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Hormonal disruption from plastic ingestion in northern fulmars: Activation and inhibition of estrogen receptors☆

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ABSTRACT

Plastic pollution is of global concern, yet documented harmful effects from plastic ingestion in wild species is limited. Procellariiformes, such as northern fulmars (*Fulmarus glacialis*, fulmar), have high levels of plastic ingestion, and we investigated their potential hormonal disruption. As human estrogen receptors (ERs) are commonly used to assess risk of hormonal disruption for wild species, we first compared human and fulmar ER responses to plastic-associated chemicals using a luciferase-reporter gene assay. ERs from both species were activated by bisphenol-A (BPA), bisphenol-S (BPS), 4-octylphenol (4-OP), and benzyl butyl phthalate (BBP), and inhibited by tetrabromobisphenol-A (TBBPA), 2,5,2′,5′tetrachloro-biphenyl (PCB-52), and 2,2′,3,4,4′,5′-hexachlorobiphenyl (PCB-138), although we observed species-specific differences in sensitivity. To assess if ingested plastic had the potential to alter fulmar hormone function, plastic recovered from fulmar stomachs ($n = 27$) were leached for 14 days with daily solvent renewal and leachates were exposed to fulmar ERs. Almost 50 % of the birds (13 out of 27) had ingested plastic that leached chemicals which caused ER activation and/or inhibition on day 1, with ~70 % of these (9 out of 13) also showing a response on day 5 and/or 14. The polymer composition of the recovered plastic pieces ($n = 142$) was identified with infrared spectroscopy. Polyethylene (PE) (60 %) was the most abundant polymer, followed by polypropylene (PP) (35 %), polyethylene terephthalate (PET) (3 %) and unidentifiable (2 %). Polymer type was not associated with ER response, suggesting that chemical additives, not polymer composition, were responsible for the observed hormonal disruption. To our knowledge, we provide the first data on a seabird's ER response to plastic-associated chemicals. Overall, we highlight the potential for plastic ingestion to disrupt fulmar hormone function, providing important information about the harmful effects of plastic pollution.

1. Introduction

An estimated 8 million tons of plastic enter the ocean annually (Jambeck et al., 2015), posing significant risks to wildlife through plastic ingestion (Kühn and van Franeker, 2020). Procellariiformes (e.g., albatrosses, petrels, shearwaters) are particularly at risk to plastic ingestion and retention due to their foraging strategy (Roman et al., 2019a) and distinctive gastric morphology (Ryan, 2019). Indeed, Procellariiformes are documented to have one of the highest incidences of plastic ingestion (63 %) (Kühn and van Franeker, 2020), with a stomach retention time of weeks to months (Ryan, 2015; Terepocki et al., 2017). Among Procellariiformes, northern fulmars (*Fulmarus glacialis*, fulmar), are widely used as bioindicators of marine plastic pollution (van Franeker et al., 2021) due to their surface-feeding behavior, which

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increases exposure to buoyant plastic debris (Hanifen et al., 2025).

Plastic ingestion poses a dual threat, causing physical harm as well as exposure to plastic-associated chemicals. Ingested plastic can cause obstruction (Pierce et al., 2004) and perforation of the digestive tract (Roman et al., 2019b; Tulatz et al., 2023), and a false sense of satiation, resulting in malnutrition (Lavers et al., 2014). Small pieces of plastic (e.g. micro- or nano-plastic) can penetrate cells, leading to inflammation and fibrosis (Charlton-Howard et al., 2023; Rivers-Auty et al., 2023; Wang et al., 2022). Plastic can also release toxic chemicals such as additives added during plastic manufacture to modify properties (e.g., plasticizers, flame retardants, antioxidants) as well as persistent organic pollutants (POPs) that sorb onto the surface of plastic in the ocean (Kühn et al., 2020; Tanaka et al., 2020; Van Hassel et al., 2024).

Plastic-associated chemicals can be absorbed by the intestinal barrier and circulate within the organism, causing physiological harm such as endocrine disruption (Bodziach et al., 2021; Maddela et al., 2023). As such, many plastic-associated chemicals are xenoestrogens [i.e., able to bind or block estrogen receptors (ERs)], and known to cause a wide-range of reproductive dysfunction in humans and wildlife (Marlatt et al., 2022). In birds specifically, studies investigating the effects of plastic ingestion on quail found an increase in reproductive abnormalities (e.g., cysts) in males and reduced estrogen levels in females (Monclús et al., 2022; Roman et al., 2019c). Overall, a large body of evidence supports the hormonal disrupting capacity of plastic-associated chemicals (Omidoyin and Jho, 2024).

In vitro ER reporter assays, predominantly with human ERs, are commonly used to assess the estrogenicity of chemicals (Rogers and Denison, 2000). However, variations in ER sequences across species can influence their sensitivity to xenoestrogens (Asnake et al., 2019; Felton et al., 2020; Matthews et al., 2000; Rider et al., 2010; Tubbs et al., 2012). For example, differences in the ER transactivation (A/B) and C-terminal (F) domains in two rhinoceros species were associated with the capacity of phytoestrogens induce an ER response, likely affecting fertility (Tubbs et al., 2012). Comparison between the ER sequences of human and fulmars (Fig. S1) also suggest potentially different sensitivity to plastic-associated chemicals, highlighting the value of assessing species-specific ER responses when investigating hormonal disruption.

We investigated the risk of hormonal disruption from plastic-associated chemicals to fulmars by i) comparing the activation and inhibition of fulmar and human ERs; ii) measuring the activation or inhibition of leachates from plastic recovered from fulmar stomachs; iii) identifying the polymer composition of the ingested plastic. To our knowledge, we provide the first comparison of human and fulmar ERs as well as the activation or inhibition of fulmar ERs to leachates from ingested plastic. Our work highlights the risk from exposure to plastic-associated chemicals, an under-studied aspect of plastic pollution.

2. Materials and methods

2.1. Chemicals and materials

17β-estradiol (E_2) was purchased from Steraloids (Newport, RI). Analytical-grade (≥98 % purity) hexanes, methanol, dimethylsulfoxide (DMSO), 4-octylphenol (4-OP), benzyl n-butyl phthalate (BBP), decabromodiphenyl ether (PBDE-209), tetrabromobisphenol A (TBBPA) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Analytical-grade (≥98 % purity) bisphenol A (BPA), bisphenol S (BPS), 2,2′,3,4,4′,5′-hexachlorobiphenyl (PCB-138) were purchased from Millipore Sigma (St. Louis, MO, USA). Analytical-grade (≥98 % purity) 2,2′,5,5′-tetrachlorobiphenyl (PCB-52) was purchased from Santa Cruz Biotechnologies, Inc. (Dallas, TX, USA).

Glass Erlenmeyer flasks (50 mL) sealed with foil-lined rubber caps and parafilm were used to leach plastics in solvents. Round glass stirring bars were purchased from Sigma-Aldrich (Overijse, Belgium). Leachates were collected with Pasteur pipets and stored in glass vials with polypropylene caps lined with aluminum foil. All glassware (e.g., pipets, flasks) was baked in a muffle furnace at 450 °C for 4 h prior to use.

2.2. Human and fulmar ER cloning

Fulmar estrogen receptor (fERα and fERβ) sequences (fESR1 and fESR2) were synthesized and cloned into pcDNA3.1+ expression vectors using IDT custom gene synthesis (https://www.idtdna.com). The fulmar ESR2 sequence was obtained from GenBank: XM_009572778.1. Fulmar ESR1 was found using BLAST and the fulmar genome (NCBI:txid30455) using chicken ESR1 as the query sequence: NM_205183.2. Human ESR1 and ESR2 in pcDNA3.1+ were purchased from the cDNA resource center (https://www.cdna.org/). Plasmids were transformed into TOP10 competent *Escherichia coli*, cultured and purified using a ZymoPureII Maxi Prep Kit (https://www.zymoresearch.com/) and sequences verified by sanger sequencing (https://retrogen.net/).

2.3. Plastic-associated chemicals used for human and fulmar ER comparison

Plasticizers, flame retardants and stabilizers are among the most common additives in plastics (Hahladakis et al., 2018). To compare the response of human and fulmar estrogen receptors to plastic-associated chemicals, we selected additives detected in marine plastic (Aminot et al., 2020; Dhavamani et al., 2022; Staniszewska et al., 2016) and known to impair reproduction (Adegoke et al., 2020; European Chemicals Agency (ECHA), 2014; Li et al., 2021; Liu et al., 2018; Santoro et al., 2019; Thoene et al., 2020; Van der Ven et al., 2008): BPA, a residual monomer of polycarbonate and used as an antioxidant; BPS, a BPA substitute; 4-OP a monomer of phenolic resins and used as an antioxidant; BBP used as a plasticizer; and PBDE-209 and TBBPA used as flame retardants (Hahladakis et al., 2018). PCB-138 and PCB-52 were selected to represent POPs commonly adsorbed to plastic (Antunes et al., 2013; Bouhroum et al., 2019). Stock solutions were concentrated at 100 mM in DMSO. E_2 was used as a positive control.

2.4. Plastic collected from fulmar stomachs

Plastic samples were collected from the stomachs of 27 fulmars (14 females and 13 males) incidentally caught as bycatch in U.S. North Pacific groundfish and halibut fisheries in Alaska in 2016 and 2017 (Table S1). Standard methods for bird necropsies and dissection followed van Franeker (2004). After removal of the stomach, contents were carefully rinsed in a sieve with a 1 mm mesh and left to air-dry in open glass vials for several days. Plastic recovered from each chamber (proventriculus and gizzard) was stored separately. In total, 25 birds had plastic in their gizzard, one bird had plastic in its proventriculus and one bird had plastic in both chambers (n = 28 plastic samples from 27 birds) (Table S2). Total mass, shape (industrial pellets, pellets other than industrial, sheet, threads, foam, fragment and "other"), number of plastic items, and their combined mass by shape were recorded (Table S2).

2.5. Plastic leaching

2.5.1. Solvents

Plastics were leached into a 40 ml mixture of methanol-hexane (1:1). Methanol is a polar solvent used for the extraction of a broad range of low hydrophobic chemicals such as bisphenol A (Bonon et al., 2016; Coffin et al., 2019; Wang and Kannan, 2023) while hexane is a non-polar solvent used for the extraction of highly hydrophobic chemicals such as phthalates (Chao et al., 2013) and brominated flame retardants (Gripon et al., 2021; Tanaka et al., 2019, 2015).

2.5.2. Leaching

Plastic collected from each stomach chamber (n = 28) was leached (stirred at 600 rpm) in a methanol-hexane mixture and renewed every 24 h for 14 days. The methanol and hexane fractions of each 24-h

leachate were recovered (Fig. 1). Daily solvent renewal allowed us to assess whether the plastic samples continued to leach sufficient amount of chemicals to induce estrogenic activity following a daily extraction. Due to logistical constraints, leaching was conducted in two equal sets of 14 samples. Solvents without plastics (vehicle controls) were included (n = 3/set) without solvent replacement for the duration of plastic leaching (14 days) (Fig. 1). Hexane and methanol leachates assessed for ER activity were filtered separately with a Whatman™ glass microfiber filter and evaporated under a gentle nitrogen stream until almost complete dryness (to avoid the loss of volatile chemicals). DMSO (50 µl) was added to the remaining leachate and the mixture was evaporated again under a gentle nitrogen stream to remove residual hexane or methanol.

To assess mass loss from the leaching process, plastic samples were weighed (with an analytical balance ± 0.0001 g) before leaching and following the final leachate collection, after being air-dried for ~ 24 h (Table S2).

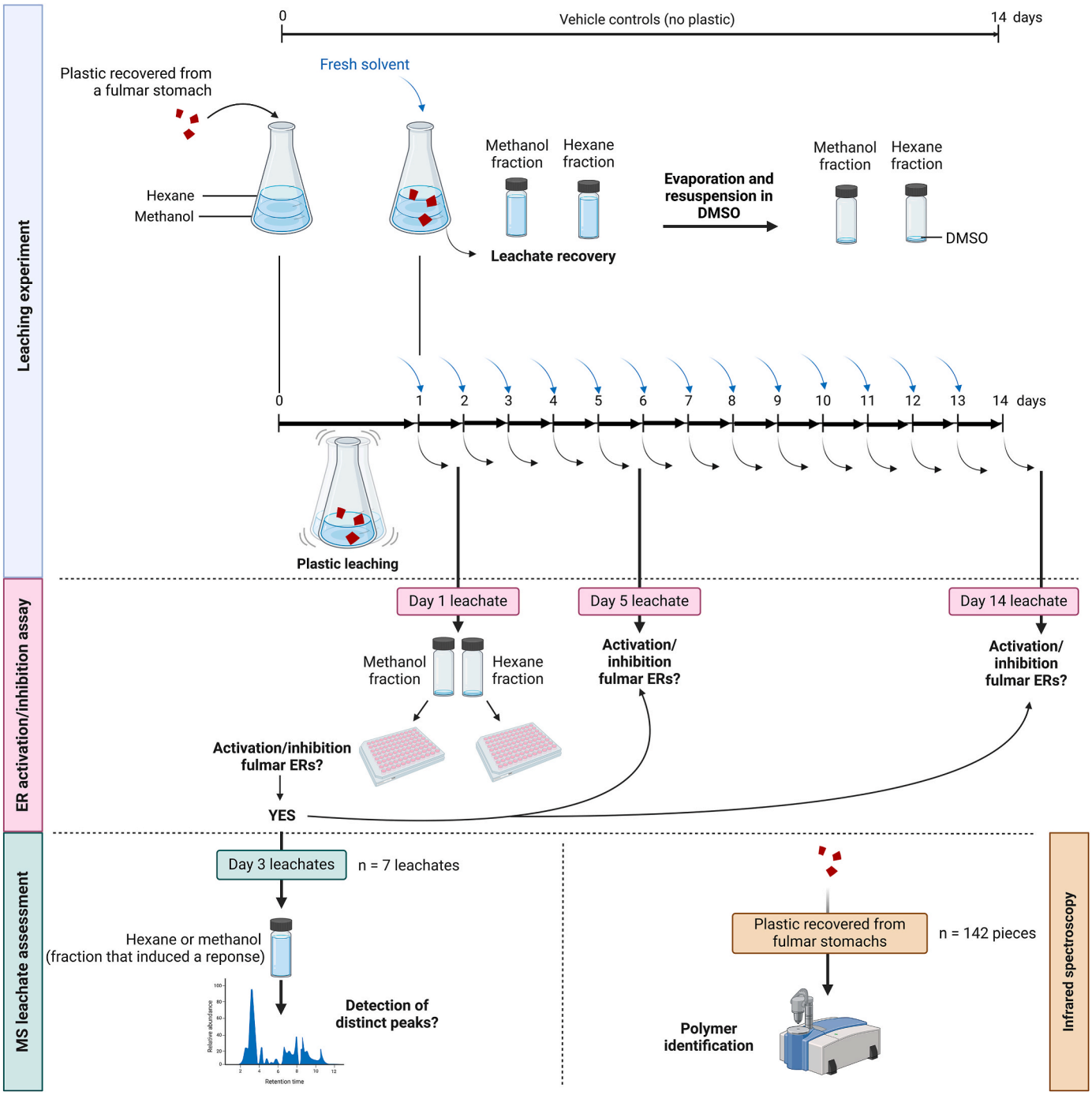

Fig. 1. Experimental design. Plastic samples were leached in a new methanol-hexane mixture every 24 h for 14 days and the methanol and hexane fractions of each 24-h leachate were recovered separately. Day 1 leachates were tested for estrogenic activity and samples showing a response were subsequently evaluated on days 5 and 14 for potential long-term effects. Vehicle controls without plastics were run in parallel without solvent replacement and recovered after 14 days. Fractions from day 3 were analyzed by mass spectrometry for selected samples showing ER response on days 1, 5, and 14 to identify compounds potentially responsible for the observed activity. Pieces recovered from fulmar stomachs were also analyzed for polymer identification using infrared spectroscopy. Created with BioRender.com.

2.6. Cell culture and receptor activation/inhibition assay

Human embryonic kidney (HEK293) cells were maintained in minimal essential medium (MEM) (Corning) supplemented with 10 % fetal bovine serum at 37 °C and 5 % CO_2. Cells were split into a new culture flask two times per week. For receptor assays, 1×10^5 cells were added to each well of a 96-well plate. After 24 h, cells were co-transfected using TransIT-2020 transfection reagent (Mirus Bio LLC) with 3 plasmids: 5-μg pCMX-β-galactosidase (β-gal), 5-μg pGL2–3xERE luciferase reporter plasmid containing three copies of chicken vitellogenin estrogen response element (Addgene plasmid 11354) and human or fulmar ESR1 or ESR2 0.5-μg ESR-pcDNA3.1 (+). After 24 h, transfection medium was removed and replaced with MEM supplemented with 10 % charcoal resin-stripped fetal bovine serum containing 10^{-14} M – 10^{-7} M endogenous hormone E_2 (0.1 % DMSO), 10^{-9} M – 10^{-4} M plastic-associated chemicals (BPA, BPS, 4-OP, BBP, PBDE-209) (0.1 % DMSO), leachates (0.25 % DMSO) or vehicle controls (0.1 % and 0.25 % DMSO). We used 0.25 % DMSO for the leachates to maximize the potential to induce an ER response, given their unknown chemical concentration. For receptor inhibition, the transfection medium was replaced with MEM supplemented with 10 % charcoal resin-stripped fetal bovine serum containing 10^{-10} M E_2 and 10^{-9} M – 10^{-4} M plastic-associated chemical (TBBPA, PCB-28, PCB-138, PBDE-209) or the leachates. We chose an E_2 concentration of 10^{-10} M for the inhibition assay to ensure sub-maximal receptor activation (~80 %), providing sufficient room for competition between the hormone and the plastic-associated chemicals (Plíšková et al., 2005). After 24 h, cells were lysed and each well assessed for luciferase and β-gal activity as described previously (Tubbs et al., 2012).

Luciferase activity per well was standardized to β-gal activity, and fold activation was determined from the normalized luciferase values relative to the vehicle control. Sigmoidal dose-response curves for ERα and ERβ activation by 10^{-12} M – 10^{-7} M E_2 were fit using GraphPad Prism software and used to calculate maximal activation by E_2. Fold activation of ERs for each plastic-associated chemical and leachate was then normalized to maximal E_2 activation of ERα or ERβ. All wells were run in triplicate and assays were conducted three times for plastic-associated chemicals (results presented as the average ± SEM) and once for leachates.

Plastic-associated chemicals (BPA, BPS, 4-OP, BBP, TBBPA, PCB-28, PCB-138 and PBDE-209) were screened for ER activation. Chemicals that showed no activation were additionally assessed for ER inhibition.

To investigate the potential for continued release of plastic-associated chemicals from ingested plastic, we tested leachates collected on day 5 and 14 for plastic samples whose leachates showed activation or inhibition on day 1 (Fig. 1). Although we recognize that testing leachates for all samples on day 5 and 14 (or all days) would have been optimal, this was beyond the scope of our study. However, our ability to test leachates from day 5 and 14 for the sub-set of samples that showed a response on day 1 does provide valuable information with respect to the potential for ingested plastic to leach endocrine-disrupting chemicals over prolonged periods of time.

To assure low cytotoxicity, the β-gal signal for treatments was compared to the β-gal signal for E_2. No treatment showed increased cytotoxicity, except for 4-OP which killed the cells at 10^{-4} M.

2.7. Identification of plastic collected from fulmars

Plastic pieces collected from fulmar stomachs (n = 142) were analyzed for polymer identification as follows (see also Table S3):

138 pieces were analyzed with hyperspectral near-infrared spectroscopy (HIS) using a Specim FX-17 hyperspectral camera (line-scan mode, 900–1700 nm) (Bruker, Belgium). Data were predicted using a discrimination model based on the chemometric tool partial least squares discriminant analysis (PLS-DA). This model was built using a set of reference polymers covering spectra of seven types [polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polystyrene (PS), nylon and nitrile butadiene rubber (NBR)]. PLS-DA results were validated by visual observation of the spectra, which was also used to differentiate between HDPE and LDPE due to limitations of the discrimination model. Plastic pieces with unidentifiable spectra (n = 9) were further analyzed using a Bruker Hyperion 3000 near-infrared microscope (NIRM) (Bruker, Belgium).

Black pieces (n = 4), due to their complete light absorption in near-infrared range, were analyzed using mid-infrared spectroscopy (MIR) with a Bruker Vertex 70 spectrometer equipped with an attenuated total reflectance (ATR) system (Bruker, Belgium).

Matlab 7.5 software with the PLS toolbox version 7.5.2. was used to identify polymers from the NIRM and MIR spectra. Spectra were also visually examined to ensure accurate identification.

2.8. Qualitative assessment of plastic leachates with mass spectrometry

A subset of leachates (n = 9) were qualitatively examined using mass spectrometry for the presence of compounds potentially responsible for ER responses (Table S2).

Methanol fractions were analyzed directly while hexane fractions were evaporated and resuspended in methanol for analysis. Samples were analyzed by direct injection from a syringe needle with 10 μL/min flow rate on a Thermo Electron Finnigan LTQ mass spectrometer (Thermo, Santa Cruz, United States) in positive and negative ion mode over a full scan range of m/z 150–2000. The mass spectrometry electrospray voltage was set to 5.0 kV and the capillary temperature was 275 °C. Data were analyzed using XCalibur software (Thermo).

2.9. Statistical analysis

2.9.1. Human and northern fulmar ER comparison

Comparison of human and fulmar ER response in the presence of plastic-associated chemicals was performed with a two-way ANOVA using GraphPad PRISM with a Bonferroni correction. Inhibition of receptors was assessed using a two-way ANOVA with a Bonferroni correction by comparing the ER activation of 10^{-10} M E_2 to the activation by 10^{-10} M E_2 plus different chemical concentrations.

2.9.2. Fulmar ER response to plastic leachates

Receptors were considered activated when the percentage of maximum E_2 activation was greater than the average + 2SD of the vehicle controls. Receptors were considered inhibited when the percentage of maximum E_2 activation was less than the average - 2SD of the vehicle controls. This method was used to reduce the likelihood of Type I errors (false positives) given the variability observed within the vehicle controls (Figs. S2 and S3).

A Spearman correlation was used to assess relationship between plastic mass and ERα response using GraphPad.

A Fisher's exact test was used to examine if there was a predictable relationship between polymer type and ERα response using JMP Pro 17.

3. Results

3.1. Fulmar and human ERs exhibited similar responses

Human and fulmar ERs were activated by BPA, BPS, 4-OP and BBP (Fig. 2A) and inhibited by TBBPA, PCB-52 and PCB-138 (Fig. 2B), while no response was observed for PBDE-209 (Fig. S4) (Table S4).

Activation – ERα. Human ERα showed a higher activation than fulmar ERα by BPA at 10^{-6} M ($P < 0.001$) and 10^{-5} M ($P = 0.032$) (up to 1.5-fold) and BPS at 10^{-5} M ($P < 0.0001$) (1.5-fold) (Fig. 2A). 4-OP at 10^{-6} M and 10^{-5} M activated fulmar ERα at a higher level than human ERα ($P < 0.0001$) (1.4-fold) (Fig. 2A). BBP activated ERα with comparable levels between fulmar and human ($P > 0.999$ for 10^{-7} M and 10^{-6} M and $P = 0.404$ for 10^{-5} M) (Fig. 2A).

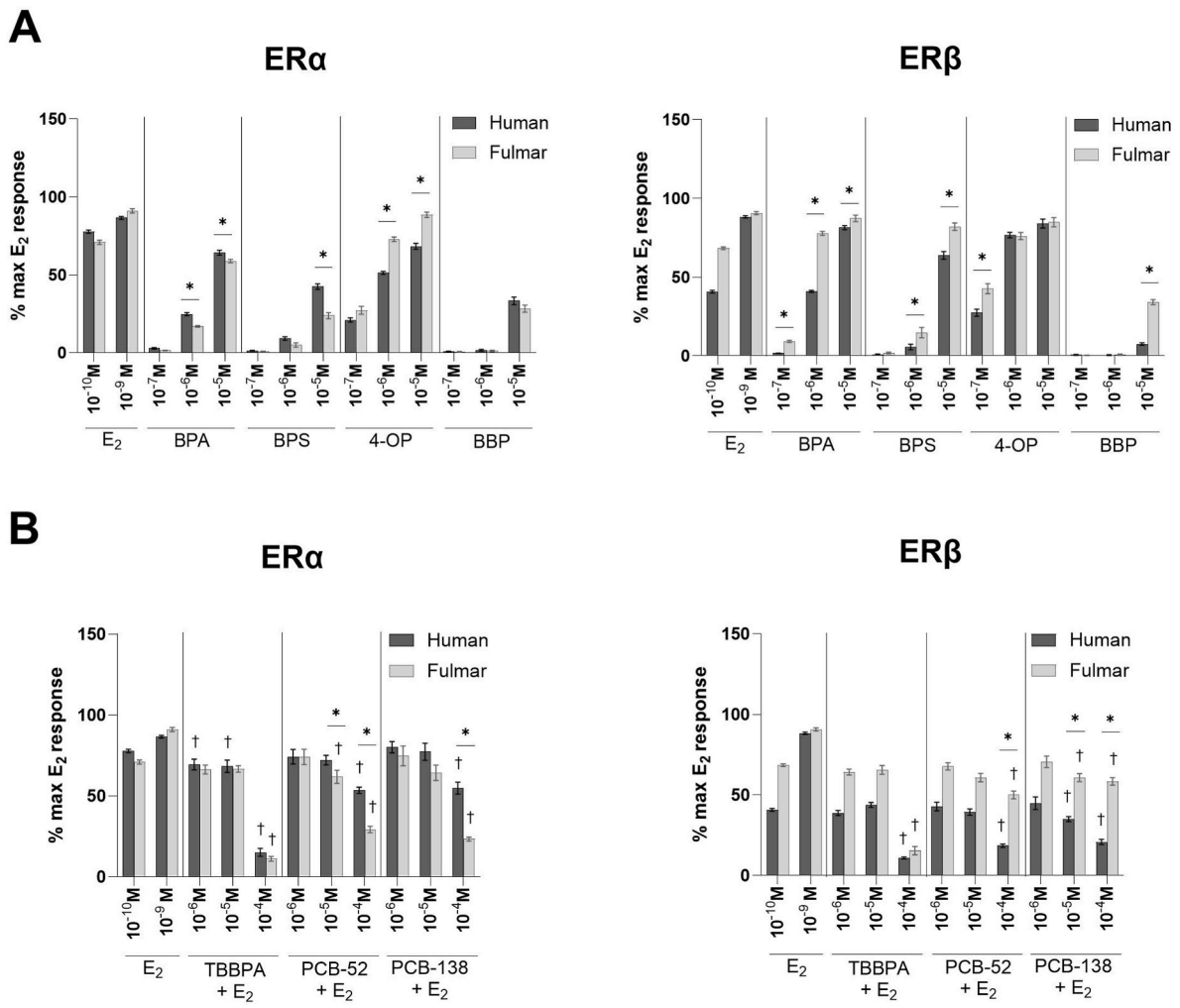

Fig. 2. Activation (A) and inhibition (B) of human and fulmar ERα and ERβ by plastic-associated chemicals. A. Treatment of BPA, BPS, 4-OP and BBP (10^{-9} M – 10^{-4} M) in 0.1 % DMSO. B. Treatment of TBBPA, PCB-52 and PCB-138 (10^{-7} M – 10^{-4} M) with 10^{-10} M E_2 in 0.1 % DMSO. Bars represent mean ± SEM of the fold activation of each treatment relative to maximal E_2 activation. Significant differences between human and fulmar were determined using a two-way ANOVA followed by individual *t*-test with Bonferroni correction ($p < 0.05$) (asterisk). Significant inhibition was determined using a two-way ANOVA with a Bonferroni correction by comparing the activation by 10^{-10} M E_2 alone to the activation by 10^{-10} M E_2 in the presence of the chemical ($p < 0.05$) (dagger).

– ***ERβ.*** Fulmar ERβ showed a higher activation than human ERβ by BPA at 10^{-7} M ($P < 0.001$), 10^{-6} M ($P < 0.0001$) and 10^{-5} M ($P = 0.012$) (up to 1.9-fold), BPS at 10^{-6} M ($P = 0.021$) and 10^{-5} M ($P < 0.0001$) (1.3-fold), 4-OP at 10^{-7} M ($P < 0.0001$) (3.4-fold) and BBP at 10^{-5} M ($P < 0.0001$) (4.6-fold) (Fig. 2A). No difference was observed at 10^{-6} and 10^{-5} M for 4-OP ($P > 0.999$).

Inhibition – ***ERα.*** For TBBPA at 10^{-4} M, comparable levels of inhibition were observed between human and fulmar ERα ($P = 0.949$). Fulmar ERα showed a higher inhibition than human ERα by PCB-52 and PCB-138 at 10^{-4} M ($P < 0.0001$) (1.8-fold) (Fig. 2B).

– ***ERβ.*** TBBPA at 10^{-4} M inhibited human and fulmar ERs at comparable levels ($P = 0.245$). Human ERβ showed a higher inhibition than fulmar ERβ by PCB-52 at 10^{-4} M ($P < 0.0001$) (2.7-fold) and PCB-138 at 10^{-5} and 10^{-4} M ($P < 0.0001$) (up to 2.8-fold) (Fig. 2B).

3.2. Plastic leachates activated and inhibited fulmar ERs

Out of 27 fulmars evaluated (Tables S1 and S2), plastic recovered from 4 birds activated while 7 inhibited ERα. Two birds had plastic leachate fractions (methanol or hexane) that activated and inhibited ERα (Table 1). For ERβ, plastic recovered from 5 birds activated while 3 inhibited ERβ. Two birds had plastic leachate fractions (methanol or hexane) that activated and inhibited ERβ (Table S5).

3.2.1. ER activation and inhibition after one, five and fourteen days of leaching

Activation – ***Day 1.*** Six leachates activated fulmar ERα (Table 1, Fig. S2). Four leachates activated ERα from the hexane fraction at 1.3 to 18-fold over the threshold (Table 1, Fig. S2). Two leachates activated ERα from both methanol and hexane fractions at 1.1- to 1.7-fold over threshold respectively. Fulmar ERβ was activated by the same leachates that activated ERα (Table S5, Fig. S2). Interestingly, fulmar ERβ was activated by both the methanol and hexane fractions of a leachate (F22) that did not activate ERα, suggesting sensitivity differences between ERα and ERβ as also observed with the plastic-associated chemicals (Table S4).

– ***Day 5 and 14.*** Activation of fulmar ERs on day 5 and 14 was assessed for plastic samples with leachates that had measurable responses (e.g., above threshold) on day 1. Of the six samples that activated ERα on day 1, five activated ERα on day 5 and 14 at 1.7- to 3.8-fold above the threshold (on day 5: n = 4 hexane fractions and n = 1 hexane and methanol fractions; on day 14: n = 3 hexane fractions and n = 2 hexane and methanol fractions) (Table 1, Fig. S5). The methanol fraction from one of these six samples exhibited ERα activation on day 14 (Table 1, Fig. S5). Fulmar ERβ was activated by the same leachates that activated ERα (Table S5, Fig. S5). In addition, the hexane fraction of the leachate that activated ERβ on day 1 (F22) activated ERβ on day 5 and

Table 1
Leachates that activated or inhibited fulmar ERα (see Table S5 for ERβ data). Sample ID ('bird'), the stomach chamber where plastic was recovered (Sto. cha.') with 'G' representing gizzard and 'P' representing proventriculus, the number of plastic pieces found per individual ('*n* pieces'), the estrogenicity of plastic leachates measured on days 1, 5, and 14 for each leachate fraction [hexane ('Hex') or methanol ('Met')] are listed. Estrogenicity was expressed as the fold change above (for activation) or below (for inhibition) the threshold. Thresholds were defined as the average vehicle control response + (activation) or - (inhibition) 2SD. Samples that exhibited both activation and inhibition are highlighted in gray.

Bird	Sto. cha.	*n* pieces	Mass (g)	Fold change above/below the threshold					
				Day 1		Day 5		Day 14	
				Hex	Met	Hex	Met	Hex	Met
Samples that activated ERα									
F13	G	1	0.018	18	NA	1.7	3.4	1.8	NA
F14	P	1	0.014	10	NA	NA	NA	NA	1.7
F15	G	4	0.010	1.6	NA	2.5	NA	2.2	NA
F17	G	2	0.023	1.2	1.7	2.0	NA	1.9	3.8
F18	G	3	0.042	1.7	NA	1.8	NA	1.8	1.2
F27	G	10	0.051	1.3	1.1	1.8	NA	1.7	NA
Samples that inhibited ERα									
F9	G	1	0.008	NA	1.1	NA	NA	NA	NA
F18	G	3	0.042	NA	1.2	NA	NA	NA	NA
F19	G	5	0.069	NA	1.4	NA	1.1	NA	NA
F20	G	2	0.089	NA	1.3	NA	1.1	NA	1.1
F22	G	4	0.090	NA	1.2	NA	NA	NA	NA
F24	G	21	0.058	NA	1.2	NA	NA	NA	NA
F25	G	1	0.010	1.1	1.1	NA	NA	NA	NA
F26	G	1	0.023	NA	1.1	NA	1.1	NA	NA
F27	G	10	0.051	NA	1.1	NA	1.1	NA	1.1

day 14 (Table S5).

Inhibition* – *Day 1. The methanol fraction from 9 plastic leachates inhibited fulmar ERα at 1.1- to 1.4-fold below the threshold (Table 1, Fig. S3). The hexane fraction from one of these 9 leachates also inhibited ERα (Table 1, Fig. S3). ERβ was inhibited by the methanol fraction of 5 plastic leachates that also inhibited fulmar ERα (Table S5, Fig. S3).

– ***Day 5 and 14.*** Out of the 9 samples that showed inhibition on day 1, the methanol fraction of four leachates inhibited fulmar ERα on day 5 with two of these also inhibiting ERα on day 14 (Table 1, Fig. S6). One of these methanol fractions inhibited Fulmar ERβ on day 14 (Table S5, Fig. S6).

3.2.2. Plastic characteristics

3.2.2.1. Mass of plastic ingested. The mass of plastic recovered from northern fulmar stomachs as well as the mass loss after leaching was not related to fulmar ERα response ($r = -0.14$, $p = 0.48$ and $r = -0.22$, $p = 0.26$, respectively) (Fig. S7). For example, one sample (F7) had the highest mass (0.555 g) but did not induce a fulmar ER response (Table S2). After 14 days of leaching with daily solvent replacement, 86 % of the samples lost mass ranging from 0.001 to 0.066 g (1.4 %–68 % of initial weight) (Table S2).

3.2.2.2. Polymer identification. Out of 142 pieces, HDPE and LDPE accounted for 25 % and 35 %, respectively (one PE piece could not be distinguished between HDPE and LDPE). PP was the third most frequent polymer identified, representing 35 %, followed by 3 % for PET. Three pieces (2 %) could not be identified as any specific polymer type. The type of polymer was not associated with ERα response ($p = 0.96$) (Fig. 3).

3.2.3. Qualitative assessment of plastic leachates via mass spectroscopy

Hexane fractions. Hexane fractions from day 3 were quantitatively screened for samples F15, F17(G), F18, and F27 that exhibited ER activation on day 1, 5, and 14 (Table 1). A distinct peak was observed at 217 m/z in positive ion mode for these four samples but not in the vehicle controls or non-responsive samples [F16 and F17(P)] (Fig. S8).

Methanol fractions. Methanol fractions from day 3 were quantitatively screened for sample F19 which inhibited ERs on days 1 and 5, and samples F20 and F27 which inhibited ERs on days 1, 5 and 14. Distinct peaks were observed at 359 m/z and 588 m/z for F19 and at 648 m/z for F20 in positive ion mode (Fig. S9). In negative ion mode, a distinct peak was also observed at 486 m/z for F19 (Fig. S9).

Our qualitative findings indicated that distinct compounds were present in the samples that induced an ER response compared to the non-responsive samples and vehicle controls. The identified peaks were not further characterized due to the need for coupling with chromatographic techniques (LC-MS or GC-MS) for complex matrices such as plastic leachates (Li et al., 2022), which was beyond the scope of our study.

4. Discussion

Our study demonstrates the endocrine disruption potential of plastic ingestion in fulmars. Both human and fulmar ERs responded similarly to plastic-associated chemicals, though with species-specific differences in sensitivity. Plastic ingested by almost half of the fulmars (n = 13 out of 27) leached chemicals that activated or inhibited fulmar ERs after one

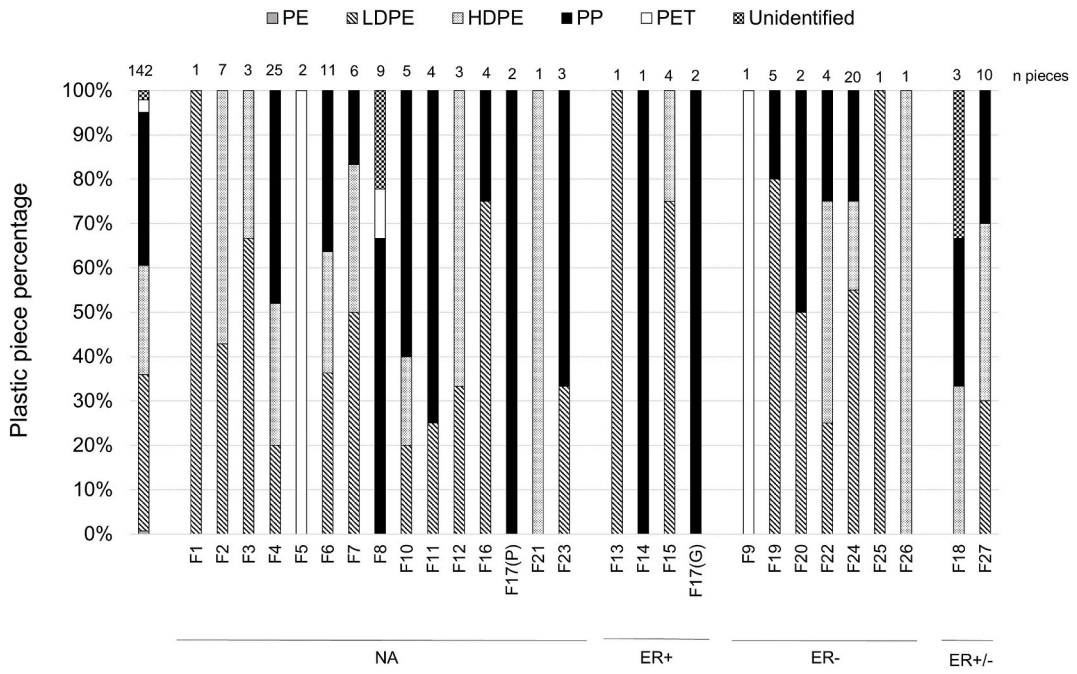

Fig. 3. Plastic polymer type. Distribution in percentage of polymer types identified among the total plastic pieces analyzed (n = 142) and among subgroups categorized by their effect on fulmar ERs: non-responsive (NA), activating (ER+), inhibiting (ER-), and both activating and inhibiting (ER+/−). The number of plastic pieces found per bird is indicated at the top of each bar. PE: light grey (when not identified as HDPE or LDPE), LDPE: diagonal stripes, HDPE: dotted pattern blue, PP: solid black, PET: white, and unidentified: crosshatch pattern. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

day of leaching, with plastic from 9 of these birds also releasing chemicals that caused an ER response after 5 and/or 14 days of subsequent leaching. Our findings highlight potential risks posed by plastic-associated chemicals from ingested plastic.

4.1. Fulmar and human ERs exhibited a similar response, with chemical and receptor type-specific sensitivity differences

Both human and northern fulmar ERs exhibited similar responses to plastic-associated chemicals. BPA, BPS, 4-OP, and BBP were potent activators of both human and fulmar ERs while TBBPA, PCB-53, and PCB-138 were potent inhibitors, consistent with previous findings for human ERs (Krivoshiev et al., 2016; Molina-Molina et al., 2013; Paris et al., 2002; Park et al., 2019; Plíšková et al., 2005). However, we found that PCB-52, which has been documented to activate human ERs (Plíšková et al., 2005; Zhang et al., 2014) inhibited human and fulmar ERs, highlighting the complexities associated with hormone receptor assessment using heterologous expression systems.

Although we observed similar responses, differences in sensitivity were detected between human and fulmar ERs depending on the chemical, the chemical's concentration, and the type of receptor (alpha or beta). ER sensitivity differences to xenoestrogens have been documented between species including humans, mice, chickens, green anoles, and rainbow trout (Matthews et al., 2000) as well as two species of rhinoceros (Tubbs et al., 2012). Differential ER activation between species is often attributed to transactivation domain (A/B and F) differences (Felton et al., 2020; Tubbs et al., 2012) of which human and fulmar ERs have between 28 and 14 % in their amino acid sequences (Fig. S1). Notably, 4-OP activated fulmar ERs at concentrations detected in the blood of razorbills (*Alca torda*), a species of seabird (Bodziach et al., 2021), indicating that environmental exposure to 4-OP could result in endocrine disruption via ER activation. The similarity of activation and inhibition of human and fulmar ERs provide important information when interpreting the hormonal disruption capacity of plastic-associated chemicals across taxa.

4.2. Leachates from ingested plastic activated and inhibited fulmar ERs

Plastic ingested by fulmars leached chemicals capable of activating or inhibiting fulmar ERs *in vitro*. Almost half of the fulmars (13 out of 27) ingested plastic that induced a hormonal response. Although others have shown that plastic recovered from common murres (*Uria aalge*) (Michishita et al., 2023) can activate human ERs, to our knowledge, we provide the first information on the ability of ingested plastic to activate and inhibit a non-human ER. Given the unknown duration of plastic retention in the birds' stomachs prior to their incidental bycatch, our findings likely provide a conservative estimate of the risk to fulmars, as many bioavailable chemicals, including POPs, may have leached from the ingested plastic in the first few days after ingestion (Van Hassel et al., 2024).

While no correlation was observed between plastic mass loss after leaching and ER response, the significant reduction of mass observed in some samples is important to note. We are unable to ascertain why some samples lost no mass, while others lost ~67 %, but the identified polymers (HDPE, LDPE, PP and PET) are resistant to hexane and methanol (Thermo Scientific). It is possible that the plastic recovered from the fulmar stomachs had a high degree of variability in their amount of weathering, causing a variation in susceptibility to solvent dissolution.

Polymer type was not associated with ER response (Fig. 3), suggesting that the endocrine-disrupting effects of plastic leachates were not related to polymer composition but more likely due to plastic additives (e.g., BPA, flame-retardants). We previously showed high concentrations of additives are released in an *in vitro* Procellariiform stomach model after 5 days (Van Hassel et al., 2024), consistent with the observed ER activation or inhibition on day 5 and/or 14 for ~ 70 % of the fulmars that showed a response on day 1 (9 out of 13). Interestingly, two samples activated ERα in the hexane fraction after the first day of leaching and in the methanol fraction on day 5 or 14 (Table 1), suggesting that different chemicals, located deeper within the plastic matrix may be released over time. Plastic weathering (e.g., cracking) may enhance solvent penetration into the polymer matrix, potentially

increasing the leaching of additives (Bridson et al., 2023). We propose additive type and initial concentration, as well as the residence time of plastic in the ocean (Koelmans et al., 2016) and/or the bird likely contributed to the observed differences in ER response among fulmars.

The use of numerous additives in plastic manufacturing poses a challenge to characterize plastic leachates, with approximately 450 compounds lacking standardized identification protocols (Ong et al., 2022). Nonetheless, our qualitative assessment confirms the presence of distinct compounds responsible for ER activation and inhibition (Figs. S8 and S9) in plastic leachates. A wide range of physico-chemical properties characterize plastic additives (Ellington, 1999), and our findings that both hexane and methanol fractions induced a response suggest that pollutants with different degrees of hydrophobicity were leached.

4.3. Biological significance

Although we used an *in vitro* system to measure hormonal disruption, the same *in vitro* expression system established a link between the ER response of southern white rhinoceros (*Ceratotherium simum simum*) to phytoestrogens and their poor reproductive success (Tubbs et al., 2016). This linkage, combined with our prior work demonstrating that chemicals are released from plastic in an *in vitro* Procellariiform stomach model (Van Hassel et al., 2024), indicates ingested plastic could cause hormonal disruption in fulmars. The similarities between ER response of humans and fulmars also suggests that other species known to ingest plastic (Kühn and van Franeker, 2020) could be at risk of hormonal disruption.

Our results might overestimate the risk from ingested plastic due the *in vitro* expression system necessitating the use of hexane and methanol as opposed to an *in vitro* Procellariiform stomach model (Van Hassel et al., 2024) for plastic leaching. In a Procellariiform stomach, highly hydrophobic chemicals (e.g., PBDE-209, PCBs) primarily leach in the presence of lipids (e.g., stomach oil), whereas less hydrophobic chemicals (e.g., BPS) leach in lipid and aqueous (acidic pepsin-protein) phases (Van Hassel et al., 2024). Due to the challenge of purifying and concentrating large volumes of these biological fluids for cell culture, we used hexane to simulate lipid-phase leaching and methanol for aqueous-phase leaching which most likely affected the release of plastic associated chemicals (Van Hassel et al., 2024).

As mentioned previously, our results might also underestimate the risk from ingested plastic as the collected pieces were in the birds' stomachs for an unknown amount of time before they were collected. Plastic can have a stomach retention time of weeks to months (Ryan, 2015; Terepocki et al., 2017) and we have shown that higher concentrations of chemicals, including sorbed POPs, are leached within 5 days (Van Hassel et al., 2024). In addition, we did not collect plastic <1 mm from the fulmar stomachs, which may also leach hormone disrupting chemicals (Michishita et al., 2023). Taken together, the use of solvents for plastic leaching which might overestimate risk, and the release of chemicals prior to collection which might underestimate risk, our results are not meant to be interpreted as a quantitative measure of hormonal disruption. Rather, we illustrate the potential harm from the release of chemicals from ingested plastic – a ubiquitous problem in marine systems worldwide.

5. Conclusion

Our study underscores the potential harmful effects of plastic ingestion in fulmars. We found that both human and fulmar ERs respond similarly to plastic-associated chemicals, albeit with sensitivity differences depending on the chemical, concentration, and receptor type. Almost half of the fulmars (13 out of 27) had ingested plastic that activated or inhibited fulmar ERs after 1 day of solvent leaching, suggesting the potential for hormonal disruption. Plastic collected from almost 70 % of these birds (9 out of 13) also elicited ER responses on day 5 or 14 of solvent leaching, illustrating the prolonged release of plastic-associated chemicals. We propose that the observed hormonal disruption was due to plastic additives, not polymer residual monomers, due to a lack of association between polymer type and ER response. Our findings highlight the potential negative consequences from plastic pollution on Procellariiform health, emphasizing the need for effective measures to reduce plastic waste in marine environments.

CRediT authorship contribution statement

Liesbeth Van Hassel: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Rachel Felton:** Writing – review & editing, Validation, Resources, Methodology. **Christopher Tubbs:** Writing – review & editing, Validation, Resources, Methodology, Conceptualization. **Jessie Beck:** Writing – review & editing, Validation, Resources, Investigation. **Maxime Albert:** Methodology, Investigation, Formal analysis. **Vincent Baeten:** Writing – review & editing, Validation, Resources, Methodology. **Juan Antonio Fernandez Pierna:** Writing – review & editing, Validation, Resources, Methodology. **Cathy Debier:** Writing – review & editing, Validation, Supervision, Resources, Methodology. **Myra Finkelstein:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Liesbeth Van Hassel reports financial support was provided by Foundation for Scientific Research. If there are other authors, they 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.2025.126145.

Data availability

Data will be made available on request.

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