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Contaminants of emerging concern in an endangered population of common eiders (*Somateria mollissima*) in the Baltic Sea ☆

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ABSTRACT

Contaminants of emerging concern (CECs) are ubiquitous in aquatic environments and pose a range of biological effects including endocrine disruption. Yet, knowledge of their occurrence in wildlife including seabirds remains scarce. We investigated the occurrence of selected bisphenols, benzophenones, phthalate metabolites, benzotriazoles, benzothiazoles, parabens, triclosan, and triclocarban in plasma of 18 breeding female common eiders (*Somateria mollissima*) from an endangered population in the Baltic Sea as most of these CECs have never before been examined in eiders. We sampled blood at the start (T1) and end (T2) of incubation to investigate concentration changes during incubation. As early- and late-breeding eiders tend to differ in how they finance reproduction (local vs stored nutrient reserves), we compared early and late breeders to assess whether CEC concentrations differed by breeding phenology. Of the 58 targeted CECs, 21 were detected in at least one female, with bisphenol A (BPA) and benzophenone-3 (BzP-3) occurring most frequently (T1: 78% and 61%; T2: 61% and 67%, respectively), while mono(2-ethyl-1-hexyl) phthalate (mEHP), BPA, and monoethyl phthalate (mEP) were detected in the highest concentrations (median concentrations 27.1, 12.7, and 11.2 ng/g wet weight, respectively, at T1). No CEC concentrations differed between early and late incubation. Late breeders had significantly higher concentrations of BzP-3, monomethyl phthalate (mMP), and mEP during early incubation (4.55 vs 1.24 ng/g ww, 7.05 vs 3.52, and 11.2 vs < limit of detection (LOD), respectively) and significantly higher concentrations of mMP and mEP during late incubation (6.16 vs <LOD and 7.51 vs <LOD, respectively) than early breeders. We showed that early and late breeders exhibited differential exposure to CECs. Our results support the need for long-term monitoring of CECs in eiders. Furthermore, it is important to examine these CECs in the eiders' prey species from their wintering and breeding grounds.

1. Introduction

Contamination of the aquatic environment by contaminants of emerging concern (CECs) is a global problem as chemicals enter the aquatic environment through municipal and industrial wastewater, urban run-off, and landfill leachates (Nanusha et al., 2023, 2022; Nilsen

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et al., 2019). The term contaminants of emerging concern encompasses a large and heterogenous group of chemicals which are often, but not necessarily, unregulated and for which there are (eco)toxicological concerns (Halden, 2015; Sauvé and Desrosiers, 2014). Here, CECs is used as a relative term to distinguish these substances from, e.g., persistent organic pollutants for which there are decades of ecotoxicological research and for which global regulations are in place. The Baltic Sea remains one of the most polluted aquatic ecosystems worldwide (HELCOM, 2023; Kanwischer et al., 2022), but the number of monitored contaminants is limited and there is a lack of knowledge regarding CECs (Kanwischer et al., 2022).

The Baltic Sea population of common eiders (*Somateria mollissima*, hereafter eider) has been declining dramatically for the past decades (Ekroos et al., 2012) and the species is listed as endangered in Europe (BirdLife International, 2021). Currently, there is very little knowledge regarding the occurrence of CECs in eiders (Huber et al., 2015). However, previous studies of eiders from the Baltic Sea have demonstrated that they are exposed to contaminants that likely affect their physiology and biochemical endpoints (e.g. Fenstad et al., 2016; Lam et al., 2020; Sonne et al., 2020). Furthermore, the extensive mass loss during incubation in this capital breeder causes re-mobilization of contaminants from lipid and, later, protein stores which may elevate contaminant levels in the blood (Bustnes et al., 2012, 2010; Criscuolo et al., 2002; McPartland et al., 2020). Thus, it is particularly important to expand the investigation of eiders' contaminant exposure as a first step in assessing whether this exposure may place an additional strain on this endangered population. Here, we focus on bisphenols, benzophenones, phthalate metabolites, benzotriazoles, benzothiazoles, parabens, triclosan, and triclocarban. These are used in large volumes and have previously been detected in the eiders' main prey species—blue mussel (*Mytilus* sp.) (Öst and Kilpi, 1998)—and/or other filter-feeding mollusks (Castro et al., 2022; García-Fernández et al., 2022; Jia et al., 2019; Lu et al., 2019; Rios-Fuster et al., 2022; Staniszewska et al., 2017), but there is very little information on these CECs in eiders. Furthermore, laboratory animal models show that these CECs are associated with adverse biological effects such as endocrine disruption (Fent et al., 2014; Halden et al., 2017; Hornung et al., 2015; Kraft et al., 2023; Lee et al., 2018; Liu et al., 2021; Mustieles et al., 2023; Nowak et al., 2018; Rochester and Bolden, 2015; Sohn et al., 2016).

Bisphenols are high production volume chemicals used in the production of, for instance, polycarbonate plastics, epoxy resins, and thermal paper (Geens et al., 2012). Scientific and public concerns over bisphenol A's (BPA) endocrine activity led to its usage being restricted (European Union, 2011) and BPA analogues are being used as substitutes with bisphenol AF (BPAF), bisphenol S (BPS), and bisphenol F (BPF) being the most frequently used substitutes (Czarny-Krzymińska et al., 2023). Of these, BPS is on the list of substances of very high concern and other analogues are also under evaluation and assessment in the European Union (ECHA, 2024).

Benzophenone (BzP) and its derivatives are organic chemicals absorbing ultraviolet (UV) radiation and therefore commonly used as sunscreen UV filters (Li and Kannan, 2022). Benzophenone-3 (BzP-3) is the main BzP-type UV filter (Li and Kannan, 2022), but benzophenone-1 (BzP-1), −2 (BzP-2), and −4 (BzP-4) are also frequently detected in surface water and wastewater plant effluents (Carstensen et al., 2023).

Phthalates are the most commonly used plasticizers globally (European Plasticisers, 2021), but they are also found in, for example, personal care products and household cleaning products (Cacho et al., 2015; Schettler, 2006). These chemicals are widespread in the environment (Net et al., 2015) with di(2-ethyl-1-hexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), di-iso-butyl phthalate (DIBP), and diethyl phthalate (DEP) being among the phthalates most frequently detected in biota (Hidalgo-Serrano et al., 2022). Indeed, phthalates are so ubiquitous that field and laboratory contamination of samples is an issue (Ikonomou et al., 2012). Therefore, their biological metabolites are preferred as biomarkers of phthalate exposure (Asimakopoulos et al., 2016).

Another group of CECs is the benzotriazoles, which are corrosion inhibitors found in, e.g., aircraft deicing fluids, dishwasher detergents, and hydraulic fluids (Janna et al., 2011; Pillard et al., 2001). Of these, 1H-benzotriazole (BTR) and tolyltriazole (TTR) have been the most investigated (Herrero et al., 2014). Another group is the benzothiazoles used as vulcanizing accelerators in rubber production (Reddy and Quinn, 1997), corrosion inhibitors (Milanova et al., 2001), and fungicides in the tanning and timber industries (Brownlee et al., 1992; Reemtsma et al., 1995). 1H-benzothiazole (BTH), 2-hydroxy-benzothiazole (2-OH-BTH), and 2-methylthio-benzothiazole (2-Me-S-BTH) are the most studied of the benzothiazoles (Herrero et al., 2014).

The last group of CECs considered in this study comprises parabens, triclosan, and triclocarban which are antimicrobial and used as preservatives in personal care products, pharmaceuticals, and food (Adolfsson-Erici et al., 2002; Chalew and Halden, 2009; Wei et al., 2021). Of the parabens, methyl (MetP) and propyl paraben (PrP) are most frequently detected in aquatic environments worldwide (Wei et al., 2021).

The aim of this study was to investigate the aforementioned CECs in plasma of eiders breeding at a Finnish colony in the Baltic Sea. Only female eiders incubate and they fast for the circa 26-day long incubation (Garbus et al., 2018; Korschgen, 1977). This fasting period has previously been shown to increase concentrations of circulating persistent organic pollutants and metals (Bustnes et al., 2012, 2010; McPartland et al., 2020), but it remains unknown how CEC concentrations may change during the course of the incubation. To examine this, we sampled females at the start and end of their incubation; as this is the first study investigating this with these CECs, we do not have grounds for predictions. A further knowledge gap is how breeding phenology may affect the CEC concentrations, as early- and late-breeding birds may differ regarding their use of local versus stored nutrient reserves for financing reproduction (Jaatinen et al., 2016; Sénéchal et al., 2011). Previous research has found the targeted CECs partitioning into tissues of birds and other wildlife (Castro et al., 2023; González-Rubio et al., 2020; Vorkamp et al., 2004; Xue and Kannan, 2016; Yao et al., 2016). Thus, the CEC signal in the early breeders' plasma may more closely reflect the environmental contamination of the wintering grounds (southern Baltic Sea and the North Sea; Laursen et al., 2019) than that of the late breeders. Hence, we predict that the concentrations of CECs will differ between early and late breeders, but the lack of information on CEC concentrations in seawater and blue mussels from these locations precludes us to predict the direction of the difference.

2. Materials and methods

2.1. Plasma samples

Breeding eider females ($n = 18$) were caught on their nest with a hand net in April–June 2021 at the Bengtskär colony (59.72 N, 22.50 E), outer Archipelago Sea, Finland. All handling procedures were approved by the Animal Experiment Board at the State Provincial Office of Southern Finland (permit no. ESAVI/9500/2021) and the Finnish Wildlife Agency (permit no. 2021-5-000-18962-8). The ringing license was issued by the Finnish Museum of Natural History to C. Arzel and access to the island for research was granted by Bengtskär Oy. All females were caught and sampled twice: once during early incubation (T1; after we had observed the female on the nest every day for 3–4 days) and again during late incubation (T2; 19.1 ± 1.43 days after the first sampling). The first observation of incubating females was on April 20 and we took the first sample from the first group of birds — termed "early breeders" ($n = 10$ females) — April 28–29 and the first sample from the second group — "late breeders" ($n = 8$ females) — May 15–16. Blood was collected from the brachial vein using an unheparinized needle (BD Microlance 3. 23G x 1″, BD, U.S.) and syringe (for the early breeders' T1 samples: Fisherbrand 10 mL, Thermo Fisher Scientific, U.S.; all other samples: BD Emerald 5 mL, BD, U.S.). The blood was immediately transferred to a BD Vacutainer blood collection tube containing lithium heparin (BD, U.S.) and centrifuged within 8 h (2500 rpm, 10 min; EBA 270, Hettich, Germany); the blood was kept cold and in the dark until centrifugation. After centrifugation, the plasma was transferred to an Eppendorf tube and frozen at −20 °C while in the field. Blanks ($n = 3$), consisting of water (molecular biology grade, Thermo Fisher Scientific, U.S.), were taken to account for contamination from sampling equipment by drawing water up in a syringe, transferred to a vacutainer, centrifuged, transferred to an Eppendorf, and finally, frozen. The plasma samples and blanks were transferred to a −80 °C freezer upon returning to the University of Turku, Finland. The samples were sent to the Norwegian University of Science and Technology (NTNU) in Trondheim on dry ice for chemical analyses. The samples were immediately transferred to a −80 °C freezer upon arrival.

2.2. Chemicals and reagents

LC-MS grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). β-Glucuronidase (type HP-2, ≥100 000 units/mL), ammonium acetate, and formic acid (98% v/v) were obtained from Sigma-Aldrich (Steinheim, Germany). Ultrapure water came from a Milli-Q grade purification system (Q-option, Elga Labwater, Veolia Water Systems LTD, UK). The hybridSPE®-Phospholipid cartridges (Supelco, bed wt. 30 mg, 1 mL) were purchased from Sigma-Aldrich (Steinheim, Germany). The supplementary material lists all the 58 chemicals we targeted and their respective CAS numbers (Table S1). Analytical standards of the phthalate metabolites were purchased from Chiron AS (Trondheim, Norway) and the analytical standards for the remaining chemicals were all purchased from Sigma-Aldrich (Steinheim, Germany). Isotopically labelled internal standards of BPA-^{13}C6, BPF-^{13}C6, BPS-^{13}C6, BPAF-^{13}C12, and BzP-3-^{13}C6 were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). The isotopically labelled internal standards of mEP-d4, mBP-d4, mNP-d4, and BTH-d4 were purchased from Chiron AS (Trondheim, Norway). Isotopically labelled standards of MetP-^{13}C6, EtP-^{13}C6, PrP-^{13}C6, and BuP-^{13}C6 were purchased from Supelco (Merck, Darmstadt, Germany).

2.3. Sample preparation for chemical analyses

Sample preparation for the phthalate metabolites followed Asimakopoulos et al. (2016) and Rian et al. (2020) with minor modifications. Briefly, 100 mg of plasma was weighed into a 1.5 mL Eppendorf tube then 10 μL of internal standards (i.e. 10 ng of each) and 50 μL of 1 M ammonium acetate (containing 22 units β-glucuronidase) were added. The samples were incubated at 37 °C and 220 rpm for 16 h. Next, 450 μL 10% formic acid in acetonitrile was added to each tube which was then vortexed for 30 s and centrifuged at 4000 rcf for 10 min. HybridSPE cartridges were conditioned with 1 mL 10% formic acid in acetonitrile before the supernatant was added to the cartridge. The sample was eluted into 15 mL polypropylene tubes and the solvent evaporated under a gentle stream of nitrogen. Finally, the samples were reconstituted with 500 μL acetonitrile:Milli-Q water (1:9 v/v), centrifuged at 4000 rcf for 5 min, and the supernatant transferred to a glass autosampler vial for ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) analysis.

The sample preparation for the other compounds (i.e. bisphenols, benzophenones, benzotriazoles, benzothiazoles, parabens, triclosan, and triclocarban) was as follows: 100 mg of plasma was weighed into a 1.5 mL Eppendorf tube, then 10 μL of internal standards (i.e. 10 ng of each) and 300 μL 1% formic acid in methanol were added. The tubes were then vortexed for 30 s before placed in the ultrasonication bath (at 30 °C) for 30 min. After ultrasonication the tubes were centrifuged at 3500 rpm for 5 min. HybridSPE cartridges were conditioned with 1 mL 1% formic acid in methanol before the supernatant was added and eluted into 15 mL polypropylene tubes. Then, 150 μL of the eluate was transferred to an amber glass vial with 150 μL glass insert for UPLC-MS/MS analysis.

2.4. UPLC-MS/MS analysis

The samples were analyzed using UPLC (Waters, Milford, U.S.) hyphenated to a triple quadrupole mass analyzer (Xevo TQ-S, Waters, Milford, U.S.). We used four different validated methods to analyze the different families of CECs: 1) bisphenols and benzophenones; 2) phthalate metabolites; 3) benzotriazoles and benzothiazoles; and 4) parabens, triclosan, and triclocarban. Further information on the instrumental analyses can be found in Table S2.

2.5. Quality assurance and quality control (QA/QC)

A calibration curve with concentrations from 0.01 to 50 ng/mL was made. The calibration curves of most compounds showed satisfactory coefficients of determination ($R^2 > 0.990$ for all compounds apart from TTR ($R^2 = 0.989$), BTH (0.985), 2-ABTH (0.984), 2-Cl-BTH (0.982), and 2-M-BTH (0.980)). However, for triclosan, PA, 4-OH-BzP, and BPF the calibration curves failed and hence these compounds have been excluded from the data analyses.

Pre- and post-extraction spiked matrix samples were used as QA/QC samples and were made by spiking a known amount of target analytes and internal standards before and after sample preparation, respectively (Table S3). For the absolute and relative recoveries, please see Table S4. The recoveries of 4-HB, 3,4-DHB, OH-EtP, BzP-8, BzP-2, and BTR-COOH were unacceptably low, and these compounds have been excluded from the data analyses. To account for the instrumental drift and carry-over, a calibration standard and a solvent blank were injected after every 25th sample injection. Four plasma samples were analyzed in duplicate and the variation ranged from 5.15 to 38.3% (Table S5).

Quantification of the target analytes was done based on the internal standard method and with relevant matrix-matched calibration standards. After calculating the limit of detection (LOD; see supplementary material equation (3)), the following compounds were detected in at least one sample and are the compounds included in the data analyses: BPA, BPAF, BPS, BzP-1, BzP-3, mMP, mEP, mBP, mIBP, mHxP, mDP, mEHP, TTR, 2-OH-BTH, 2-S-BTH, 2-SCNMeS-BTH, MetP, EtP, PrP, BuP, and BezP. The calculated LOD and limit of quantification (LOQ) values per compound are presented in Table 1 and Table S6, respectively. Blanks were analyzed to account for background contamination coming from the field and laboratory and the concentrations are listed in Table S7. Due to analytical issues, mMP and mEP were re-analyzed but not all samples could be re-analyzed due to plasma volume constraints leading to a lower sample size for these compounds ($n = 14$ females, as opposed to $n = 18$ for all other CECs).

2.6. Data analysis

Data analysis was performed using Python (v. 3.9.12, Python Software Foundation) and R (v. 4.1.1, R Core Team, 2024). The dataset is available on Zenodo (https://doi.org/10.5281/zenodo.10405104) and the scripts are available on GitHub (https://github.com/amalieask/EiderContaminantsAnalysis). The sample concentrations were blank-corrected by subtracting the mean concentration of the blanks from each of the sample concentrations.

For the summary statistics presented in Table 1, only observations above the LOD were included in the calculations and all compounds for which there was at least one sample above LOD were included following González-Rubio et al. (2020). However, to facilitate literature comparisons we also calculated the median using all observations. The profile plot was created using the median concentrations of only those observations which were above the LOD (as per González-Rubio et al., 2020). To examine whether CEC concentrations changed over the incubation and/or across the breeding season, we included only those compounds

Table 1

Occurrence of contaminants of emerging concern in paired plasma samples of incubating female common eiders from Bengtskär, Finland, 2021. The first and second samples were taken at approx. day 4 (T1) and day 24 (T2) of the incubation, respectively. Median$_{\text{all}}$ is calculated using all observations, while median $_{> \text{LOD}}$, minimum (Min), and maximum (Max) are calculated using only observations above the limit of detection (LOD). Concentrations are given in ng/g wet weight. Detection rate (DR) presents the number of samples above LOD out of the total number of samples analyzed for that compound.

Compound	LOD	First plasma sample (T1)					Second plasma sample (T2)				
		DR	Median$_{\text{all}}$	Median $_{> \text{LOD}}$	Min	Max	DR	Median$_{\text{all}}$	Median $_{> \text{LOD}}$	Min	Max
BPA	6.21	14/18	10.8	12.7	7.85	46.6	11/18	10.2	17.9	8.05	87.8
BPS	0.06	0/18					6/18	<0.06	0.19	0.11	0.36
BPAF	0.02	2/18	<0.02	0.07	0.04	0.09	5/18	<0.02	0.04	0.03	0.06
BzP-1	0.66	2/18	<0.66	0.97	0.70	1.24	3/18	<0.66	0.90	0.68	2.30
BzP-3	0.87	11/18	1.81	2.56	1.23	9.58	12/18	1.43	2.55	1.01	14.8
mMP	1.79	7/14	<1.79	4.12	2.04	8.85	4/14	<1.79	6.16	4.73	7.33
mEP	2.08	4/14	<2.08	11.2	6.23	25.2	4/14	<2.08	7.51	5.54	10.5
mBP	3.61	1/18	<3.61	6.42	6.42	6.42	0/18				
mIBP	1.81	1/18	<1.81	1.89	1.89	1.89	0/18				
mHxP	0.45	0/18					1/18	<0.45	0.73	0.73	0.73
mDP	1.33	3/18	<1.33	5.70	1.35	6.60	2/18	<1.33	3.47	1.59	5.35
mEHP	10.1	4/18	<10.1	27.1	11.9	52.2	5/18	<10.1	17.8	10.1	25.0
TTR	0.61	0/18					1/18	<0.61	0.85	0.85	0.85
2-OH-BTH	0.60	1/18	<0.60	1.57	1.57	1.57	0/18				
2-S-BTH	0.29	1/18	<0.29	0.58	0.58	0.58	2/18	<0.29	3.15	0.34	5.96
2-SCNMeS-BTH	0.05	1/18	<0.05	0.10	0.10	0.10	0/18				
MetP	0.21	0/18					2/18	<0.21	0.68	0.51	0.85
EtP	0.03	3/18	<0.03	0.06	0.03	0.07	1/18	<0.03	0.03	0.03	0.03
PrP	0.01	3/18	<0.01	0.03	0.02	0.05	3/18	<0.01	0.05	0.03	0.09
BuP	0.01	3/18	<0.01	0.02	0.02	0.02	0/18				
BezP	0.06	0/18					2/18	<0.06	0.11	0.09	0.13

which had at least 60% of observations above LOD in at least one group. The observations below LOD were replaced by 1/2LOD. All observations (not just those above LOD) were used when testing these differences. The Shapiro-Wilk and Levene's tests were used to test the assumptions of normality and homoscedasticity, respectively, and depending on the results concentration differences between T1 and T2 were tested with either the paired *t*-test or Wilcoxon signed-rank test. Likewise, concentration differences between early and late breeders were tested with either the independent *t*-test or the Mann-Whitney *U* test. The absolute mean difference is given as an unstandardized effect size, as most compounds were not normally distributed with equal variance which precluded using, e.g., Cohen's *d* or Hedge's *g* (Nakagawa and Cuthill, 2007; Peng and Chen, 2014). To account for multiple testing, the *p*-values were adjusted using the Benjamini-Hochberg false discovery rate. The results of the statistical tests are interpreted using the language of evidence (Muff et al., 2022), wherein little or no evidence corresponds to *p*-values above 0.1, weak evidence to *p*-values between 0.05 and 0.1, moderate evidence to *p*-values between 0.01 and 0.05, and strong evidence to *p*-values below 0.01.

3. Results and discussion

3.1. CEC concentrations and profiles

We detected 21 of the 58 targeted CECs in female eiders during early and late incubation (Tables 1, S8, and S9). Considering only samples above the LOD for early and late breeders together, BPA, mEHP, and mEP were the three CECs with highest median concentrations at both timepoints (T1: 12.7 ng/g ww, 27.1, and 11.2, respectively; T2: 17.9, 17.8, and 7.51, respectively). The most frequently detected CECs at both timepoints were BPA (T1: 14/18 females; T2: 11/18) and BzP-3 (T1: 11/18; T2: 12/18). In addition, for the late breeders only, BPS was detected in 5 out of 8 females at T2 (it was not detected at T1) and mMP and mEP were detected in all analyzed samples for both T1 (4/4 females) and T2 (4/4 females).

Based on the 2021 data reported to KemiDigi (Table S10) — a registry of chemicals on the Finnish market — BPA was the chemical registered for use in the greatest number of products and at the highest mass (89 products and 7.12 tonnes, respectively) of the 58 CECs investigated in our study. This may help explain why BPA was the most frequently occurring CEC as well as among the CECs occurring at the highest concentrations. mEHP and mEP are biological metabolites whose parent compounds are DEHP and DEP, respectively (Table S11). In 2021, DEP was used in 70 products with 1.56 tonnes being placed on the market. Whereas, for DEHP, the information is categorized as "confidential" meaning that either less than 4 products contained DEHP, or less than 3 companies had registered the chemical. While the prevalence of BPA and, to a lesser extent, mEP in the eiders is reflected in the KemiDigi data (DEP instead of mEP), we do not see this for DEHP – likely due to the restrictions placed on DEHP (ECHA, 2023). However, contaminant discharges into the Baltic Sea is an international problem (HELCOM, 2023) and Finnish eiders are also exposed at their Danish wintering sites (Lehikoinen et al., 2008), as well as on their passage to and from these wintering sites, which could explain the apparent discrepancy between the occasionally high concentrations of mEHP in the plasma and Finland's apparent modest use of DEHP.

3.1.1. Bisphenols

In addition to BPA and BPS we also detected BPAF in the eiders (Table 1), and this is the first-time bisphenols have been reported for this species. BPA has been detected in plasma of bald eagle chicks (*Haliaeetus leucocephalus*), European starlings (*Sturnus vulgaris*), tree swallows (*Tachycineta bicolor*), and double-crested cormorants (*Phalacrocorax auritus*) from North America at median concentrations of around 0.50–1.0 ng/mL (Elliott et al., 2019; Gewurtz et al., 2021). These concentrations are an order of magnitude lower than in the eiders (median$_{\text{all}}$ concentrations 10.2–10.8 ng/g ww) — assuming 1 mL plasma weighs approximately 1 g — potentially reflecting the contaminated state of the Baltic Sea. In a report by Ruus et al. (2018) one out of 30 herring gulls (*Larus argentatus*) from the Oslofjord, Norway had BPA above LOD, at 82 ng/g ww blood, which is similar to the maximum BPA concentration of 87.8 ng/g ww in the current study. The rank order of median concentrations (above LOD) for detected bisphenols is BPA (12.7 ng/g ww) > BPAF (0.07) for early incubation and BPA (17.9) > BPS (0.19) > BPAF (0.04) for late incubation (Fig. 1). The three bisphenols which were detected in the eiders (i.e. BPA, BPAF, and BPS) were also the only three bisphenols placed on the Finnish market in 2021 (Table S10). González-Rubio et al. (2020) found a median rank order of BPA (67.5

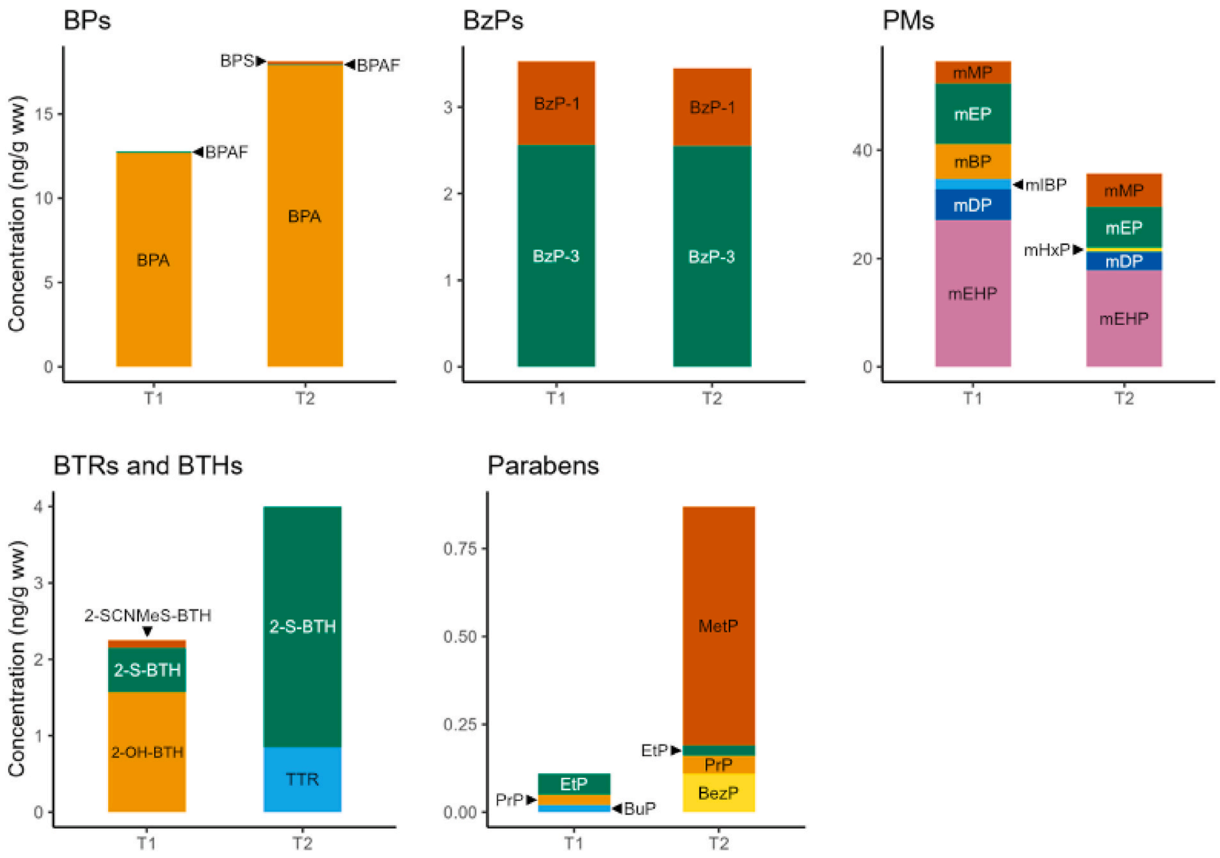

Fig. 1. Profiles of the detected compounds in plasma samples from early (T1) and late (T2) incubation of female eiders ($n = 18$) from Bengtskär, Finland, 2021. The data are shown per compound class, i.e. bisphenols (BPs), benzophenones (BzPs), phthalate metabolites (PMs), benzotriazoles and benzothiazoles (BTRs and BTHs), and parabens. Median concentrations (only observations > LOD) were used in the figure. Note the difference in scale for the y-axes.

ng/g ww) > BPF (3.01) > BPB (1.60) > BPAP (0.81) > BPS (0.65) > BPM/BPP (0.08) > BPAF (not detected) in various tissues from raptors while Oró-Nolla et al. (2021) found BPA (6.37 ng/g ww) > BPAF (2.52) > BPS (not detected) in white-tailed eagle livers. Direct comparison between our study and these two studies is hampered by differences in species, trophic position, sample matrices, sampling years, and locations. Nevertheless, BPA is the bisphenol with highest median concentration across all three studies, likely attributable to BPA remaining the dominant bisphenol in terms of production volume.

3.1.2. *Benzophenones*

We detected BzP-3 and BzP-1 in the eiders (Table 1). Apart from one study of BzP-3 in herring gull blood — which did not detect BzP-3 in any of the 30 gulls sampled (Ruus et al., 2018) — we could not find any other study investigating benzophenones in plasma or blood of wild birds, but they have been detected in tissues of birds of prey (González-Rubio et al., 2020; Oró-Nolla et al., 2021). For the benzophenones, the rank order in the eiders is BzP-3 (2.56 ng/g ww) > BzP-1 (0.97) for early and BzP-3 (2.55) > BzP-1 (0.90) for late incubation (Fig. 1). The dominance of BzP-3 over BzP-1 may be due to its higher production volume in Finland (Table S10), but BzP-1 is, in addition to being manufactured, a major metabolite of BzP-3 whose other metabolites include BzP-2, BzP-8, and 4-OH-BzP (Mutlu et al., 2020). Indeed, in the studies by González-Rubio et al. (2020) and Oró-Nolla et al. (2021), the benzophenones found in various tissues at highest median concentrations were metabolites of BzP-3: BzP-8 (27.1 ng/g ww) > 4-OH-BzP (9.70) > BzP-1 (0.95) > BzP-3 (0.47) > BzP-2 (not detected) (González-Rubio et al., 2020) and BzP-8 (6.32 ng/g ww) > BzP-1 (2.89) > BzP-2 (2.17) > 4-OH-BzP (0.38) (Oró-Nolla et al., 2021), but note that Oró-Nolla et al. (2021) did not actually target BzP-3 itself. Unfortunately, as explained earlier, BzP-2, BzP-8, and 4-OH-BzP had to be excluded from our analysis (Table S4) hindering further comparison.

3.1.3. *Phthalate metabolites*

We found mEHP, mMP, mEP, mBP, mIBP, mDP, and mHxP in the eiders (Table 1). In birds, while parent phthalates have been investigated in different tissues (Hardesty et al., 2015; Huber et al., 2015; Sühring et al., 2022), as far as we know only two studies have examined phthalate metabolites in plasma, namely, in northern fulmar (*Fulmarus glacialis*) fledglings from Svalbard (Collard et al., 2024) and bald eagle chicks from USA (Elliott et al., 2019). In the fulmar fledglings' plasma, mBP was detected most frequently (5/15 fledglings; maximum concentration of 2.31 ng/mL) whereas, in the eiders, mBP was detected in only one plasma sample (T1) but at higher maximum concentration (6.42 ng/g ww). Overall, mEP was the chemical with highest maximum concentration in the fulmars (6.99 ng/mL; detected in 1/15 fledglings), which, in the eiders, reached 25.2 ng/g ww. The higher concentrations found in the Baltic eiders likely reflect differences in environmental contamination as the fulmars were caught in the Arctic. The bald eagle chicks had a median plasma mEHP concentration of 1.2 ng/mL whereas, when calculating the median for all observations in the eiders to match the method by Elliott et al. (2019), the concentrations were below the LOD at both timepoints in the eiders (Table 1, $\text{median}_{\text{all}}$). This lower concentration in eiders compared to the bald eagle chicks likely reflects the lower trophic position of eiders. In addition, the LOD for mEHP in our study was high (10.1 ng/g) while the lowest detected concentration was 0.27 ng/mL in Elliott et al. (2019), and thus we probably underestimate the contamination in the eiders. The median rank order of phthalate metabolites in the eiders is mEHP (27.1 ng/g ww) > mEP (11.2) > mBP (6.42) > mDP (5.70) > mMP (4.12) > mIBP (1.89) for early incubation and mEHP (17.8) > mEP (7.51) > mMP (6.16) > mDP (3.47) > mHxP (0.73) for late incubation (Fig. 1). Comparing these values to the information on parent phthalates on the Finnish market in 2021 (Table S10), the prevalence of mEP and mMP is consistent with their parent phthalate's positions as the third (1.56 tonnes, DEP) and second (1.88 tonnes, DMP) highest market volume, respectively. Only dicyclohexyl phthalate was placed on the market in higher quantities (3.65 tonnes) in 2021 but, interestingly, its metabolite, mCHP, was not detected in any of the eider samples. The entries for the parent phthalates of mBP (di-n-butyl phthalate and benzyl butyl phthalate) and mIBP (di-iso-butyl phthalate) are marked "confidential" (Table S10), whereas the parent phthalates of mDP and mHxP were not registered on the Finnish market at all in 2021. Yet, the detection of mBP and mIBP in polar bears (*Ursus maritimus*) from Svalbard demonstrates that these compounds may be detected in wildlife even without any immediate (known) point sources (Routti et al., 2021). Lastly, it is interesting that mEOHP and mEHHP were not detected in the eiders as, like mEHP, they are metabolites of DEHP (Table S11). However, previous research demonstrates that there are notable species differences in the biotransformation of DEHP with regards to the prevalence of which metabolites are formed (Albro et al., 1982) which may explain why mEOHP and mEHHP were not detected in the eiders if DEHP is primarily metabolized into mEHP.

3.1.4. Benzotriazoles and benzothiazoles

We detected TTR in one sample at 0.85 ng/g ww (Table 1). To our knowledge there is only one other study on benzotriazoles in birds: Gkotsis et al. (2023) detected TTR in one egg from Eurasian curlew (*Numenius arquata*), however at a concentration below the LOQ (4.64 ng/g ww). As for the benzothiazoles, we detected 2-OH-BTH (median concentration: T1 = 1.57 ng/g ww and T2 = not detected), 2-S-BTH (T1 = 0.58 and T2 = 3.15), and 2-SCNMeS-BTH (T1 = 0.10 and T2 = not detected) in one, three, and one plasma sample(s), respectively (Table 1). This is, to the best of our knowledge, the first time 2-OH-BTH and 2-SCNMeS-BTH are reported for a bird precluding comparison with previous avian studies, but they have previously been detected in marine invertebrates and fish (Castro et al., 2023; Jia et al., 2019). A non-target screening study tentatively identified 2-S-BTH in common guillemot (*Uria aalge*) eggs from the Baltic Sea, but as it was a non-targeted approach, no concentrations are available to compare with (Rebryk et al., 2022). The eiders' median rank order for benzotriazoles and benzothiazoles is 2-OH-BTH (1.57 ng/g ww) > 2-S-BTH (0.58) > 2-SCNMeS-BTH (0.10) for early incubation and 2-S-BTH (3.15) > TTR (0.85) for late incubation (Fig. 1). Only 2-S-BTH and BTR were registered on the Finnish market in 2021 (Table S10) at 6.04 and 3.43 tonnes each. We observed an increase in the concentration of 2-S-BTH from early to late incubation and previous research on rats has shown that 2-SCNMeS-BTH is primarily metabolized into 2-S-BTH (Manninen et al., 1996). However, biotransformation of 2-SCNMeS-BTH into 2-S-BTH is not a likely explanation for the increase in 2-S-BTH in the eiders as no eiders had detectable concentrations of 2-SCNMeS-BTH in their first plasma sample, and the eiders who had detectable concentrations of 2-SCNMeS-BTH in their second plasma sample had no detectable concentrations of 2-S-BTH. The reason for the increase of 2-S-BTH from early to late incubation could be due to its re-distribution from tissues as the female fasts or local exposure and needs to be studied further. There are extremely few studies of benzotriazoles and benzothiazoles in wildlife and there is an urgent need to examine these CECs in more species from more locations across the world to assess the risks they may pose to wildlife and humans alike.

3.1.5. Parabens and triclocarban

We detected MetP, EtP, PrP, BuP, and BezP in the eiders, for the first time in this species. The parabens were found at low detection rates and in low concentrations (ranging from 0.02 to 0.85 ng/g ww) (Table 1). MetP, PrP, BuP, and the metabolite 4-HB have previously been found in plasma from bald eagle chicks (Elliott et al., 2019; Xue and Kannan, 2016). While the median concentrations of PrP and BuP were an order of magnitude higher in the bald eagle chicks (0.73 ng/mL and 0.27, respectively) (Elliott et al., 2019) than in the eiders, Xue and Kannan reported a mean concentration of 0.09 ng/mL for MetP which is slightly lower than the concentrations we detected in the two eiders which had MetP > LOD (0.51 ng/g ww and 0.85). Interestingly, Xue and Kannan found that the concentration of 4-HB (28.6 ng/mL) was much higher than MetP and this pattern was also observed in white-tailed eagle livers from the Baltic Sea (mean concentration MetP: 112 ng/g ww, 4-HB: 11500) and other birds. The high concentration of 4-HB demonstrates the importance of also targeting metabolites when assessing the exposure of wildlife to these compounds and it is unfortunate that the recovery of 4-HB (and the other metabolites) was too low to be included in our study. EtP and BuP have been detected in a few liver samples of white-tailed eagles (maximum concentrations of 81.8 ng/g ww and 24, respectively; Xue and Kannan, 2016) and black-tailed godwits (*Limosa limosa*) (maximum concentrations of 7.36 and 65.2 ng/g ww, respectively; Movalli et al., 2023). All were found at considerably higher concentrations than those found in the eiders, probably due to the difference in trophic position in the case of the eagles, but also possibly the role of the liver in biotransformation. The eiders' median rank order of parabens is EtP (0.06 ng/g ww) > PrP (0.03) > BuP (0.02) for early incubation and MetP (0.68) > BezP (0.11) > Prp (0.05) > EtP (0.03) for late incubation (Fig. 1). In 2021, 2.74 tonnes of MetP was placed on the Finnish market and <4 products contained or <3 companies registered EtP, PrP, and BuP while there were no registrations for BezP and HeP (Table S10). In white-tailed eagle livers from the Baltic Sea collected between 1979 and 1999, Xue and Kannan (2016) found 4-HB > MetP > HeP > EtP > BuP > OH-MetP > PrP, OH-EtP, 3,4-DHB (not detected) from highest to lowest concentration. Their results illustrate the interrelationships between parabens and their metabolites with the metabolite 4-HB being the most prolific compound detected. Finally, while triclocarban has been detected in birds previously (Elliott et al., 2019), we did not detect it in any of the samples in this study which is consistent with no registrations of triclocarban in Finland in 2021 (Table S10).

3.2. CEC concentrations in relation to incubation stage

Only compounds which occurred above LOD in ≥60% of samples in at least one group were statistically compared. For the early breeders, only BPA occurred above LOD in ≥60% of the T1 plasma samples and no CEC was above LOD in ≥60% of their T2 plasma samples (Fig. 2). As for the late breeders, BPA, BzP-3, mMP, and mEP occurred above LOD in ≥60% of the T1 plasma samples and BPS, in addition to BPA, BzP-3, mMP, and mEP, occurred above LOD in ≥60% of their T2 plasma samples (Fig. 2). For BPA there was no evidence that concentrations differed between early and late incubation for the early breeders (paired *t*-test, mean difference = 6.43 ng/g ww, adjusted $p = 0.29$), whereas there was weak evidence that the concentration increased at late incubation for the late breeders (paired *t*-test, mean difference = 18.7 ng/g ww, adjusted $p = 0.10$). Concentration differences in BPS, BzP-3, mMP, and mEP between early and late incubation were only tested for late breeders: there was weak evidence that BPS increased in concentration from early to late incubation (Wilcoxon, mean difference = 0.12 ng/g ww, adjusted $p = 0.07$), but no evidence for differing concentrations for BzP-3 (Wilcoxon, mean difference = 0.65 ng/g ww, adjusted $p = 0.68$), mMP (Wilcoxon, mean difference = 0.38 ng/g ww, adjusted $p = 0.15$), nor mEP (Wilcoxon, mean difference = 5.66 ng/g ww, adjusted $p = 0.36$). At first sight, it may seem surprising that there were no strong differences between the two timepoints. On the one hand, given that studies in humans, rodents, and fish report that many of our target CECs are relatively quickly metabolized and/or excreted (e.g. Asimakopoulos et al., 2016; Aubert et al., 2012; Frederiksen et al., 2007; Kadry et al., 1995; Lindholst et al., 2003) and given that incubating eiders fast, thus

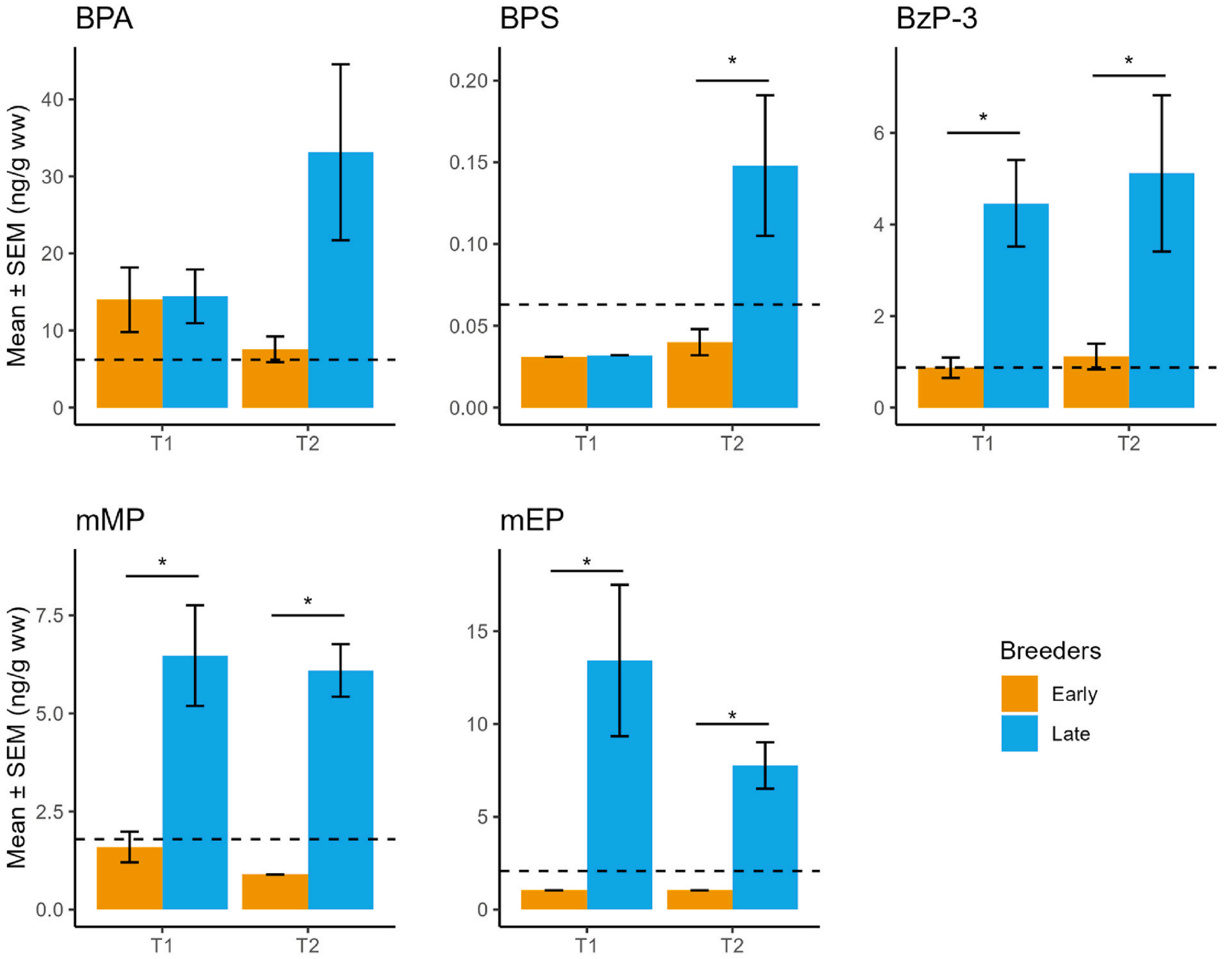

Fig. 2. Plasma concentrations (ng/g wet weight (ww)) of bisphenol A (BPA), bisphenol S (BPS), benzophenone-3 (BzP-3), monomethyl phthalate (mMP), and monoethyl phthalate (mEP) at early (T1) and late (T2) incubation for female eiders from Bengtskär, Finland, 2021. The concentrations are shown separately for early ($n = 10$) and late ($n = 8$) breeders. Note the difference in scale for the y-axes. The dashed line in the plots shows the compound-specific limit of detection (LOD). The mean concentrations plotted here have been calculated using all observations (i.e. including the observations which have been replaced by 1/2LOD). The asterisks denote when there was strong or moderate evidence of a difference in concentrations.

reducing their oral exposure to CECs, one might expect higher concentrations at T1 compared to T2. However, we lack knowledge on the toxicokinetics of the targeted CECs in birds and there are likely considerable interspecific differences in the metabolism and excretion efficiencies (e.g. Hanioka et al., 2022; Ito et al., 2005). As the CECs have been found in tissues of birds and other wildlife (Castro et al., 2023; González-Rubio et al., 2020; Rian et al., 2020; Vorkamp et al., 2004; Xue and Kannan, 2016), the rates of metabolism and excretion are at least sufficiently slow to allow tissue accumulation. On the other hand, therefore, the eiders' mass loss could cause re-distribution of stored CECs, leading to higher plasma concentrations at T2 compared to T1: this increase in circulating contaminants has been shown for persistent organic pollutants and metals (Bustnes et al., 2012, 2010; McPartland et al., 2020). It may be that both of these mechanisms are co-occurring and cancelling each other out, with the observed result of little to no change in concentrations between early and late incubation. Given the dearth of information on the toxicokinetics and toxicodynamics of these CECs in birds, we call for future research on this important topic. Another consideration is maternal transfer of CECs into eggs; the T1 sample was taken when the females had laid most or all of their eggs and may thus have excreted a notable amount of CECs into the eggs (Allen et al., 2021; Molins-Delgado et al., 2017). However, in our study there was no relationship between clutch size and concentration of ΣCECs at T1 (Fig. S1). Nevertheless, eiders can exhibit a high degree of conspecific brood parasitism in dense colonies (Waldeck et al., 2004) such as this one; indeed, a concurrent parallel study from Bengtskär found that three (30%) of the 10 study nests contained at least one parasitic egg and of the 44 investigated eggs, seven (15.9%) were parasitic (unpublished data). Thus, it is likely that some of the clutches of the early and late breeders include parasitic eggs and this, in turn, may mask a link between clutch size and plasma contaminant concentrations. The reason for the similar concentrations at both timepoints is most likely a combination of several factors, including biotransformation, re-distribution from tissues, and maternal transfer, calling for further studies with larger sample sizes in different tissues as well as in the eggs.

3.3. *CEC concentrations in relation to breeding phenology*

Resource allocation strategies for reproduction in female eiders may differ depending on breeding phenology (Jaatinen et al., 2016; Sénéchal et al., 2011) wherein early breeders rely essentially on endogenous resources and late breeders partly on exogenous resources — which may be reflected in differential concentrations of CECs in the plasma if the local environmental contamination in the wintering and breeding areas differ. Consistent with this, there was strong evidence that late breeders had higher concentrations of BzP-3 (Mann-Whitney, mean difference = 3.59 ng/g ww, adjusted $p = 0.003$) and mEP (Mann-Whitney, mean difference = 12.4 ng/g ww, adjusted $p = 0.003$) during early incubation (T1) than their early-breeding counterparts (Fig. 2). Furthermore, we found moderate evidence that the late breeders had higher concentrations of mMP (Mann-Whitney, mean difference = 4.88 ng/g ww, adjusted p = 0.026) during early incubation compared to early breeders. We found no evidence that BPA differed between early and late breeders at early incubation (Mann-Whitney, mean difference = 0.44 ng/g ww, adjusted p = 0.93). During late incubation (T2), we found strong evidence that late breeders had higher concentrations of mMP (Mann-Whitney, mean difference = 5.20 ng/g ww, adjusted p = 0.003) than early breeders (Fig. 2). There was moderate evidence for late breeders having higher concentrations of BPS (Mann-Whitney, mean difference = 0.11 ng/g ww, adjusted p = 0.045), BzP-3 (Mann-Whitney, mean difference = 4.00 ng/g ww, adjusted p = 0.045), and mEP (Mann-Whitney, mean difference = 6.73 ng/g ww, adjusted p = 0.004). Finally, for BPA there was weak evidence that late breeders had higher concentration during late incubation (Mann-Whitney, mean difference = 25.6 ng/g ww, adjusted p = 0.10) than early breeders. The watersheds draining into the wintering area of the eiders in our study has a higher human population density (Bollmann et al., 2019) which may indicate a higher concentration of CECs in the environment. However, we found that late breeders had higher concentrations than early breeders. This seemingly unexpected finding might reflect an association between breeding phenology and individual quality, potentially affecting circulating contaminant concentrations during incubation. In eiders, females in poor condition tend to breed later (Descamps et al., 2011; Jaatinen and Öst, 2016). However, the body condition of early and late breeders was comparable (Fig. S2), so we do not consider differences in body condition to be a contributing factor in our study. Altogether, there is a lack of knowledge on our target CECs in the Baltic Sea making it an important avenue for future research. Besides potential breeding phenology-related differences in resource allocation strategies, late breeders lay smaller clutches (Coulson, 1999; Descamps et al., 2011; Hermansson et al., 2023). Thus, the higher concentrations of BzP-3, mMP, and mEP in late breeders could be explained by lower transfer of contaminant body burden into eggs. In our study, at the time of the first sampling, the early and late breeders had a clutch size of 7.20 ± 2.57 (mean ± standard deviation) and 5.38 ± 2.07 eggs, respectively. Although numerically in the expected direction, there was little evidence that these clutch sizes differed (t-test, mean difference = 1.83 eggs, adjusted p = 0.16). However, direct comparison is complicated by the likely occurrence of conspecific brood parasitism and differential heat stress experienced by early and late breeders. With respect to the latter, core body temperature affects the absorption, distribution, metabolism, and excretion of contaminants (reviewed by Leon, 2008), likely reflected in contaminant concentrations.

4. Conclusion

Incubating female eiders are exposed to a cocktail of CECs, some previously undocumented in birds. Of the CEC families we investigated, bisphenols, phthalate metabolites, and benzophenones are those detected most frequently and/or in highest concentrations, whereas parabens and triclocarban appear to be of minor concern in our study colony. While their detection frequencies and concentrations are low, we report the presence of benzotriazoles and benzothiazoles which are two severely understudied contaminant families. While there was no evidence of changing concentrations from early to late incubation within individuals, future studies should take into consideration that early and late breeders may have different concentrations of circulating CECs due to their resource allocation strategies. Future research should examine the concentrations of these CECs in the eiders' prey species to elucidate exposure vectors. Finally, our results raise concern regarding maternal transfer of CECs to eggs with potential implications for embryo development, which could possibly scale up to population-level effects. In eggs, only a few of the CECs targeted in this study have been investigated so far, which should be urgently addressed.

CRediT authorship contribution statement

Amalie V. Ask: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Veerle L.B. Jaspers:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Junjie Zhang:** Writing – review & editing, Supervision, Investigation. **Alexandros G. Asimakopoulos:** Writing – review & editing, Supervision, Resources, Methodology. **Sunniva H. Frøyland:** Writing – review & editing, Investigation. **Juho Jolkkonen:** Writing – review & editing, Investigation. **Wasique Z. Prian:** Writing – review & editing, Investigation. **Nora M. Wilson:** Writing – review & editing, Resources, Investigation. **Christian Sonne:** Writing – review & editing, Conceptualization. **Martin Hansen:** Writing – review & editing, Conceptualization. **Markus Öst:** Writing – review & editing, Conceptualization. **Sanna Koivisto:** Writing – review & editing. **Tapio Eeva:** Writing – review & editing, Conceptualization. **Farshad S. Vakili:** Writing – review & editing. **Céline Arzel:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125409.

Data availability

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