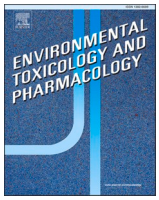
Environmental Toxicology and Pharmacology 116 (2025) 104703

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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



Environmental assessment of steroid endocrine disruptors in stranded cetaceans: A methodological approach to detection and analysis

Adrián Bullón-Téllez [a], Zoraida Sosa-Ferrera [a], José Juan Santana-Rodríguez [a], Natalia Garcia-Álvarez [b], Jesús De la Fuente [b], Manuel Arbelo [b], Antonio Fernández [b], Pedro López-Suárez [c], Carolina Oujo [c], Katia Freire-Lopes [c], Rayco Guedes-Alonso [a,*]

[a] Instituto Universitario de Estudios Ambientales y Recursos Naturales (i-UNAT), Universidad de Las Palmas de Gran Canaria, 35017, Las Palmas de Gran Canaria, Spain

[b] Veterinary Histology and Pathology, Institute of Animal Health and Food Safety (IUSA), Centro Atlántico de Investigación de Cetáceos, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

[c] BIOS.CV, Association for Environmental Conservation and Sustainable Development, Sal Rei, Boa Vista, Cabo Verde

ARTICLE INFO

Keywords:
Steroid hormones
Cetacean blubber
Stranded cetaceans
QuEChERS
Ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS)

ABSTRACT

Exploring the presence of endocrine disrupting compounds in marine organisms, particularly cetaceans, is crucial to evaluate contamination within the ocean's food web and the detection of synthetic hormones in cetaceans is a strong indication of anthropogenic pollution in marine ecosystems. Due to their characteristics, blubber samples are a key component in assessing the analysis of cetaceans, but this type of sample require precise and sensitive analytical methods. Despite some methodologies have been developed for the analysis of natural steroid hormones in cetacean blubber, a significant gap persists in the comprehensive analysis of synthetic steroids within these samples. In this work, a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction combined with UHPLC-MS/MS (ultra-high-performance liquid chromatography with tandem mass spectrometry) analysis was performed to determine six synthetic steroid hormones (nandrolone, prednisolone, prednisone, megestrol acetate, norethisterone, and norgestrel) in 11 stranded cetacean blubber samples. Despite the complex nature of blubber samples, the developed methodology showed promising results, with recoveries ranging from 70 % to 120 % for most target compounds at low concentration levels (150 $ng \cdot g^{-1}$). The method demonstrated high precision with relative standard deviations under 20 % in both intra- and inter-day experiments. Regarding the matrix effect, ion suppressions of about 50 % were also calculated for those samples spiked at concentration levels of 150 $ng \cdot g^{-1}$, nevertheless, all compounds were still able to be detected, in most cases below quantification limits. Notably, one sample presented a quantified concentration of megestrol acetate, a steroid hormone used in contraceptives and cancer treatments, at 26.98 ± 2.62 $ng \cdot g^{-1}$. The results affirm the methodology's effectiveness for analyzing synthetic steroid hormone levels in cetacean blubber, providing a valuable tool for assessing environmental concentrations of these anthropogenic endocrine disruptors and their impact on cetacean welfare and conservation.

1. Introduction

Hormone pollution is becoming an environmental problem of emerging relevance since the 1980s, when deformities in male fish from British STW laggons were observed and identified as consequence of the estrogenic compounds presence in STWs effluents (Purdom et al., 1994). Desbrow et al. (1998) report 2 natural (17ß-estradiol and estrone) and 1 synthetic hormones (ethinylestradiol) as the causative of these events. Male fish were able to respond to very low concentrations of these compounds, which induce to a vitellogenin production, a protein associated with reproducing female, causing the "feminization" (Routledge et al., 1998). Most of the studies performed about the presence of hormones in marine environment have focused in estrogens (Mezzelani and Regoli, 2022); however, "masculinization" of female fish caused by exposure to androgens in effluents have been proved (Parks, 2001). By following this path, goal number 14 of the 2030 Agenda for Sustainable

* Corresponding author.
E-mail address: rayco.guedes@ulpgc.es (R. Guedes-Alonso).

https://doi.org/10.1016/j.etap.2025.104703
Received 13 November 2024; Received in revised form 22 March 2025; Accepted 18 April 2025
Available online 23 April 2025

Development of the United Nations, titled "Conserve and sustainably use the oceans, seas and marine resources for sustainable development", proposes as a target "increase scientific knowledge, develop research capacity and transfer marine technology" (United Nations General Assembly, 2015).

Oceans consist on 71 % of the Earth's surface and contain much of its biodiversity, where marine mammals are major consumers at most trophic levels, from primary production to other mammals (Bowen, 1997; Costello and Chaudhary, 2017). Human impact affects 74 % marine mammal species at high levels (Davidson et al., 2012). Cetaceans represent 70 % of the species of marine mammals and play an important role on marine ecosystems, as predators, prey, carcasses scavenged and nutrient transference vector (Quaggiotto et al., 2022; Roman et al., 2017; Tobeña et al., 2016). As predators, cetaceans consume between 200 and 400 million tons of squid and fish per year. For example, sperm whales consume 100 million tons of squid while small odontocetes consume between 100 and 300 million tons of fish each year (Kanwisher and Ridgway, 1983). This means a large difference compared to capture production in marine fishing areas, which is estimated to be 80,4 million tons by the Food and Agriculture Organization of the United Nations (FAO, 2021). In this context, studies about human impact on cetaceans become relevant as representative results of ocean conditions (Bellante et al., 2012; Chen et al., 2017; López-Berenguer et al., 2020; Zhu et al., 2019) as well as it has been proved that marine mammals present significant concentrations of anthropogenic substances considered as persistent organic pollutants (POPs) such as pesticides, polychlorinated biphenyls or polycyclic aromatic hydrocarbons (Bartalini et al., 2022) and emerging contaminants (Andvik et al., 2021). Several effects of endocrine disruptors in cetaceans have been reported, including cancer, immunosuppression, reduced reproductive success and diseases (Fossi and Marsili, 2003).

Steroid hormones, natural or synthetic, anthropogenically discharged into the marine environment, act as endocrine disruptors, even at very low levels (Ting and Praveena, 2017). The study of these compounds in ocean mammals is fundamental to analyze the anthropogenic impact on this environment. Different studies have focused on the determination of sexual maturity (Mello et al., 2017; Mello and Alvarenga, 2016; Wittmaack et al., 2022), pregnancy (Atkinson et al., 2019; Kellar et al., 2006; Mansour et al., 2002; Pérez-Jorge et al., 2011) and hormone profiles (Boggs et al., 2017; Dalle Luche et al., 2019; Galligan et al., 2018), analyzing endogenous hormones contained in blubber of cetaceans but the studies about the presence of synthetic steroid hormones in cetacean tissue are scarce. Blubber is a specialized hypodermic adipose tissue found in marine mammals (Galligan et al., 2018). Most organic contaminants are incorporated into the body of mammals via food (Aguilar et al., 1999). Fat-soluble pollutants, such as organochlorines, dioxins or polychlorinated biphenyls are transported by lipoproteins and accumulate in blubber (Lind and Lind, 2020; Lydersen et al., 2002). During fasting periods, a very common process in the life cycle of marine mammals, lipophilic pollutants are liberated from the blubber into the blood circulation (Debier et al., 2003; Vanden Berghe et al., 2012) which could induce physiological and health problems (Debier et al., 2006). Steroid hormones are lipophilic compounds that are accumulated in cetaceans blubber (Dalle Luche et al., 2019), so synthetic steroid hormones could present a risk for marine mammals health. No information about effects of synthetic hormones in marine mammals exist in the published literature. Monitor mammals population is required to determine at which concentrations of chemical pollutants, as synthetic hormones, different adverse effects occur, and to use these to improve the health of individuals or populations (Reijnders et al., 1999). Blubber collection is guaranteed in stranded or hunted cetaceans and biopsies appear as a moderately invasive technique, non-lethal, on free-ranging animals. It is a good alternative specially in large cetaceans in which is difficult to collect other matrixes in live animals (Mello and Alvarenga, 2016), as blood (Temte and Spielvogel, 1985), saliva (Rickert et al., 2021), urine (Muraco et al., 2009), faeces (Miller et al., 2021), ocular secretions (Atkinson et al., 1999), milk (West et al., 2007) or blow (Hogg et al., 2005). In addition, the possibility of using the same technique for live and dead animals, makes the blubber sample a very attractive matrix to determine, by hormone analysis, the health or welfare status, reproductive stage, and pregnancy of live animals, allowing to monitor cetacean population groups.

Adeel et al. (2017) developed a study on environmental impacts of estrogens in which the authors determined that one of the main sources of anthropogenic steroid hormones were sewage treatment plants and effluent from livestock feedlots. Hormones contained in hospital, industrial and domestic waste are not completely removed through sewage plants processes, so significant concentrations of these compounds contained in bio-solids and wastewater may be discharged to the natural environment (Guedes-Alonso et al., 2014; Robinson et al., 2007). Moreover, hormones and other contaminants contained in water from irrigation of agricultural lands, with fertilizers, or in leachates from municipal landfills, can arrive to groundwaters through infiltration. In this case, mega farms or concentrated animal feeding operations (CAFOs), due to the infiltration of the animal waste into the groundwater, are a great source of synthetic and natural hormones. Main hormones measured in tissues of marine organisms are 17β-estradiol, 17α-ethynylestradiol, estrone, diethylstilbestrol, estriol, norgestrel, norethisterone, megestrol acetate, progesterone, testosterone, boldenone, nandrolone, cortisone, prednisone, and prednisolone, 9 synthetic and 6 natural hormones (Mezzelani and Regoli, 2022). In the present study 6 synthetic hormones were selected for analyse, based on the presence of them in the aquatic environments of Macaronesia region.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) has been applied to the analysis of steroid hormones in environmental and biological matrices due to its selectivity, sensitivity, analytical throughput and compatibility with physico-chemical properties of compounds (Guedes-Alonso et al., 2016a). However, the study in complex biological matrices such as blubber is a challenge due to multiple variables such as the difficulty of homogenizing the sample due to its viscosity or the high lipid content that can affect the selectivity of the method, so existing methodologies for its analysis for different compounds show a lower accuracy and precision than analyses performed on simpler matrixes such as water or sediments (González-Bareiro et al., 2023; Huysman et al., 2017; Liu et al., 2022). In this sense Boggs et al. (2017) developed an LC-MS/MS method for the analysis of multiple natural hormones in bottlenose dolphin blubber using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) as extraction method.

The purpose of this study was to evaluate the performance of a method for analyzing synthetic steroids in bottlenose dolphin blubber (*Tursiops truncatus*) and to apply this method to determine these hormones profile in two cetacean species (*Tursiops truncatus* and *Globicephala macrorhynchus*) stranded in Macaronesia region. The chosen synthetic steroid hormones were detected previously in waters and biota from Macaronesia region (Guedes-Alonso et al., 2017; Torres-Padrón et al., 2020) and this assessment could be useful to evaluate possible bioaccumulation and biomagnification effects. Also this study introduces a novel modification of the QuEChERS extraction method, as proposed by (Boggs et al., 2017)., making it more accessible for regions with large cetacean populations but limited scientific resources. The methodology not only ensures cost-effectiveness and ease of use but also allows for the analysis of small sample sizes, which can be obtained through non-lethal biopsy darting. This adaptation provides a practical and scalable solution for monitoring synthetic steroid pollution in marine mammals as well as it addresses the determination of synthetic hormones, a significantly less explored area compared to the analysis of endogenous hormones.

2. Materials and methods

2.1. Material, and reagents

HPLC-grade methanol and acetonitrile, and LC/MS-grade methanol and water used in the extraction and in the chromatographic methods were obtained from Panreac Química (Barcelona, Spain), while ultrapure water was provided by a Milli-Q system (Millipore, Bedford, MA). Nandrolone (NAN), prednisolone (PRDNL), prednisone (PRD), megestrol acetate (MGA), norethisterone (NORET) and norgestrel (NOR) shown in Table 1, were purchased from Sigma–Aldrich (Madrid, Spain) and showed purity over 99 %. Stock solutions (1000 mg·L^{-1}) of target analytes were prepared by dissolving the compound in LC/MS-grade methanol, and the solutions were stored in glass-stoppered bottles, wrapped in aluminum foil to block light exposure, at −20 °C prior to use. A working solution consisting of a mixture of target compounds at a concentration of 10 mμL^{-1} was prepared also in LC-MS methanol and conserved at −20 °C.

2.2. Sample collection

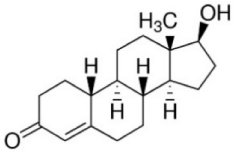
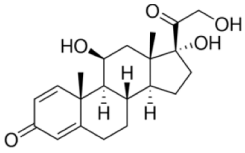
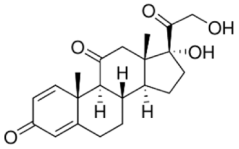
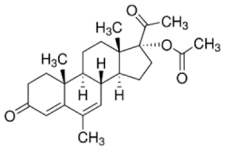
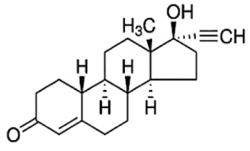
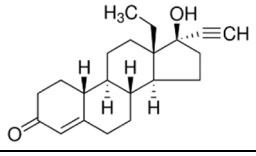
For the validation and application of the method and in order to obtain a large amount of dolphin blubber, samples were collected from dead bottlenose dolphins (*Tursiops truncatus*), corresponding CET0696; CET1042; CET1133; CET1191; CET1200 and CET1209 codes, and short-finned pilot whales (*Globicephala macrorhynchus*), corresponding CET0880; CVGM2_18; CVGM4_18; CVGM6_18 and CVGM7_18 codes, stranded in Macaronesia, specifically in the Canary Islands and Cabo Verde Islands (Table 2). All cetaceans sampling were histologically mature. According to the sampling protocol, samples were collected from the dorsal area of cetaceans.

Blubber samples were stored at −80 °C, minimally thawed for sectioning, and stored again at −20 °C until sample pretreatment and extraction procedures.

2.3. Pretreatment and extraction procedures

The sample preparation followed the work of Boggs et al. on dolphin

Table 1
List of studied hormones, class, structures, retention times (TR), n-octanol-water partition coefficient (Kow) and negative base-10 logarithm of acid dissociation constant (pKa).

Compound	Class[a]	Structure[b]	K_{ow}[c]	pK_a[d]	T_R (min)
Nandrolone (NAN)	Androgen		2.62	15.06 ± 0.40	2.94 ± 0.39
Prednisolone (PRDLN)	Glucocorticoid		1.62	12.46 ± 0.70	2.48 ± 0.38
Prednisone (PRD)	Glucocorticoid		1.46	12.36 ± 0.60	2.37 ± 0.41
Megestrol acetate (MGA)	Progestogen		4.00	n.a[e]	3.53 ± 0.32
Norethisterone (NORET)	Progestogen		2.97	13.09 ± 0.40	2.98 ± 0.34
Norgestrel (NOR)	Progestogen		3.48	13.09 ± 0.40	3.25 ± 0.37

Abbreviations for each compound are indicated in parentheses.
[a] From reference (Guedes-Alonso et al., 2014)
[b] From reference (Sigma-Aldrich, 2022)
[c] from EPI (Estimation Programs Interface) Suite™
[d] from Scifinder database
[e] not available.

Table 2
Sampled animals for hormone analysis.

Stranded code	Species	Sex	Stranded date	Island	Region
CET0696	*Tursiops truncatus*	Male	12/02/2014	Fuerteventura	Canary Islands
CET1042	*Tursiops truncatus*	Male	23/11/2019	Tenerife	Canary Islands
CET1133	*Tursiops truncatus*	Male	31/10/2020	Fuerteventura	Canary Islands
CET1191	*Tursiops truncatus*	Male	26/08/2021	La Gomera	Canary Islands
CET1200	*Tursiops truncatus*	Male	04/11/2021	Gran Canaria	Canary Islands
CET1209	*Tursiops truncatus*	Male	31/01/2022	Tenerife	Canary Islands
CET0880	*Globicephala macrorhynchus*	Male	15/12/2017	Lanzarote	Canary Islands
CVGM2_18	*Globicephala macrorhynchus*	Female	06/09/2018	Boa Vista	Cabo Verde Islands
CVGM4_18	*Globicephala macrorhynchus*	Female	06/09/2018	Boa Vista	Cabo Verde Islands
CVGM6_18	*Globicephala macrorhynchus*	Male	06/09/2018	Boa Vista	Cabo Verde Islands
CVGM7_18	*Globicephala macrorhynchus*	Male	06/09/2018	Boa Vista	Cabo Verde Islands

blubber (Boggs et al., 2017). Scapels were used to remove superficial layers of skin and muscle from samples in a sterile Petri dish and blubber were minced (<3 mm) to provide as much homogeneity as possible without compressing the tissue, which can result in the loss of oil. Then the sample was evaporated under stream of N_2 to remove water content during congelation. 100 mg of sample weighed in an analytical balance with a precision of 0.1 mg (VWR lnternational Eurolab, Spain) were transferred into a 50 mL Falcon tube, adding 4 mL of ultrapure water and shaking for 10 seconds. 10 mL of acetonitrile (ACN) were then added, and the Falcon tube was vortexed for 30 seconds. Following a QuEChERS-based procedure, some salts (4 g $MgSO_4$; 1 g NaCl; 1 g sodium citrate; 0.5 g disodium citrate sesquihydrate) were added to the sample, and the Falcon tube was again hand shaken for 1 min. Samples were sonicated for 1 min and centrifuged at 4000 rpm for 5 min. 7 mL of the supernatant were transferred to a clean glass test tube and 100 mg of octadecyl silica (C_{18}), weight optimized with high lipid matrixes, were added to perform dispersive solid phase extraction (dSPE) for lipid removal, vortexed during 1 min and then centrifuged at 4000 rpm for 10 min. Finally, in order to achieve greater preconcentration, the samples were transferred into a glass bottle and evaporated under stream of N_2 and reconstituted in 300 µL of MeOH, which means a preconcentration of the extract of 23.3 times.

2.4. Chromatographic and detection conditions

The analysis of 6 synthetic steroid compounds was undertaken on an ultra-high performance liquid chromatography system coupled to a triple quadrupole detector (UHPLC–MS/MS) with Electrospray Ionization Interface (ESI) from Waters (Barcelona, Spain). Components were managed with MassLynx mass spectrometry software also from Waters. Fig. 1 shows the chromatogram obtained from a sample spiked at 150 $ng{\cdot}g^{-1}$.

Chromatographic separation was performed with a Phenomenex EVO column C_{18} (50 × 2.1 mm) with a particle size of 1.7 µm (Phenomenex, California, USA) at a temperature of 35 °C. The sample volume injected was 10 µL, using water with 0.1 % (v/v) of ammonia and methanol without additives at a flow rate of 0.4 mL min^{-1} in gradient mode for the chromatographic separation. Mass spectrometry parameters for the detection and identification of hormone compounds are shown in Table 3.

2.5. Method validation

The analytical parameters evaluated in this study were linearity, detection and quantification limits (instrumental and method), recoveries, repeatability, and reproducibility for blubber samples.

External calibration curves were built with 9 points in the range from 1 to 800 $ng{\cdot}mL^{-1}$ which correspond to the theoretical concentrations in blubber samples between 1.5 and 1200 $ng{\cdot}g^{-1}$ of target analytes. Instrumental detection and quantification limits (IDLs and IQLs) were calculated as the concentrations that showed a signal-to-noise ratio equal to 3 and 10, respectively.

The relative recoveries (Fig. 2) were studied by calculating the ratio

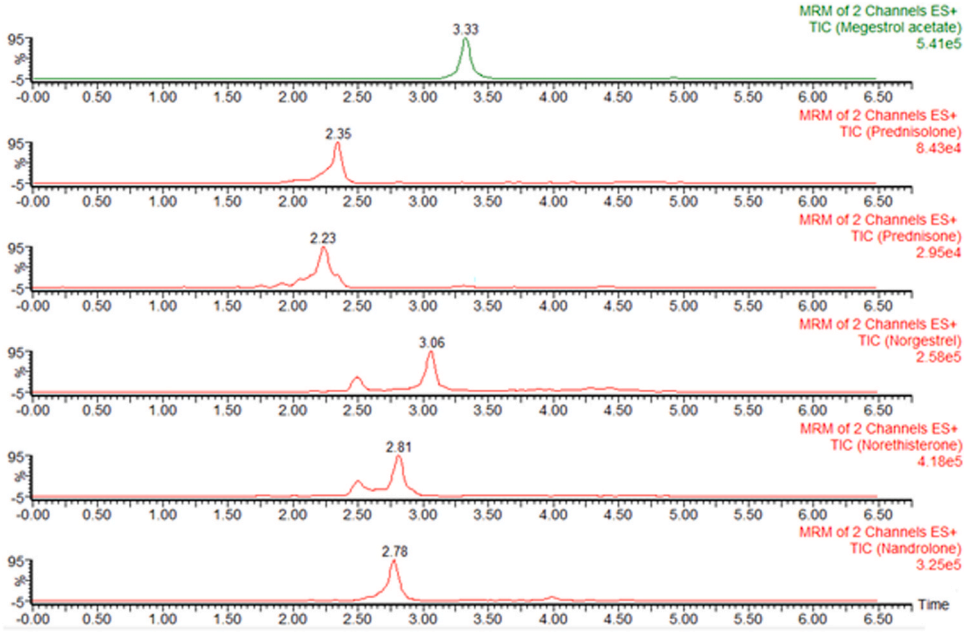


Fig. 1. Chromatograms of the target synthetic steroid hormones obtained after QuEChERS extraction of a blubber sample spiked at 150 $ng{\cdot}g^{-1}$.

Table 3
Mass spectrometer parameters for the determination of target analytes (Guedes-Alonso et al., 2016b).

Compound	Precursor ion (*m/z*)	Cone voltage (ion mode)	Quantification ion, *m/z* (collision potential, V)	Confirmation ion, *m/z* (collision potential, V)
NAN	275.2	35 V (ESI+)	109.1 (20)	83.0 (30)
PRDNL	361.3	20 V (ESI+)	147.1 (20)	173.1 (25)
PRD	359.3	30 V (ESI+)	147.0 (15)	237.0 (20)
MGA	385.5	30 V (ESI+)	267.3 (15)	224.2 (30)
NORET	299.2	30 V (ESI+)	109.1 (25)	91.0 (40)
NOR	313.2	38 V (ESI+)	109.0 (26)	245.1 (18)

between the response of the extracted sample spiked with the target compounds and the response of post-extracted spiked samples. According to the expected concentration of hormones in real samples, triplicate samples were spiked at three concentration levels (150, 600 and 1200 $ng{\cdot}g^{-1}$ which correspond to 35, 140 and 280 $ng{\cdot}mL^{-1}$ in final extract).

Matrix effect is another important analytical parameter, especially in the analysis of biological samples. These matrices are considered very complex ones due to the multiple interferences such as lipids and proteins that could affect the ionization during the detection step. In this study, the matrix effect was calculated as the percentage of signal suppression/enhancement by comparing the difference of a QuEChERS extract of a sample spiked post-extraction and a standard at the same concentration prepared in a pure solvent, in this case, methanol with the signal of standard in pure solvent. Equation of signal change presented in Fig. 2 was used to evaluate signal suppression/enhancement. Positive values of the analytical signal change indicated signal suppression while negative values indicate signal enhancement.

Method detection and quantification limits (MDLs and MQLs) were calculated as the concentrations that showed a signal-to-noise ratio equal to 3 and 10, respectively considering the extraction efficiency and matrix effect produced in the obtained extracts after QuEChERS extraction.

The repeatability of the method was studied intra-day (samples triplicated on the same day) and reproducibility was inter-day (samples triplicated on different days), calculating relative standard deviation (RSD) values at the same three concentration levels as in recovery calculations (Table 4).

3. Results and discussion

3.1. Method validation

The results showed a great linearity in the studied range and for all compounds, with correlation coefficients (r^2) higher than 0.990 (Fig. S1). Regarding instrumental detection limits (ILODs) were in the range of 0.017–0.21 $ng{\cdot}mL^{-1}$. Instrumental quantification limits (ILOQs) were the lowest point of the calibration curve that was fixed in 1 $ng{\cdot}mL^{-1}$ for all compounds.

Extraction efficiencies (Fig. 3) show differences between spiked concentration levels and compounds. In this study, recoveries in spiked samples at 150 $ng{\cdot}g^{-1}$ ranged between 105 % and 124 %. In spiked samples at 600 $ng{\cdot}g^{-1}$, recoveries ranged between 64 % and 71 % for all compounds. Finally, in spiked blubber at the most elevated concentration level, 1200 $ng{\cdot}g^{-1}$, recoveries ranged between 78 % and 92 %. Boggs et al. proposed recovery bounds between 70 % and 120 % as acceptable, range slightly exceeded by some compounds evaluated in the present study (Boggs et al., 2017). The expected concentrations in the subsequent analysis of the samples were closer to the lowest spiked concentration, for which acceptable recovery values were obtained.

Regarding matrix effect, the study was focused on gaining a comprehensive understanding of their behavior of the synthetic during extraction and analysis. For this reason, instead of using isotopically labeled internal standards to overcome matrix effects, signal suppression/enhancement was assessed for each compound under study. This

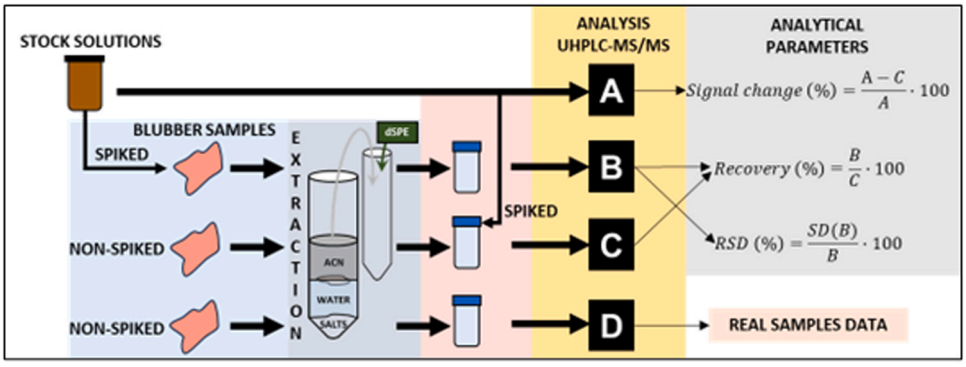


Fig. 2. Scheme of methodology for analytical parameters determination. RSD: Relative standard deviation. SD: Standard deviation.

Table 4
Method detection limit (MDL), method quantification limit (MQL), repeatability (intra-day RSD) and reproducibility (inter-day) of the validated method.

Compound	MDL ($ng{\cdot}g^{-1}$)	MQL ($ng{\cdot}g^{-1}$)	Intra-day RSD (%) n = 3			Inter-day RSD (%) n = 3 × 3		
			150 ($ng{\cdot}g^{-1}$)	600	1200	150 ($ng{\cdot}g^{-1}$)	600	1200
NAN	2.28	7.61	14.73	9.88	8.78	12.80	5.43	7.05
PRDNL	9.48	31.61	13.14	8.60	8.75	6.35	10.67	5.04
PRD	11.29	37.64	16.06	1.81	6.66	20.00	14.76	6.70
MGA	2.13	7.11	19.26	9.69	10.47	9.70	6.14	6.65
NORET	6.24	20.80	9.76	2.84	8.48	14.33	3.70	9.25
NOR	37.16	123.86	7.88	12.48	8.32	21.39	14.06	19.48

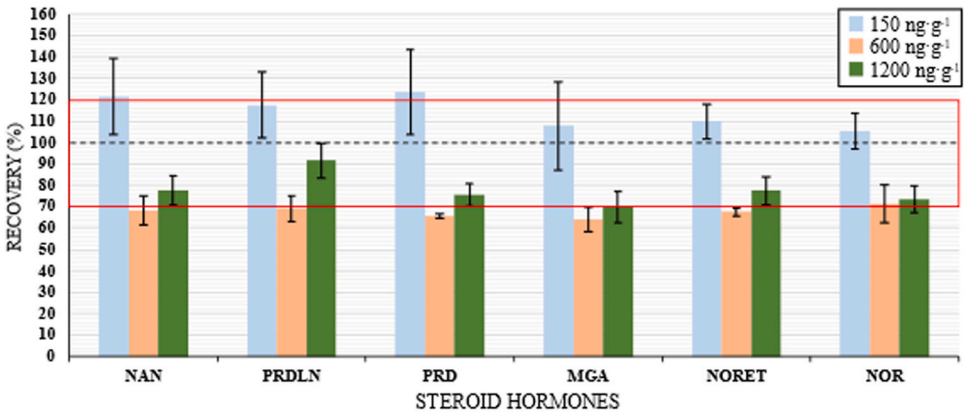


Fig. 3. Relative recoveries of steroids from Tursiops truncatus blubber (n = 3) spiked at three different concentration levels (150, 600 and 1200 ng·g^{-1}). The red box delimits the range of acceptable recovery values according to Boggs et al. (2017). The dotted line denotes 100 % recovery value. Error bars represent ± standard deviations.

approach also ensures that the methodology remains accessible to laboratories with limited resources, facilitating its implementation in future research. All the target analytes presented a suppression of the signal due to the complexity of the sample (Fig. 4). Signal suppressions of most of the steroid hormones analysed from samples spiked with 600 and 1200 ng·g^{-1} were below 50 %, proposed as an acceptable limit. In contrast, this effect is more pronounced at the lower spiked concentration (150 ng·g^{-1}), because the analytical signals are also lower. This effect may be related to the interferences that are extracted regardless of the concentration at which the sample is spiked. The lower the concentration, the greater the effect of these interferences on the determination.

The method detection and quantification limits show a significant variability between compounds (Table 4). NOR presented highest values (MQL were between 61.32 and 130.69 ng·g^{-1}) compared to other compounds with MQLs that ranged between 7.11 and 37.64 ng·g^{-1}. This could be explained because NOR showed greater matrix effects than rest of target analytes.

RSDs are presented in Table 4. Values from intra-day evaluation ranged between 1.81 % and 19.26 %, generally showing a tendency to decrease with increasing concentration level. RSD from inter-day study ranged between 3.70 % and 20 %, except for NOR at 150 ng·g^{-1} (21.39 %), which is the compound with the highest LOQ. Generally, inter-day RSD values indicate slight lower reproducibility at the lowest concentration, with a mean RSD of 14.10 % at 150 ng·g^{-1}, 9.13 % at 600 ng·g^{-1}, and 9.03 % at 1200 ng·g^{-1}, confirming this trend. Considering an acceptable RSD limit of 20 %, due to matrix complexity and interferences that are not removed in the clean-up process through C_{18}, the repeatability and reproducibility of the methodology could be considered adequate.

Wittmaack et al. (2022) performed a blubber homogenization optimization for hormones extraction by comparing bead beating and blade dispersion, resulting in suitable recovery and RSD values through bead beating with dry ice technique, while homogenization through blade dispersion, more similar to the homogenization performed in this study, indicates unacceptable parameters at 75 % of samples. In addition to its automation capability, which results in a more stable error, bead beating provides blubber minced to smaller size, more homogenous, and dry ice reduces compress the tissue, minimizing the loss of oil (Dalle Luche et al., 2019). In the present study, homogenization was performed using scapels, which limits the minced ability and increases the possibility of human error but reducing the cost and maintaining the philosophy of the QuEChERS method. Despite this, in contrast to the study of (Wittmaack et al., 2022)., repeatability and reproducibility presented acceptable values (recoveries between 70 % and 120 % and reproducibilities under 20 %).

3.2. Analysis of synthetic steroid hormones in blubber samples

Fig. 5 shows the target hormone compounds detected in the blubber samples analysed as well as those compounds for which the concentration exceeded the limit of quantification. In this study, the sampling body location was the dorsal region, which is a relevant factor for the determination of hormones because blubber presents differences across the body, therefore, a location with high lipid concentration and an elevated number of adipocytes could present higher hormones

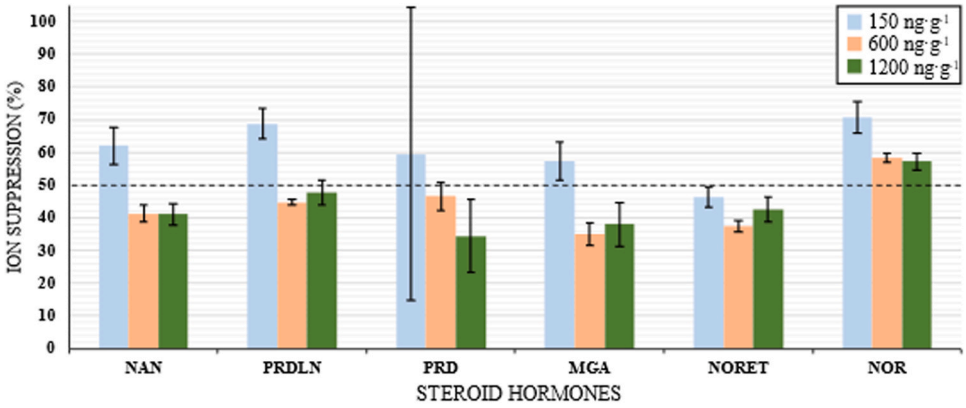


Fig. 4. Ion suppressions of steroids from Tursiops truncatus blubber (n = 3) spiked at three different concentration levels (150, 600 and 1200 ng·g^{-1}). The dotted line denotes 50 % matrix effect. Error bars represent ± standard deviations.

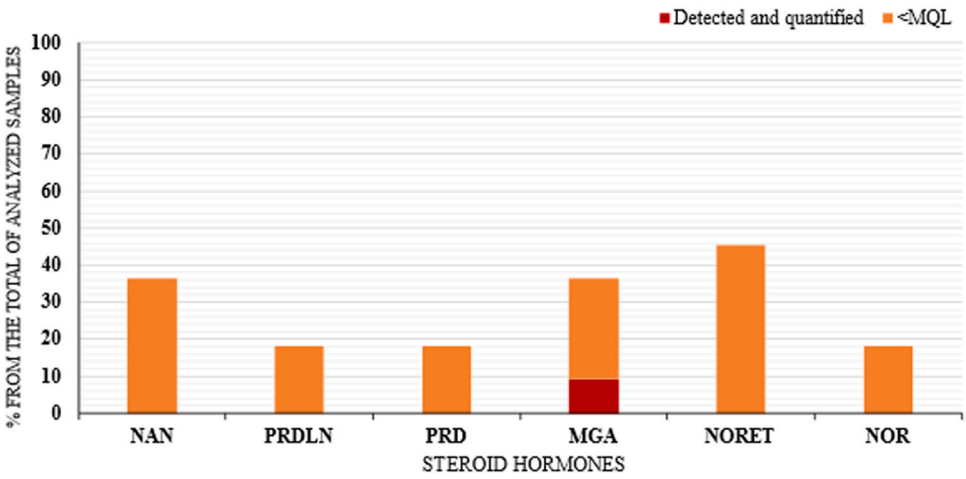


Fig. 5. Distribution of the detection of the target analytes in the studied samples (n = 11).

concentrations, such as the ventral region. However, dorsal area is the region with lower lipid concentration and number of adipocytes across all body of cetaceans (Carbajal et al., 2022). In the same way, (Kellar et al., 2006). compared three different layers as a function of blubber depth (outer, middle and inner) and obtained higher values of progesterone in the middle layer. The degree of blubber stratification varies among marine mammals (Guerrero and Rogers, 2017). Aguilar & Borrell (Aguilar and Borrell, 1991) performed a study about the distribution of organochlorine contaminants in the blubber layers of baleen whales, and concluded that pollutant analyses should include all layers in order to be representative of total pollution load into the cetacean. (Krahn et al., 2004) found that the variation in organochlorine compounds with depth and body region in each whale analysed was small relative to the differences between whales, such that each animal could be distinguished from the others. Pedro et al. (2017) suggest that changes in the stratification of blubber in females could be associated with the lactation period. Analyse full thickness of blubber from stranded cetaceans could be of special interest for comparison with the analysis of samples collected by biopsy darts from free-ranging cetaceans, to know if this biopsy system could be representative of the total. In most cases collecting only the outermost layer of the blubber, being impossible to divide into layers (Koopman, 2007). The average weight of blubber collected by (Kellar et al., 2006). using biopsy darts was 150 mg. Although the size of the dart used varies according to the size of the animal (Sinclair et al., 2015). However, there are factors that can alter the weight collected, such as the distance the dart is thrown (Mijele et al., 2016). In the present study, full thickness were analysed trying to ensure that the minced sample was representative of the total, using a sample weight that is practically guaranteed in samples collected by biopsy darts. In this way, the method can be applied to biopsies, obtaining results that could be compared with the full-thickness blubber. In addition, the limited access to samples from stranded cetaceans makes it impossible to have enough weight of each layer to make a triplicate of the sample.

As can be seen in Table S1, norethisterone was presented in 45.5 % of the analysed samples but were not appeared in sufficient quantity to be quantified. Nandrolone and megestrol acetate appeared in 36.4 % of samples. Megestrol acetate was quantified in one sample (CET1200) at a concentration of 27.0 ± 2.6 $ng{\cdot}g^{-1}$. Prednisolone and norgestrel were detected in the same samples but there was not sufficient information to make a relation between them. Prednisone appears in only two samples (18.1 %) with concentration below MQL too. The six synthetic hormones analyzed were detected in at least two samples each one. Galligan et al. (2020) determined natural hormone profiles of free-ranging bottlenose dolphins from blubber, obtaining a range between 1.08 and 63.3 $ng{\cdot}g^{-1}$ of androgens in adult males and up to 174 $ng{\cdot}g^{-1}$ of progestogens as progesterone in female animals. Considering the MQLs of the detected hormones, most of the compounds detected in the present study are included in this ranges, which could indicate similar concentrations of natural and synthetic hormones, a relevant information that indicates the human impact on the ocean. Other studies of natural hormones from cetaceans blubber presented concentrations ranges that includes the values obtained in the present study too (Boggs et al., 2017; Dalle Luche et al., 2019).

Some studies have found synthetic hormones in fish tissues in the marine area where the studied cetaceans were stranded. Guedes-Alonso et al. (2017) studied the hormones presence on fishes captured in the proximity of the outfall of a wastewater treatment plant (WWTP) in Canary Islands, detecting all hormones detected in the present study except PRD, and obtaining some values at $\mu g{\cdot}g^{-1}$ level in the case of NAN and NOR, differing with the study performed by Torres-Padrón et al. using the same methodology, in which PRD was quantified at $\mu g{\cdot}g^{-1}$ level (Torres-Padrón et al., 2020). This case could show the large variability between samples, including with the same sampling areas, in studies about marine pollution based on the presence in organisms, as in the present study, in which detected hormones are different in each sample.

Results obtained from cetacean samples show the potential for detecting synthetic hormones through these animals, being an available bioindicator for the study of pollution in marine environment, apart of sediments (Liu et al., 2022) and seawater (Huysman et al., 2017), and its impact in trophic chain and biological processes. Studies about effects of some synthetic hormones as endocrine disruptors are focused in fishes samples (Hicks et al., 2017; Hua et al., 2018; Li et al., 2021), performed through the exposure to these hormones, which is impossible to perform in cetaceans, so it is difficult to know the exact impact that could be taking in marine mammals these compounds.

4. Conclusions and future trends

The results presented in this study indicated that QuEChERS extraction combined with UHPLC-MS/MS analysis could efficiently detect synthetic steroid hormones in blubber samples. Also, the analysis is innovative, providing an insight into the anthropogenic impact on the marine environment with the first detection and quantification of synthetic hormones, contained in drugs and other products. Apart from providing information on the pre-death health status of stranded cetaceans, blubber sampling is not lethal, so it seems a good alternative for monitoring the health or welfare status of free-ranging cetaceans. Hormone profiles may be relevant to take more effective measures in the case of protected species, obtaining information about health, reproductive stage or pregnancy.

While the current methodology meets the study's accessibility goals, future research might explore automated homogenization techniques to further enhance sample consistency. Additionally, refining sample cleanup steps could help minimize the matrix effects observed at low analyte concentrations, supporting even greater analytical precision. Finally, expanding this approach to include biopsy samples from live animals could broaden the method's applicability, and additional analyses of different blubber zones in specimens with significant hormone concentrations would deepen understanding of these emerging contaminants' impact on marine mammals. Relating these concentration values to necropsy findings could also provide a more comprehensive health assessment, contributing to the conservation and welfare of cetacean populations.

CRediT authorship contribution statement

De la Fuente Jesús: Writing – original draft, Validation, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Bullón-Téllez Adrián:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation. **Sosa-Ferrera Zoraida:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Santana-Rodríguez José:** Writing – review & editing, Supervision, Resources, Conceptualization. **Garcia-Álvarez Natalia:** Writing – review & editing, Resources, Investigation, Conceptualization. **López-Suárez Pedro:** Writing – review & editing, Resources, Investigation, Conceptualization. **Oujo Carolina:** Writing – review & editing, Resources, Investigation, Conceptualization. **Freire-Lopes Katia:** Writing – review & editing, Resources, Investigation, Conceptualization. **Guedes-Alonso Rayco:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Arbelo Manuel:** Writing – review & editing, Resources, Investigation, Funding acquisition, Conceptualization. **Fernández Antonio:** Writing – review & editing, Resources, Project administration, 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.

Acknowledgements

This study is a result of the MARCET II Project (MAC2/4.6c/392), co-financed by the Interreg Madeira-Azores-Canarias (MAC) Territorial Cooperation Program 2014–2020, and coordinated by the University of Las Palmas de Gran Canaria.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.etap.2025.104703.

Data availability

The data that has been used is confidential.

References

Adeel, M., Song, X., Wang, Y., Francis, D., Yang, Y., 2017. Environmental impact of estrogens on human, animal and plant life: a critical review. Environ. Int. 99, 107–119. https://doi.org/10.1016/j.envint.2016.12.010.

Aguilar, A., Borrell, A., Pastor, T., 1999. Biological factors affecting variability of persistent pollutant levels in cetaceans. J. Cetacea Res. Manag. 83–116. https://doi.org/10.47536/jcrm.v1i1.264.

Aguilar, A., Borrell, A., 1991. Heterogeneous distribution of organochlorine contaminants in the blubber of baleen whales: implications for sampling procedures. Mar. Environ. Res. 31, 275–286. https://doi.org/10.1016/0141-1136(91)90017-3.

Andvik, C., Jourdain, E., Lyche, J.L., Karoliussen, R., Borgå, K., 2021. High levels of legacy and emerging contaminants in killer Whales (*Orcinus orca*) from Norway, 2015 to 2017. Enviro Toxic. Chem. 40, 1848–1858. https://doi.org/10.1002/etc.5064.

Atkinson, S., Combelles, C., Vincent, D., Nachtigall, P., Pawloski, J., Breese, M., 1999. Monitoring of progesterone in captive female false killer Whales, Pseudorca crassidens. Gen. Comp. Endocrinol. 115, 323–332. https://doi.org/10.1006/gcen.1999.7319.

Atkinson, S., Gendron, D., Branch, T., Mashburn, K., Melica, V., Enriquez-Paredes, L., Brownell, R., 2019. Pregnancy rate and biomarker validations from the blubber of eastern North Pacific blue whales. Mar. Mammal. Sci. 36. https://doi.org/10.1111/mms.12616.

Bartalini, A., Muñoz-Arnanz, J., García-Álvarez, N., Fernández, A., Jiménez, B., 2022. Global PBDE contamination in cetaceans. A critical review. Environ. Pollut. 308, 119670. https://doi.org/10.1016/j.envpol.2022.119670.

Bellante, A., Sprovieri, M., Buscaino, G., Buffa, G., Di Stefano, V., Salvagio Manta, D., Barra, M., Filiciotto, F., Bonanno, A., Giacoma, C., Mazzola, S., 2012. Stranded cetaceans as indicators of mercury pollution in the Mediterranean Sea. Ital. J. Zool. 79, 151–160. https://doi.org/10.1080/11250003.2011.621072.

Boggs, A.S.P., Schock, T.B., Schwacke, L.H., Galligan, T.M., Morey, J.S., McFee, W.E., Kucklick, J.R., 2017. Rapid and reliable steroid hormone profiling in Tursiops truncatus blubber using liquid chromatography tandem mass spectrometry (LC-MS/MS). Anal. Bioanal. Chem. 409, 5019–5029. https://doi.org/10.1007/s00216-017-0446-z.

Bowen, W., 1997. Role of marine mammals in aquatic ecosystems. MAR. ECOL. -Prog. SER. - MAR ECOL-PROGR SER 158, 267–274. https://doi.org/10.3354/meps158267.

Carbajal, A., Agustí, C., Domingo, M., Olvera-Maneu, S., Lopez-Bejar, M., 2022. Influence of sample location on blubber cortisol concentration in striped dolphins (Stenella coeruleoalba): The importance of the reference denominator. Mar. Mammal. Sci. 38, 756–764. https://doi.org/10.1111/mms.12862.

Chen, M.-H., Zhuang, M.-F., Chou, L.-S., Liu, J.-Y., Shih, C.-C., Chen, C.-Y., 2017. Tissue concentrations of four Taiwanese toothed cetaceans indicating the silver and cadmium pollution in the western Pacific Ocean. Mar. Pollut. Bull. 124, 993–1000. https://doi.org/10.1016/j.marpolbul.2017.03.028.

Costello, M.J., Chaudhary, C., 2017. Marine biodiversity, biogeography, deep-sea gradients, and conservation. Curr. Biol. 27, R511–R527. https://doi.org/10.1016/j.cub.2017.04.060.

Dalle Luche, G., Bengtson Nash, S., Kucklick, J.R., Mingramm, F.M.J., Boggs, A.S.P., 2019. Liquid chromatography tandem mass spectrometry for the quantification of steroid hormone profiles in blubber from stranded humpback whales (Megaptera novaeangliae). Conserv. Physiol. 7, coz030. https://doi.org/10.1093/conphys/coz030.

Davidson, A.D., Boyer, A.G., Kim, H., Pompa-Mansilla, S., Hamilton, M.J., Costa, D.P., Ceballos, G., Brown, J.H., 2012. Drivers and hotspots of extinction risk in marine mammals. Proc. Natl. Acad. Sci. 109, 3395–3400. https://doi.org/10.1073/pnas.1121469109.

Debier, C., Chalon, C., Le Bœuf, B.J., de Tillesse, T., Larondelle, Y., Thomé, J.-P., 2006. Mobilization of PCBs from blubber to blood in northern elephant seals (Mirounga angustirostris) during the post-weaning fast. Aquat. Toxicol. 80, 149–157. https://doi.org/10.1016/j.aquatox.2006.08.002.

Debier, C., Pomeroy, P.P., Dupont, C., Joiris, C., Comblin, V., Le Boulengé, E., Larondelle, Y., Thomé, J.-P., 2003. Quantitative dynamics of PCB transfer from mother to pup during lactation in UK grey seals Halichoerus grypus. Mar. Ecol. Prog. Ser. 247, 237–248.

Desbrow, C., Routledge, E.J., Brighty, G.C., Sumpter, J.P., Waldock, M., 1998. Identification of Estrogenic Chemicals in STW Effluent. 1. Chemical fractionation and in vitro biological screening. Environ. Sci. Technol. 32, 1549–1558. https://doi.org/10.1021/es9707973.

FAO, FAO Yearbook. Fishery and Aquaculture Statistics 2019/FAO annuaire. Statistiques des pêches et de l'aquaculture 2019/FAO anuario. Estadísticas de pesca y acuicultura 2019. Rome/Roma., (2021). ⟨https://www.fao.org/fishery/en/publications/287024⟩ (accessed July 4, 2022).

Fossi, M.C., Marsili, L., 2003. Effects of endocrine disruptors in aquatic mammals. Pure Appl. Chem. 75, 2235–2247. https://doi.org/10.1351/pac200375112235.

Galligan, T.M., Boggs, A.S.P., Balmer, B.C., Rowles, T., Smith, C.R., Townsend, F., Wells, R.S., Kellar, N.M., Zolman, E.S., Schwacke, L.H., 2020. Blubber steroid hormone profiles as indicators of physiological state in free-ranging common bottlenose dolphins (Tursiops truncatus). Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. 239, 110583. https://doi.org/10.1016/j.cbpa.2019.110583.

Galligan, T.M., Schwacke, L.H., McFee, W.E., Boggs, A.S.P., 2018. Evidence for cortisol–cortisone metabolism by marine mammal blubber. Mar. Biol. 165, 114. https://doi.org/10.1007/s00227-018-3373-4.

González-Bareiro, E., Montesdeoca-Esponda, S., De la Fuente, J., Sosa-Ferrera, Z., Arbelo, M., Fernández, A., Santana-Rodríguez, J.J., 2023. Assessment of the presence of UV filters and UV stabilizers in stranded dolphin blubber. Sci. Total Environ. 895, 165041. https://doi.org/10.1016/j.scitotenv.2023.165041.

Guedes-Alonso, R., Ciofi, L., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., Bubba, M.D., Kabir, A., Furton, K.G., 2016a. Determination of androgens and progestogens in environmental and biological samples using fabric phase sorptive extraction coupled to ultra-high performance liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1437, 116–126. https://doi.org/10.1016/j.chroma.2016.01.077.

Guedes-Alonso, R., Montesdeoca-Esponda, S., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2014. Liquid chromatography methodologies for the determination of steroid hormones in aquatic environmental systems. Trends Environ. Anal. Chem. 3–4, 14–27. https://doi.org/10.1016/j.teac.2014.10.001.

Guedes-Alonso, R., Santana-Viera, S., Montesdeoca-Esponda, S., Afonso-Olivares, C., Sosa-Ferrera, Z., Santana-Rodriguez, J.J., 2016b. Application of microwave-assisted extraction and ultra-high performance liquid chromatography-tandem mass spectrometry for the analysis of sex hormones and corticosteroids in sewage sludge samples. Anal. Bioanal. Chem. 408, 6833–6845.

Guedes-Alonso, R., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2017. Determination of steroid hormones in fish tissues by microwave-assisted extraction coupled to ultra-high performance liquid chromatography tandem mass spectrometry. Food Chem. 237, 1012–1020. https://doi.org/10.1016/j.foodchem.2017.06.065.

Guerrero, A.I., Rogers, T.L., 2017. Blubber fatty acid composition and stratification in the crabeater seal, Lobodon carcinophaga. J. Exp. Mar. Biol. Ecol. 491, 51–57. https://doi.org/10.1016/j.jembe.2017.03.004.

Hicks, K.A., Fuzzen, M.L.M., McCann, E.K., Arlos, M.J., Bragg, L.M., Kleywegt, S., Tetreault, G.R., McMaster, M.E., Servos, M.R., 2017. Reduction of intersex in a wild fish population in response to major municipal wastewater treatment plant upgrades. Environ. Sci. Technol. 51, 1811–1819. https://doi.org/10.1021/acs.est.6b05370.

Hogg, C.J., Vickers, E.R., Rogers, T.L., 2005. Determination of testosterone in saliva and blow of bottlenose dolphins (Tursiops truncatus) using liquid chromatography–mass spectrometry. J. Chromatogr. B 814, 339–346. https://doi.org/10.1016/j.jchromb.2004.10.058.

Hua, J., Han, J., Guo, Y., Zhou, B., 2018. Endocrine disruption in Chinese rare minnow (Gobiocypris rarus) after long-term exposure to low environmental concentrations of progestin megestrol acetate. Ecotoxicol. Environ. Saf. 163, 289–297. https://doi.org/10.1016/j.ecoenv.2018.07.049.

Huysman, S., Van Meulebroek, L., Vanryckeghem, F., Van Langenhove, H., Demeestere, K., Vanhaecke, L., 2017. Development and validation of an ultra-high performance liquid chromatographic high resolution Q-Orbitrap mass spectrometric method for the simultaneous determination of steroidal endocrine disrupting compounds in aquatic matrices. Anal. Chim. Acta 984, 140–150. https://doi.org/10.1016/j.aca.2017.07.001.

Kanwisher, J., Ridgway, S., 1983. The physiological ecology of Whales and porpoises. SCI. Am. - SCI Am. 248, 110–120. https://doi.org/10.1038/scientificamerican0683-110.

Kellar, N., Trego, M., Marks, C., Dizon, A., 2006. Determining pregnancy from blubber in three species of delphinids. Mar. Mammal. Sci. 22, 1–16. https://doi.org/10.1111/j.1748-7692.2006.00001.x.

Koopman, H.N., 2007. Phylogenetic, ecological, and ontogenetic factors influencing the biochemical structure of the blubber of odontocetes. Mar. Biol. 151, 277–291. https://doi.org/10.1007/s00227-006-0489-8.

Krahn, M.M., Herman, D.P., Ylitalo, G.M., Sloan, C.A., Burrows, D.G., Hobbs, R.C., Mahoney, B.A., Yanagida, G.K., Calambokidis, J., Moore, S.E., 2004. Stratification of lipids, fatty acids and organochlorine contaminants in blubber of white whales and killer whales. J. Cetacea Res. Manag. 6, 175–189. https://doi.org/10.47536/jcrm.v6i2.781.

Li, X., Lin, X., Chen, Y., Wang, Z., Guo, Y., Afriyie, G., Zhang, N., Dong, Z., 2021. Norethisterone exposure alters the transcriptome of Marine Medaka (Oryzias melastigma) larvae. Chem. Ecol. 37, 767–779. https://doi.org/10.1080/02757540.2021.1981299.

Lind, P.M., Lind, L., 2020. Are persistent organic pollutants linked to lipid abnormalities, atherosclerosis and cardiovascular disease? A review. J. Lipid Atheroscler. 9, 334–348. https://doi.org/10.12997/jla.2020.9.3.334.

Liu, S., Tian, F., Pan, Y.-F., Li, H.-X., Lin, L., Hou, R., Zhang, L.-B., Zhang, Z., Liu, S.-S., Xu, X.-R., Cheng, Y.-Y., Chen, H.-G., 2022. Contamination and ecological risks of steroid metabolites require more attention in the environment: evidence from the fishing ports. Sci. Total Environ. 807, 150814. https://doi.org/10.1016/j.scitotenv.2021.150814.

López-Berenguer, G., Bossi, R., Eulaers, I., Dietz, R., Peñalver, J., Schulz, R., Zubrod, J., Sonne, C., Martínez-López, E., 2020. Stranded cetaceans warn of high perfluoroalkyl substance pollution in the western Mediterranean Sea. Environ. Pollut. 267, 115367. https://doi.org/10.1016/j.envpol.2020.115367.

Lydersen, C., Wolkers, H., Severinsen, T., Kleivane, L., Nordoy, E., Skaare, J., 2002. Blood is a poor substrate for monitoring pollution burdens in phocid seals. Sci. Total Environ. 292, 193–203. https://doi.org/10.1016/S0048-9697(01)01121-4.

Mansour, A.A.H., Mkay, D.W., Lien, J., Orr, J.C., Banoub, J.H., Ølen, N., Stenson, G., 2002. Determination of pregnancy status from blubber samples in minke Whales (Balaenoptera acutorostrata). Mar. Mammal. Sci. 18, 112–120. https://doi.org/10.1111/j.1748-7692.2002.tb01022.x.

Mello, D., Alvarenga, C., 2016. Biological matrices for sampling free-ranging cetaceans and the implications of their use for reproductive endocrine monitoring (n/a-n/a). Mammal. Rev. 46. https://doi.org/10.1111/mam.12055.

Mello, D., Colosio, A., Marcondes, M., Viau, P., Oliveira, C., 2017. Feasibility of using humpback whale blubber to measure sex hormones. J. Exp. Mar. Biol. Ecol. 486, 32–41. https://doi.org/10.1016/j.jembe.2016.09.010.

Mezzelani, M., Regoli, F., 2022. The biological effects of pharmaceuticals in the marine environment. Ann. Rev. Mar. Sci. 14, 105–128. https://doi.org/10.1146/annurev-marine-040821-075606.

Mijele, D., Omondi, P., Gakuya, F., Rossi, L., Chiyo, P.I., Soriguer, R.C., Angelone-Alasaad, S., 2016. A practical guideline to remote biopsy darting of wildebeests for genetic sampling. Int. J. Vet. Sci. Med. 4, 27–32. https://doi.org/10.1016/j.ijvsm.2016.10.004.

Miller, L.J., Lauderdale, L.K., Walsh, M.T., Bryant, J.L., Mitchell, K.A., Granger, D.A., Mellen, J.D., 2021. Reference intervals and values for fecal cortisol, aldosterone, and the ratio of cortisol to dehydroepiandrosterone metabolites in four species of cetaceans. PLOS ONE 16, e0250331. https://doi.org/10.1371/journal.pone.0250331.

Muraco, H., Cheng, A., Ravida, N., Arn, D., Hudson, J., Durrant, B., 2009. A new approach to detection of luteinizing hormone in a bottlenose dolphin (Tursiops truncatus). Aquat. Mamm. 35, 386–393. https://doi.org/10.1578/AM.35.3.2009.386.

Parks, L.G., 2001. Masculinization of female mosquitofish in kraft mill effluent-contaminated fenholloway river water is associated with androgen receptor agonist activity. Toxicol. Sci. 62, 257–267. https://doi.org/10.1093/toxsci/62.2.257.

Pedro, S., Boba, C., Dietz, R., Sonne, C., Rosing-Asvid, A., Hansen, M., Provatas, A., McKinney, M.A., 2017. Blubber-depth distribution and bioaccumulation of PCBs and organochlorine pesticides in Arctic-invading killer whales. Sci. Total Environ. 601–602, 237–246. https://doi.org/10.1016/j.scitotenv.2017.05.193.

Pérez-Jorge, S., García-López, Á., Stephanis, R., Giménez, J., García-Tiscar, S., Verborgh, P., Mancera, J., Martínez-Rodríguez, G., 2011. Use of blubber levels of progesterone to determine pregnancy in free-ranging live cetaceans. Mar. Biol. 158. https://doi.org/10.1007/s00227-011-1676-9.

Purdom, C.E., Hardiman, P.A., Bye, V.V.J., Eno, N.C., Tyler, C.R., Sumpter, J.P., 1994. Estrogenic effects of effluents from sewage treatment works. Chem. Ecol. 8, 275–285. https://doi.org/10.1080/02757549408038554.

Quaggiotto, M.-M., Sánchez-Zapata, J.A., Bailey, D.M., Payo-Payo, A., Navarro, J., Brownlow, A., Deaville, R., Lambertucci, S.A., Selva, N., Cortés-Avizanda, A., Hiraldo, F., Donázar, J.A., Moleón, M., 2022. Past, present and future of the ecosystem services provided by cetacean carcasses. Ecosyst. Serv. 54, 101406. https://doi.org/10.1016/j.ecoser.2022.101406.

Reijnders, P.J.H., Donovan, G.P., Aguilar, A., Bjørge, A., 1999. Report of the workshop on chemical pollution and cetaceans. J. Cetacea Res. Manag. 1, 1–42. https://doi.org/10.47536/jcrm.v1i1.253.

Rickert, D., Simon, R., von Fersen, L., Baumgartner, K., Bertsch, T., Kirschbaum, C., Erhard, M., 2021. Saliva and blood cortisol measurement in bottlenose dolphins (Tursiops truncatus): methodology, application, and limitations. Animals 12, 22. https://doi.org/10.3390/ani12010022.

Robinson, I., Junqua, G., Van Coillie, R., Thomas, O., 2007. Trends in the detection of pharmaceutical products, and their impact and mitigation in water and wastewater in North America. Anal. Bioanal. Chem. 387, 1143–1151. https://doi.org/10.1007/s00216-006-0951-y.

Roman, J., Fisher, S., Schteinbarg, R., Galletti, B., 2017. Role of cetaceans in ecosystem functioning: defining marine conservation policies in the 21st century. 28th Int. Congr. Conserv. Biol.

Routledge, E.J., Sheahan, D., Desbrow, C., Brighty, G.C., Waldock, M., Sumpter, J.P., 1998. Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach. Environ. Sci. Technol. 32, 1559–1565. https://doi.org/10.1021/es970796a.

Sigma-Aldrich, Sigma-Aldrich, (2022). ⟨https://www.sigmaaldrich.com/ES/es⟩ (accessed July 23, 2022).

C. Sinclair, J. Sinclair, E. Zolman, A. Martinez, B. Balmer, K. Barry, Remote biopsy sampling field procedures for cetaceans used during the Natural Resource Damage Assessment of the MSC252 Deepwater Horizon oil spill, 2015.

Temte, J.L., Spielvogel, S., 1985. Serum progesterone levels and reproductive status of incidentally killed female dall porpoises. J. Wildl. Manag. 49, 51–54. https://doi.org/10.2307/3801842.

Ting, Y.F., Praveena, S.M., 2017. Sources, mechanisms, and fate of steroid estrogens in wastewater treatment plants: a mini review. Environ. Monit. Assess. 189, 178. https://doi.org/10.1007/s10661-017-5890-x.

Tobeña, M., Prieto, R., Machete, M., Silva, M.A., 2016. Modeling the potential distribution and richness of cetaceans in the azores from fisheries observer program data. Front. Mar. Sci. 3. https://doi.org/10.3389/fmars.2016.00202 (accessed July 4, 2022).

Torres-Padrón, M.E., Montesdeoca-Esponda, S., Santana-Viera, S., Guedes-Alonso, R., Herrera-Melián, J.A., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2020. An update of the occurrence of organic contaminants of emerging concern in the Canary Islands (Spain). Water 12, 2548. https://doi.org/10.3390/w12092548.

United Nations General Assembly, Resolution adopted by the General Assembly on 25 September 2015 (Resolution No. A/RES/70/1), (2015) 35.

Vanden Berghe, M., Weijs, L., Habran, S., Das, K., Bugli, C., Rees, J.-F., Pomeroy, P., Covaci, A., Debier, C., 2012. Selective transfer of persistent organic pollutants and their metabolites in grey seals during lactation. Environ. Int. 46, 6–15. https://doi.org/10.1016/j.envint.2012.04.011.

West, K., Oftedal, O., Carpenter, J., Krames, B., Campbell, M., Sweeney, J., 2007. Effect of lactation stage and concurrent pregnancy on milk composition in the bottlenose dolphin. J. Zool. 273, 148–160. https://doi.org/10.1111/j.1469-7998.2007.00309.x.

Wittmaack, C., Urbán Ramírez, J., Bernot-Simon, D., Martínez-Aguilar, S., Subbiah, S., Surles, J.G., Looney, M., Kumar, N., Halaska, B., Duignan, P.J., Knauss, M., Burns, K., Godard-Codding, C.A.J., 2022. Small blubber samples (50 mg) sufficient for analyses of 10 stress and reproductive steroid hormones in gray and fin whales via liquid chromatography mass spectrometry. Front. Mar. Sci. 8. https://doi.org/10.3389/fmars.2021.808764 (accessed July 2, 2022).

Zhu, J., Yu, X., Zhang, Q., Li, Y., Tan, S., Li, D., Yang, Z., Wang, J., 2019. Cetaceans and microplastics: first report of microplastic ingestion by a coastal delphinid, Sousa chinensis. Sci. Total Environ. 659, 649–654. https://doi.org/10.1016/j.scitotenv.2018.12.389.