol L^{-1})	55.1 ± 9.6 (41–74)	147 ± 29 (101–238)€	↓: The birds are starving
CK (U L^{-1})	28.4 ± 19.4 (5–65)	550 ± 479 (226–1100)¥	↓: The birds are starving
CREA (μmol L^{-1})	5.4 ± 5.4 (0–14)	6.3 ± 3.7 (0–17)€	Normal
UREA (mmol L^{-1})	2.73 ± 1.3 (1.1–5.6)	0.4 ± 0.2 (0–0.9)€	↑: The birds are starving and have likely both pre-renal dehydration as well as renal insufficiency.
UA (μmol L^{-1})	1895 ± 661 (1100–3499)	488 ± 190 (274–904)¥	↑: The birds are starving and have likely both pre-renal dehydration as well as renal insufficiency.
CHOL (mmol L^{-1})	1.0 ± 0.2 (0.7–1.4)	8.2 ± 2.4 (4.6–13.8)¥	↓: The birds are starving
CHE (k U L^{-1})	1.1 ± 0.3 (0.6–1.5)	1.2 ± 0.2 (0.8–1.7)€	Normal
Fe (μmol L^{-1})	10.3 ± 5.2 (5–24)	54.1 ± 3 (15–309)€	↓: The birds are starving and have signs of anemia
PHOS (mmol L^{-1})	1.8 ± 1.2 (1.0–5.6)	1.2 ± 0.6 (0.5–2.2)¥	↑: The birds remodel bones, have calcium deficiency and kidney disorders
Ca (mmol L^{-1})	1.3 ± 0.2 (1.1–1.6)	2.7 ± 0.2 (2.3–3.1)¥	↓: The birds are starving and have calcium deficiency
Mg (mmol L^{-1})	1.1 ± 0.1 (0.8–1.2)	1.2 ± 0.1 (0.9–1.5)€	Normal
Na (mmol L^{-1})	149 ± 3.46 (142–155)	163 ± 6 (148–178)¥	Normal
K (mmol L^{-1})	6.3 ± 1.3 (4.2–9.3)	2.1 ± 0.4 (1.6–3.2)¥	↑: Renal insufficiency or congestive heart failure
Cl (mmol L^{-1})	115 ± 5.6 (105–124)	118 ± 5 (108–128)¥	Normal

2.6. Statistical analyses

Microsoft Excel was used as database and for the calculations of basic statistics.

3. Results

3.1. Body mass

Physical examination of the 39 male eiders showed that the eiders had reduced pectoral musculature and "long legs" due to the relatively smaller breast and were unresponsive having difficulty standing and holding up their beak. The average body mass of all the collected subadult and adult males was 1266–1340 g (Table 1).

3.2. Clinical examination and necropsy

Visual inspection showed intact body cavity without traces of contamination such as oil or presence of visible ectoparasites. On visual examination and palpation, the skin appeared pale but intact. The eiders were emaciated with empty crop, prominent keel and greatly reduced pectoral muscles. No skeletal deformity or fractures were observed except for one eider with a deform keel. The bill appeared normal and the inside of the mouth and tongue were without any lesions. Body orifices and mucous membranes were in all cases pale without discharge, ulceration, plaques or foreign objects. Dissection showed atrophy of pectoral muscles and depletion of subcutaneous and coelomic fat resources.

In general, the organs appeared pale and shriveled without any lesions, hemorrhages or signs of bacterial or viral infection or state of toxicological poisoning. Air sacs appeared transparent and glistening

Table 2
Complete blood count (mean ± SD [Min-Max]) from six of the 11 male eiders from which blood was collected at Christianø in 2016. All birds were moribund. For clinical interpretation reference values are shown (ISIS, 2015). Abbreviations are found in materials and methods. Blank: information not available.

	This study (n = 6)	Reference	Clinical interpretation
WBC	14 ± 6.8 (6.2–23)	13 ± 8.9 (2.5–43)	Normal
LYM%	6 ± 3 (3−11)	–	
MONO%	4 ± 2 (1–6)	–	
HET%	85 ± 10 (70–97)	–	
EOS%	4 ± 3 (1−11)	–	
BAS%	<1 ± 0 (0–0.01)	–	
LYM#	0.93 ± 0.66 (0.3–2.1)	5.3 ± 4.6 (0.3–25)	↓: The birds are starving and have infections
MONO#	0.59 ± 0.41 (0.1–1.2)	1.15 ± 1.6 (0.05–7.5)	Normal
HET#	12 ± 5.7 (5.27–19.2)	6.1 ± 6.4 (1.1–42.2)	↑: The birds are stressed and have infections
EOS#	0.46 ± 0.24 (0.06–0.82)	0.68 ± 0.89 (0.04–4.6)	Normal
BAS#	0.04 ± 0.09 (0–0.23)	0.35 ± 0.14 (0.18–0.59)	Normal
H:L index	13	1.2	↑: The birds are stressed and have immune supression

Table 3
Biometrics [mean ± SD (min-max, n)] and prevalence of pathology and infections of 39 dead male eiders collected during the mortality event at Christiansø 2016. For clinical interpretation, reference values of healthy eiders are included (Laursen and Frikke, 2008). Blank cells: data not available.

	Christiansø 2016		Wadden Sea
	Adult males (n = 37)	Subadult males (n = 2)	Adult male
Body weight (g)	1341 ± 118 (1180–1600, 36)	1267 ± 20 (1100–1450, 3)	2494 (79)
Breast muscle (g)	52.8 ± 8.5 (38.1–70.9, 24)	49.9 ± 0.3 (49.6–50.2, 2)	
Gizzard (g)	51.7 ± 8,4 (40.5–67.8, 14)	36.9 ± 6.5 (30.4–43.5, 2)	
Heart (g)	12.7 ± 3.1 (7.4–22.9, 34)	11.4 ± 3.9 (7.9–16.9, 3)	
Liver (g)	36.1 ± 8.8 (23.9–56.4, 31)	42 ± 13.5 (24.0–56.5, 3)	
Total body (cm)	65.6 ± 1.0 (63.6–68.4, 23)	64 ± 0.6 (63.4–64.5, 2)	
Tarsus total (mm)	66.0 ± 1.8 (61.5–69.0, 23)	65.7 ± 3 (62.7–68.7, 2)	
Wingcord flat (mm)	301 ± 4.4 (292–312, 22)	285 ± 7 (278–292, 2)	
Head total (mm)	131 ± 3 (123–136, 22)	130 (1)	
Keel (mm)	130 ± 3.8 (121–136, 23)	129 ± 3 (126–132, 2)	
Hydropericardium	26	3	
Hunger edema	12	3	
Airsac infection	1	0	
Enteritis	3	0	

except one bird with an aspergillus infection in the left proximal air sac. The pericardial sac was thin, pale and transparent containing non-viscous clear serous fluid in excess. In general, a build-up of fluid (serous transudate) was observed in most of the birds in the thoracic and coelom cavity. Of the 39 eiders, 29 had hydropericardium, one with dilated right heart chamber, 15 had a combined hunger edema i.e. excessive fluid volume from loss of proteins (Table 3). In addition, the liver appeared pale-yellow with enlarged gall bladder. The gizzards were empty and contained only small pieces of granite and marine litter (glass) for grinding up food. Also the gastrointestinal tracts were empty except for parasites causing some intestinal dilation. Twenty-nine eiders were diagnosed with hydropericardium, three birds were found with enteritis, two of these had a dilated colon and one bird had sand in the intestine (Fig. 2).

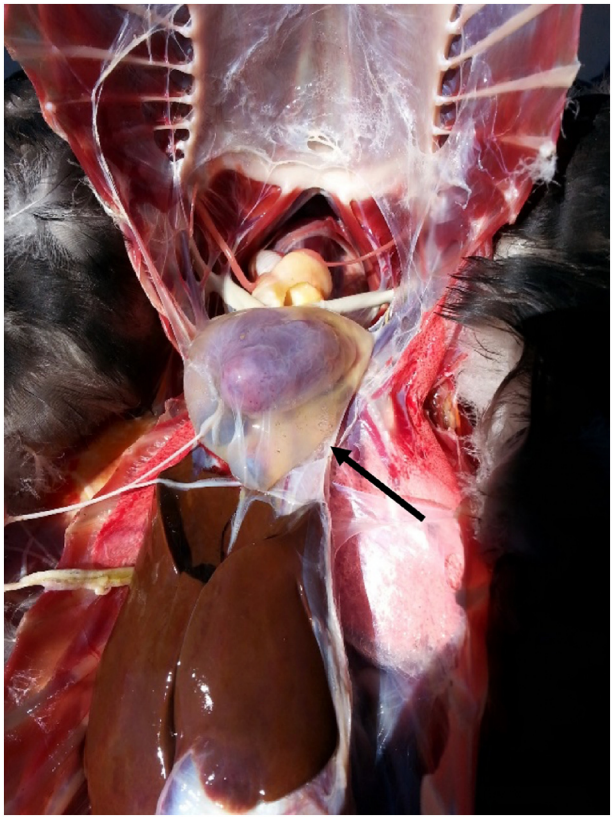


Fig. 2. Hydropericardium (black arrow) was observed combined with hunger edema i.e. excessive fluid volume from loss of proteins.

3.3. Parasitic investigations

Moderate to heavy presence of the intestinal acanthocephalan parasite *Polymorphus minutus* was observed in all 39 eiders collected in 2016 (Fig. 5, Table 4). On average, the birds contained 1102 (range: 115–3839) *P. minutus* individuals (Table 4). In the most heavily infected birds, the mucosa of the middle part of the intestine was completely covered. Presence of *Echinostoma spp.* (intestinal flukes) was detected as well. Twenty of 32 tested eiders showed positive for *Echinostomidae* eggs, one positive for *Acanthocephalan* eggs but none for nematode eggs. Microscopic parasitic investigations confirmed acanthocephalan *Polymorphus minutus* and identified endoparasitic trematodes of the families *Bucephhalidae*, *Echinostomidae*, *Notocotyluridae* and *Levinsiniella*.

3.4. Biochemistry and haematology

A panel of 28 biochemical profiles was obtained from 11 eiders in total and from these a complete blood count was obtained from 6 individuals (Tables 1, 2). Glucose and the glucose biomarker fructosamine both decreased up to 3- folds. The digestive enzyme amylase also decreased along with plasma protein, creatinine kinase and creatinine while and increase was found for lipase (Table 1). The albumin:globulin (A:G) ratio decreased 4-fold. Dramatic increases in concentrations of alkaline phosphatase, aspartate transaminase, alanine-transaminase, glutamate-dehydrogenase, lactate-dehydrogenase and bile acid was also observed (Table 1). In addition uric acid, urea, phosphor and potassium increased while iron decreased by 5-folds on average (Table 1). Erythrocyte morphology included polychromasia, hypochromasia, anicytosis and poikilolycytosis with loss of nucleus (Fig. 4). A white blood cell count (WBC) of 14.27 10^9/L performed on six eiders with a slight lymphopenia and heterophilia in addition to a decreased A:G ratio (Table 1, Fig. 3). In addition the H:L index was elevated (Table 2).

Table 4
Number of parasites in dead male eiders (n = 39) collected during the mortality event at Christiansø in 2016. €: data from Garbus et al. (2018a). §: data from Buchmann (2010, 2016).

	Healthy eiders§		Mortality Christiansø€	Present study
	May 2010–2011	Nov 2010–2011	June 2015	May 2016
N	4	10	3	17
Mean	86	287	723	1102
SD	77	142	467	1040
Range	3–319	1–1153	330–1240	115–3839

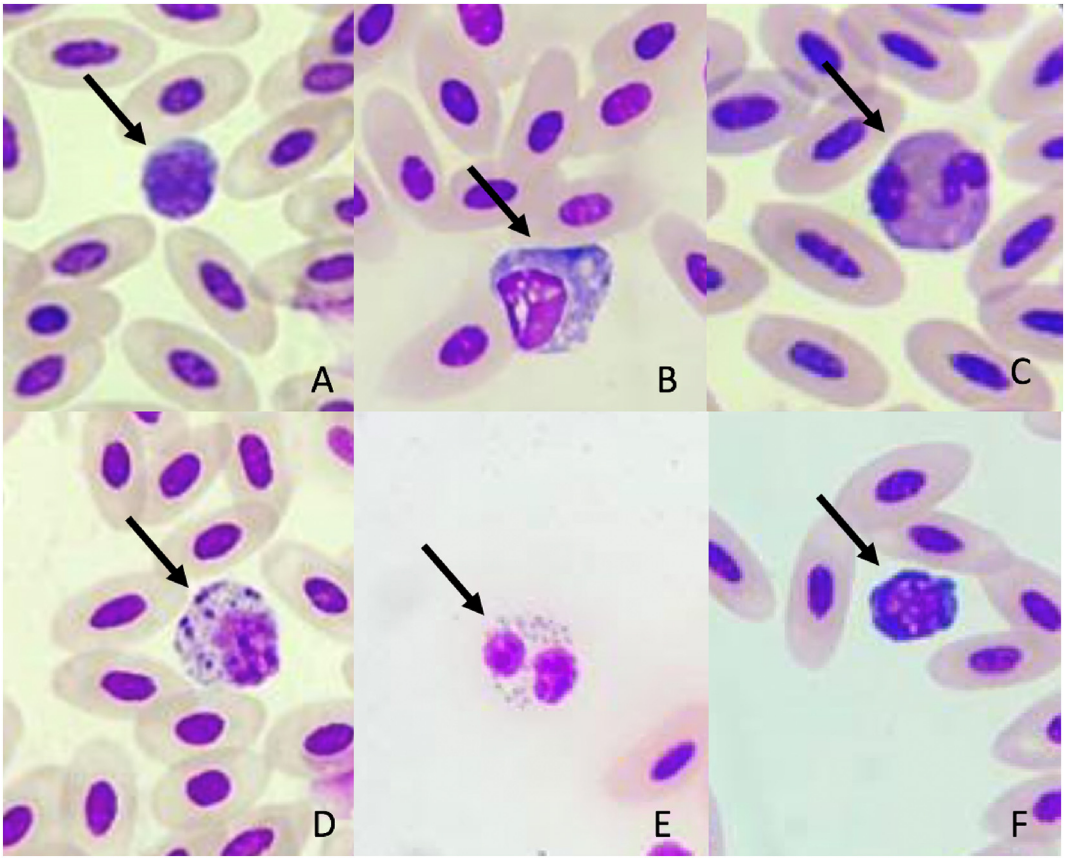


Fig. 3. Example of blood smear leukocyte haematology from one of the eleven male eiders from which blood samples were obtained at Christiansø 2016. A: Lymphocyte. B: Monocyte. C: Heterophil. D: Toxic heterophil. E: Eosinophil. F: Basophil. Stain: Hemacolor.

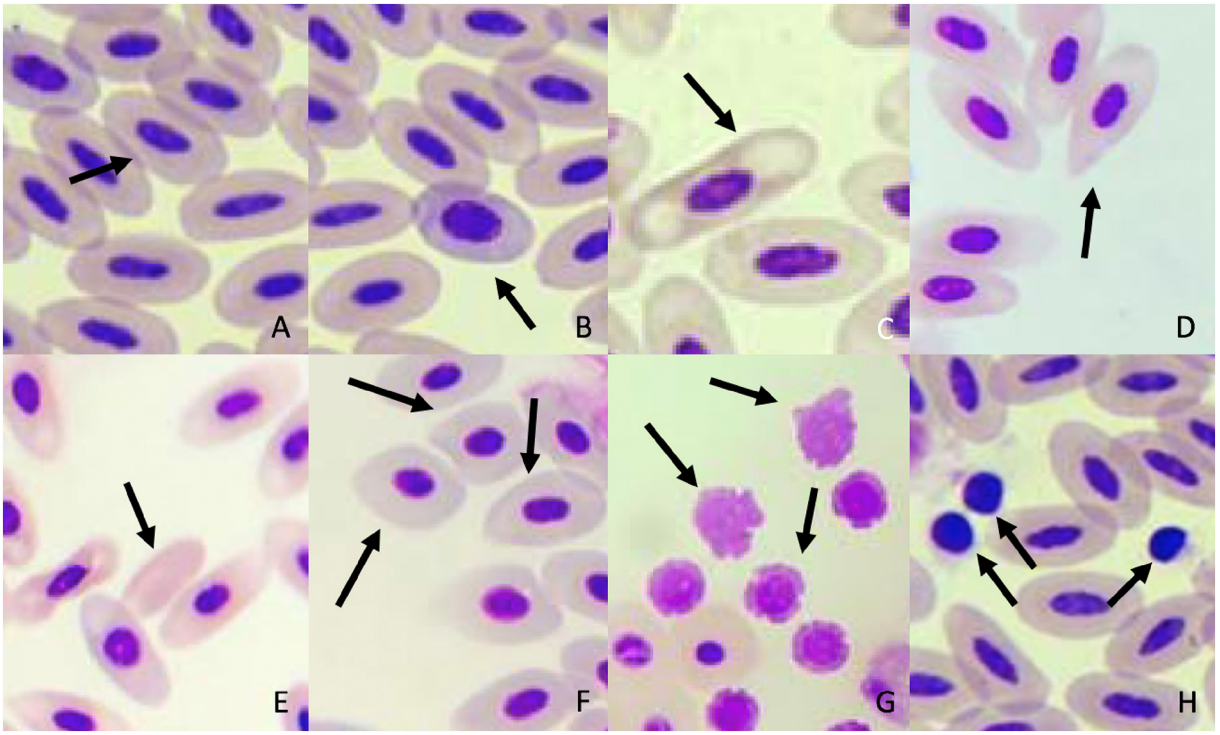


Fig. 4. Example of blood smear erythrocyte haematology from one of the eleven male eiders from which blood samples were obtained at Christiansø 2016. A: Normal erythrocyte. B: Young erythrocyte (polychromasia). C: Erythrocyte with vacuoles. D: Poicolycyte. E: Enucleated erythrocyte F: Anicytose and hypochromasia. G. Partly haemolysed red blood cells. H: Thrombocytes. Stain: Hemacolor.

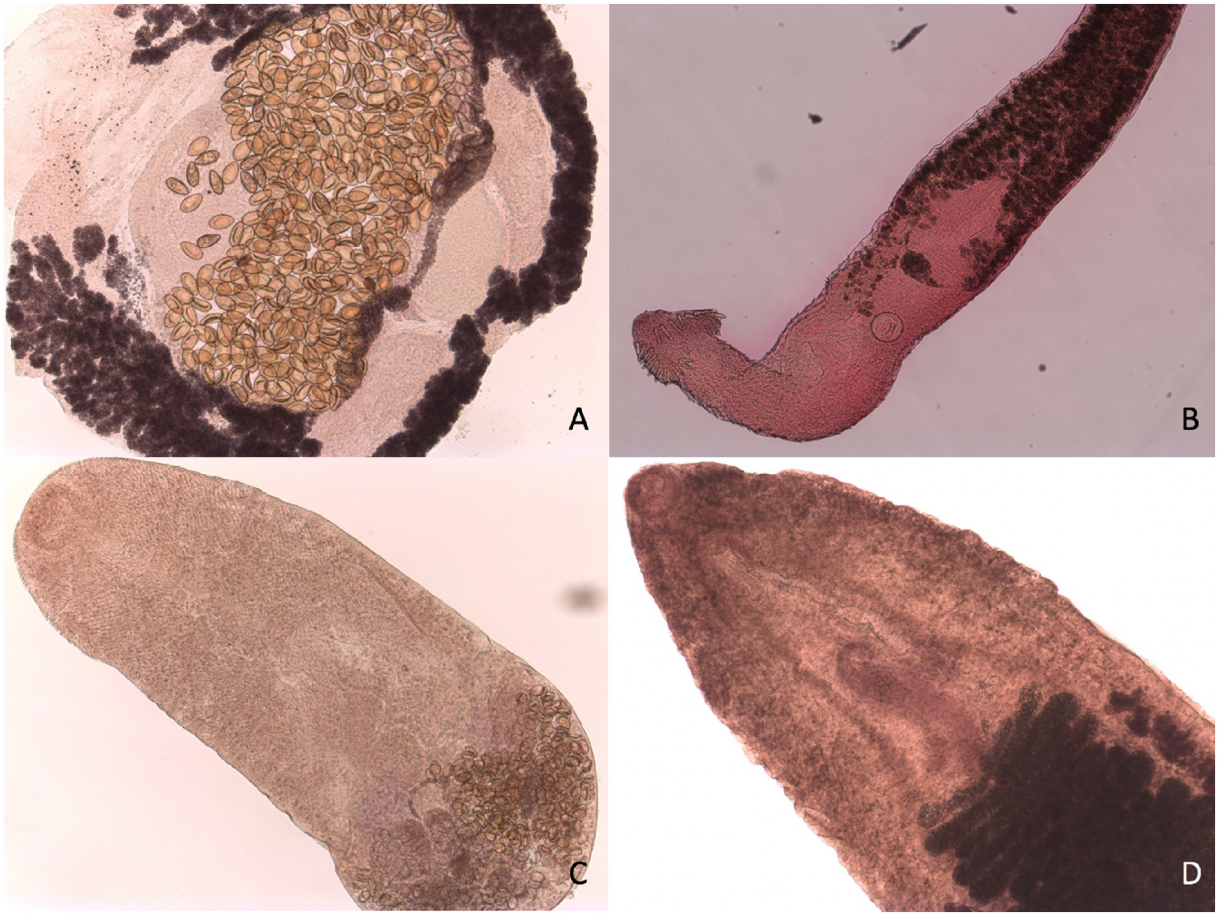


Fig. 5. Endoparasitical flukes from the intestines of one of the 39 eiders collected at Christiansø in 2016. A: *Fam. Bucephalidae* B: *Fam. Echinostomidae* C: *Fam. Notocotylurida* D: *Fam. Levinsiniella.*

4. Discussion

The mortality events in 2007, 2015 and 2016 raises multiple questions regarding food access and composition, parasite burdens and other environmental stressors such as infectious diseases (Camphuysen et al., 2002; Garbus et al., 2018a). The findings of the present study were with great certainty not caused by for example botulism, avian cholera or influenza (Alexander, 2008; Christensen and Bisgaard, 1997; Christensen et al., 2008; Gough, 2008; Kaldhusdal and Jordan, 2008). The heavy loss of body mass of ca. 50% and decreases in plasma glucose and fructosamine show that the birds were starving, low in energy and were malnourished for weeks prior to the event (Garbus et al., 2018a; Harr, 2006; Hochleithner, 1994; Lumeij and Westerhof, 1987, Lumeij, 2008; Williams, 2012). Biochemical changes showed a clear phase III starvation with catabolism of adipose tissue into protein as well as dehydration leading to multi organ failure including congestive heart failure with hydropericardium (Garbus et al., 2018a; Hollmén et al., 2001; Lierz, 2003; Thrall et al., 2006).

4.1. Parasites

In this study the acanthocephalan *Polymorphus minutus* was confirmed while also trematodes of the families *Bucephhalidae*, *Echinostomidae*, *Notocotyluridae* and *Levinsiniella* were found. In previous studies, *acanthocephalans and trematodes were* identified (Borgsteede et al., 2005; Camphuysen et al., 2002), although echinostomid and bucephalid trematodes were rarely recorded, have been found in a case-study (Buchmann, 2010) and in a mortality event from 2015 (Garbus et al., 2018a). The *Acanthocephalans* seem to harm the eiders more than other parasites as this specific parasite need to adhere to the intestinal mucosa with its spiky proboscis, which cause immense damage. While the tegument of the worm corpus absorbs nutritional elements from the intestinal lumen, e.g. albumin, and takes energy from the eider which cannot excrete this parasite. It is shown by microscopical investigations of the intestinal mucosa that *Echinostomes*, also adhering to the intestinal mucosa with a spiky head, causes great harm to the intestinal mucosa with acute hemorrhage and a more chronic but pronounced deterioration of intestinal tissue (Buchmann, 2010) which might lead to protein-losing enteropathy (Kendal, 2006).

4.2. Haematology and immune system

The white blood cell count (WBC) performed on six eiders was within the reference range while the high H:L index caused by heterophilia and lymphopenia and the decreased A:G ratio points toward stress, immune suppression and inflammatory reactions (Averbeck, 1992; Bienzle et al., 1997; Kendal, 2006; Campbell, 1995) caused by starvation (Krams et al., 2010, 2011; Thrall et al., 2006). This suggests that the birds were not able to mount a fully immunological response likely due to metabolic costs (Ben-Nathan et al., 1981).

4.3. Considerations

The present finding is possibly due to a combination of multiple stress from reduced food quality and quantity and a warmer climate that require energy for temperature regulation. Overall, it is difficult to investigate the exact reasons and the combined effects, however, this

present study together with the multiple other reports on eider health from Christiansø add to the puzzle and help to establish the larger picture of cause-and-effects. We recommend to map the food web of the Baltic eiders and to conduct telemetry which will add to the understanding of the biology of the eiders and how winter and summer conditions affect the health of the flyway. The present study show that male eiders undergo similar long-term starvation as the incubating females and future studies should focus on food composition, migration patterns and environmental changes including global warming.

5. Conclusions

The birds were emaciated and cachexic showing hydropericardium, hunger edema and enteritis. All birds were infected with endoparasites, and white blood cell count showed slight heterophilia and lymphopenia indicating stress, immune supression and infections. This was supported by the decrease in albumin:globulin ratio and increase in heterophil:lymphocyte ratio. Blood biochemistry all showed starvation and dehydration and bone, hearth, liver and kidney disorders. These findings show that male eiders undergo similar long-term starvation similar to that of incubating females and we recommend future studies to focus on food composition, migration patterns and environmental changes including parasitic infections and global warming.

Acknowledgements

For Funding of the study, we acknowledge The Danish Environmental Protection Agency, The Danish Hunters' Association, Hunters' Nature Fund, 15. Juni Fonden and BONUS BALTHEALTH that has received funding from BONUS (Art. 185), funded jointly by the EU, Innovation Fund Denmark (grants 6180-00001B and 6180-00002B), Forschungszentrum Jülich GmbH, German Federal Ministry of Education and Research (grant FKZ 03F0767A), Academy of Finland (grant 311966) and Swedish Foundation for Strategic Environmental Research (MISTRA). We also acknowledge the Christiansø Administration and the people of Christiansø for their invaluable help and kind hospitality on the project.

References

Alexander, D.J., 2008. Orthomyxoviridae – Avian influenza. In: Jordan, F.T.W., Pattison, M., Alexander, D., Faragher, T. (Eds.), Poultry Diseases, 6th edition W.B. Saunders, London, pp. 317–332.
Averbeck, C., 1992. Haematology and blood chemistry of healthy and clinically abnormal great black backed gulls (Larus Marinus) and herring gulls (Larus Argentatus). Avian Pathol. 21, 215–223.
Ben-Nathan, B., Drabkin, N., Heller, D., 1981. The effect of starvation on the immune response of chickens. Avian Dis. 25, 214–217.
Bienzle, D., Pare, J.A., Smith, D.A., 1997. Leukocyte changes in diseased non-domestic birds. Vet. Clin. Pathol. 26, 76–84.
Boismenu, C., Gauthier, G., Larochelle, J., 1992. Physiology of prolonged fasting in greater snow geese (Chen caerulensis atlantica). Auk 109, 511–521.
Bønløkke, J., Madsen, J.J., Thorup, K., Pedersen, K.T., Bjerrum, M., Rahbek, C., 2006. Dansk Trækfugleatlas (The Danish Bird Migration Atlas). . 880 pp. Forlaget Rhodos, Humlebæk.
Borgsteede, F.H.M., Okulewicz, A., Zoun, P.E.F., Okulewicz, J., 2005. The gastro-intestinal helminth fauna of the eider duck (Somateria mollissiuma L.) in the Netherlands. Helminthologia 42, 83.
Buchmann, K., 2010. Døde edderfuglene af et parasitangreb? (Did the eiders die from parasitic infections?) Natur på Bornholm. vol. 8, pp. 18–21 (In Danish)
Buchmann, K., 2016. Parasitter i ederfugle – fundet I voksne fugle på Christiansø 2015 (Parasites in eiders – found in adult birds on Christiansø 2015). Natur på Bornholm 14, 24–27 (In Danish).
Campbell, T.W., 1995. Avian Hematology and Cytology. 2nd ed. Iowa State University Press, USA.
Camphuysen, C.J., Berrevoets, C.M., Cremers, H.J.W.M., Dekinga, A., Dekker, R., Ens, B.J., Van der Have, T.M., Kats, R.K.H., Kuiken, T., Leopold, M.F., van der Meer, J., Piersma, T., 2002. Mass mortality of common eiders (Somateria mollissima) in the Dutch Wadden Sea, winter 1999/2000: starvation in a commercially exploited wetland of international importance. Biol. Conserv. 106, 303–317.
Christensen, J.P., Bisgaard, M., 1997. Avian pasteurellosis: taxonomy of the organisms involved and aspects of pathogenesis. Avian Pathol. 26, 461–483.
Christensen, T.K., Bregnballe, T., 2011. Status of the Danish breeding population of eiders Somateria mollissima 2010. Dansk Orn Foren Tidsskr. vol. 105, pp. 195–205.
Christensen, P., Bojesen, M.A., Bisgaard, M., Pattison, M., 2008. Fowl cholera. In: Jordan, F.T.W., Alexander, D., Faragher, T. (Eds.), Poultry Diseases, 6th edition W.B. Saunders, London, pp. 149–154.
Cramp, S., Simmons, K.E.L., 1977. Tribe SOMATERIINI Eiders. In: Cramp, S., Simmons, K.E.L. (Eds.), Handbook of the Birds of Europe, the Middle East and North Africa: The Birds of the Western Palearctic. Volume I: Ostrich to Ducks. Oxford University Press, Oxford, pp. 593–618.
Fudge, A.M. (Ed.), 2000. Laboratory Medicine: Avian and Exotic Pets. W.B. Saunders, Philadelphia, PA U.S.A.
Garbus, S.E., Lyngs, P., Christensen, J.P., Buchmann, K., Eulaers, I., Mosbech, A., Dietz, R., Gilchrist, H.G., Sonne, C., 2018a. Common eider (Somateria mollissima) body condition and parasitic load during a mortality event in the Baltic proper. Avian Biol. Res. 11, 167–172.
Garbus, S.E., Lyngs, P., Popp Thyme, A., Christensen, J.P., Sonne, C., 2018b. Candling and field atlas of early egg development in common eiders Somateria mollisima. Acrocephalus 39, 85–126.
Garbus, S.E., Lyngs, P., Garbus, M., Garbus, P., Eulaers, I., Mosbech, A., Dietz, R., Gilchrist, H.G., Huusmann, R., Christensen, J.P., Sonne, C., 2018c. Incubation behaviour of common eiders Somateria mollisima in the central Baltic. Acrocephalus 39, 91–100.
Gough, R.E., 2008. Duck virus enteritis. In: Jordan, F.T.W., Pattison, M., Alexander, D., Faragher, T. (Eds.), Poultry Diseases, 6th edition W.B. Saunders, London, pp. 272–275.
Groscolas, R., 1986. Changes in body mass, body temperature and plasma fuel levels during the natural breeding fast in male and female emperor penguins Aptenodytes forsteri. J. Comp. Physiol. B. 156, 521–527.
Harr, K.E., 2002. Clinical chemistry of companion avian species: a review. Vet. Clin. Pathol. 31, 140–151.
Harr, K.E., 2006. Diagnostic value of biochemistry. In: Harrison, J.G., Lightfoot, T. (Eds.), Clinical Avian Medicine. vol. Volume 2. Florida Spix Publishing (611-629 pp).
Hochleithner, M., 1994. Biochemistries. In: Ritchie, B.W., Harrison, G.J., Harrison, L.R. (Eds.), Avian Medicine: Principles and Applications. Wingers, LakeWorth, Fla, pp. 223–245.
Hollmén, T., Franson, J.C., Hario, M., Sankari, S., Kilpi, M., Lindström, K., 2001. Use of serum biochemistry to evaluate nutritional status and health of incubating common eiders (Somateria mollissima) in Finland. Physiol. Biochem. Zool. 74, 333–342.
ISIS, 2015. International species information system. Common eider. www.isis.org (Retrieved December 2015).
Kaldhusdal, M., Jordan, F.T.W., 2008. Clostridia. In: Jordan, F.T.W., Pattison, M., Alexander, D., Faragher, T. (Eds.), Poultry Diseases, 6th edition W.B. Saunders, London, pp. 200–214.
Kendal, E.H., 2006. Diagnostic value of biochemistry. Clin. Avian Med. 2, 611–629.
Kollias N. 2017. Avian euthanasia. Cornell University. Institutional Animal Care and Use Committee. Document: ACUP308.07. html: https://ras.reasearch.cornell.edu/care/documents.
Kolmstetter, C.M., Ramsay, E.R., 2000. Effects of feeding on plasma uric acid and urea concentrations in blackfooted penguins (Spheniscus demersus). J. Avian Med. Surg. 14, 177–179.
Krams, I., Cirule, D., Suraka, V., Krama, T., Ramtala, M.J., Ramey, G., 2010. Fattening strategies of wintering great tits support the optimal body mass hypothesis under conditions of extremely low ambient temperature. Funct. Ecol. 24, 172–177.
Krams, I., Cirule, D., Krama, T., Vrublevska, J., 2011. Extremely low ambient temperature affects haematological parameters and body condition in wintering great tits (Parus major). J. Ornithol. 152, 889–895.
Laursen, K., Frikke, J., 2008. Hunting from motorboats displace Wadden Sea Eiders Somateria mollissima from their favoured feeding distribution. Wildl. Biol. 14, 423–433.
Laursen, K., Møller, A.P., 2014. Long-term changes in nutrients and mussel stocks are related to numbers of breeding eiders Somateria mollissima at a large Baltic colony. PLoS One 9, e95851.
Le Maho, Y., Vu Van Kha, H., Koubi, H., Dewasmes, G., Girard, J., Ferré, P., Cagnard, M., 1981. Body composition, energy expenditure, and plasma metabolites in long-term fasting geese. Am. J. Phys. 241, E342–E354.
Lierz, M., 2003. Avian renal disease: pathogenesis, diagnosis, and therapy. Vet. Clin. North Am. Exot. Anim. Pract. 6, 29–55.
Lumeij, J.T., 2008. Avian clinical biochemistry. In: Kaneko, J.J., Harvey, J.W., Bruss, M.L. (Eds.), Clinical Biochemistry of Domestic Animals, 6th edition Academic Press, San Diego, California, USA (839-872 pp).
Lumeij, J.T., Westerhof, I., 1987. Blood chemistry for the diagnosis of hepatobiliary disease in birds. A review. Vet. Q. 9, 255–261.
Lyngs, P., 1992. Ynglefuglene på Græsholm 1925–1990. vol. 94. Dansk Orn Foren Tidsskr, pp. 12–18 (In Danish with an English summary).
Lyngs, P., 2014. Christiansø Fieldstation. http://www.chnf.dk/aktuelt/edf14/edfugl14.php (In Danish).
Madin, K., 2009. Scientists investigate mysterious duck die-offs: what caused five mass mortalities of northern eiders on Cape Cod since 2006? Oceanus 47, 34–36.
Robin, J.P., Cherel, Y., Girard, H., Géloen, A., Le Maho, Y., 1987. Uric acid and urea in relation to protein catabolism in long-term fasting geese. J. Comp. Physiol. B. 157, 491–499.
Robin, J.P., Frain, M., Sardet, C., Groscolas, R., Le Maho, Y., 1988. Protein and lipid utilization during long-term fasting in emperor penguins. Am. J. Phys. 254, R61–R68.
Samour, J., 2006. Diagnostic value of hematology. In: Harrison, J.G., Lightfoot, T. (Eds.), Clinical Avian Medicine. vol. Volume 2. Florida Spix Publishing, pp. 587–610.
Smith, S.A., 1993. Diagnosis and treatment of hemints in birds of prey. Raptor Biomededicin, pp. 21–27.

Sonne, C., Rigét, F.F., Leat, E.H.K., Bourgeon, S., Borgå, K., Strøm, H., Hanssen, S.A., Gabrielsen, G.W., Petersen, Æ., Olafsdottir, K., Magnusdottir, E., Bustnes, J.O., Furness, R.W., Kjelgaard-Hansen, M., 2013. Organohalogen contaminants and blood plasma clinical-chemical parameters in three colonies of North Atlantic Great skua (Stercorarius skua). Ecotoxicol. Environ. Saf. 92, 245–251.

Stevens, L., 1996. Metabolic adaptation in avian species. In: Stevens, L. (Ed.), Avian Biochemistry and Molecular Biology. University Press, Cambridge. Cambridge. United Kingdom (82–99 pp).

Thrall, M.A., Baker, D.C., Campbell, T.W., DeNicola, D., Fettman, M.J., Lassen, E.D., Rebar, A., Weiser, A., 2006. Veterinary Hematology and Clinical Chemistry: Text and Clinical Case Presentations Set. Blackwell Publishing, Iowa, USA, p. 618.

Williams, T.D., 2012. Physiological Adaptations for Breeding in Birds. Princeton University Press (368 pp., illus).