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Steroid Fingerprint Analysis of Endangered Caspian Seal (*Pusa caspica*) through the Gorgan Bay (Caspian Sea)

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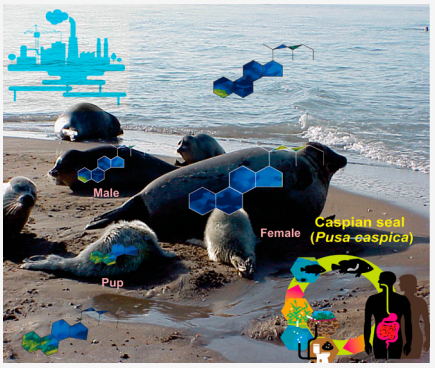
ABSTRACT: The profile of steroid congeners was evaluated in Caspian seals *Pusa caspica* by age, sex, and tissue-specific bioaccumulation, and compared with that of abiotic matrices (seawater, surface sediment, and suspended particulate materials, SPMs) from Miankaleh Wildlife/Gorgan Bay, (Caspian Sea, Iran). To identify the level of human fecal contamination, ∑25 sterol congeners were measured in all abiotic/biotic samples, revealing coprostanol, a proxy for human feces, as the most abundant sterol (seawater: 45.1−20.3 ng L^{-1}; surface sediment: 90.2−70.3 ng g^{-1} dw; SPMs: 187.7−157.6 ng g^{-1} dw). The quantification of ∑25 sterols in seals followed the order of brain > liver > kidney > heart > blood > spleen > muscle > intestine > blubber > fur, and in both sexes coprostanol level (8.95−21.01% of ∑25s) was higher in blubber and fur, followed by cholesterol in brain, liver, kidney, heart, and blood, cholestanone in intestine and muscle, and β-sitosterol in spleen. Though no age/sex differentiation was observed, the mean concentration of ∑25s was higher in male than females and pup. Different diagnostic ratios revealed sterols originating from human and nonhuman sewage sources. Findings pinpoint the urgent necessity to investigate the ecotoxicity of fecal sterols in mammals, and consequent implications for human health.



1. INTRODUCTION

Considering the increase in population in the world and consequent higher amounts of wastewater, the demand for maintaining water quality and pollution control is one of the most important challenges worldwide.[1] It is necessary that an accurate identification is made of waste load allocations from point/nonpoint sources such as land-use changes and human/animal/plant wastes that remarkably contribute to the contamination, oxygen depletion, and finally deterioration of water and sediment quality in seas, rivers and lakes, which can critically challenge the sustainable aquatic resources management. In aquatic environments, the conceptual model of dynamics of organic matter (OM)[2] plays important roles in bioavailability, distribution, and toxicity of pollutants in sewage sludge.[3,4]

Sewage pollution (including feces, urine, and laundry waste) is one of the main causes of declining water quality and increased risk of infectious diseases in aquatic ecosystems.[5−7] For an effective control of environmental pollution, simultaneous analysis of total coliforms and *Escherichia coli* in water serves as a conventional method for detection of fecal contamination.[8] However, there are many chemical/molecular biomarkers (i.e., sterols and stanols) for source tracking and tracing anthropogenic inputs, and for predicting the fate of fecal pollution in natural environments.[9−11]

Fecal sterols and stanols, unsaturated steroid alcohols, are ubiquitous stable organic compounds with a fused ring structure. In aquatic systems, due to their hydrophobic nature, sterols are easily transferred from aqueous to organic phases, which are resistant to degradation in the environmental matrices, that is, sediments, suspended particulate materials (SPMs), and water,[12−15] and can also effect on metabolic and reproductive potential of aquatic organisms, that is, marine mammals.[16] Sterols and their ratios (human/animal and plant steroids) have been used as reliable chemical markers to source assignment of long-term contamination of natural OM (e.g., fecal pollution), to the health state of current and past environmental systems as well as indicators of the level of sewage treatment and/or human interference.[17,2,9,15]

Sterol biomarkers are based on the high specificity of some steroid compounds to feces of humans/higher vertebrates (it depends on their diet), microbial contamination of their gastrointestinal tract (presence/absence of anaerobic microflora), and subsequent bacterial biosynthesis/biohydrogenation products of reduction sterols to stanols.[2,9,18] Coprostanol (5β-cholestan-3β-ol) (constitute ∼40−60% of the total sterols), epicoprostanol (5β-cholestan-3α-ol), cholesterol, 24-ethyl-

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coprostanol, campesterol, sitosterol, and sitostanol have been widely used as main sterol markers of sewage pollution, and their steroid ratios are generally assigned to distinguish the origin of sterols.[18] In aquatic systems, sterols and stanols can also be used as ideal indicators to trace specific sources. Indeed, coprostanol is a dominant sterol in humans and higher vertebrates feces;[19] epicoprostanol is strongly related to human sewage, livestock/poultry waste, and anaerobic treatments on wastewater;[18,20] cholesterol is an essential structural component in eukaryotic cell membrane lipids and a dominant sterol in marine organisms such as zooplankton and invertebrates;[21] phytosterols such as campesterol, sitosterol, and stigmasterol are predominant sterols in terrestrial vascular higher plants (terrigenous OM), and campesterol and β-sitosterol can be found in ruminant organisms (cows and sheep) feces.[22] Since the absolute sterols levels are not reliable enough as indicators of fecal contamination, different sterol-based ratios are usually applied for the assessment of sewage contamination.[22,23,9]

In aqua-inland systems such as wetlands, dynamics of OM and water/sediment chemistry is still challenging due to the complexity of anthropogenic contamination and the diverse resources.[24] Nonetheless, the current knowledge on sewage sludge rate to wetlands and their prominent negative impacts on the environment in multiple scales are still limited.[25,26]

Miankaleh Peninsula, located at the southeast of the Caspian Sea, is a protected region of Iran (area of about 263 mi^2), and one of the richest ecosystems in West Asia. Based on the cut off the long and narrow Miankaleh wetlands from the Caspian Sea by brackish water of semienclosed Gorgan Bay as an elongated barrier system, it is vulnerable to eutrophication, persistence of industrial pollution, longer water residence times, and also land degradation.[27] In the recent years, the number of migratory animals such as birds and marine mammals has significantly fallen due to extensive fishing and hunting, and decline of the water quality of Miankaleh wetlands.[27] Consequently, marine mammals in high trophic levels, such as the Caspian seal *Pusa caspica*, through the food chain are exposed to fecal contaminants, toxins, and other sources of pollution that can be biomagnified in their tissues. In this line, phocids could be considered as bioindicators to screening pollution in aquatic ecosystems.[32−34] The objectives of the current study were to quantify the profile of steroid congeners in endangered Caspian seals (as biota) by age (adult and pup), sex, and tissue-specific bioaccumulation, and compare the sterols patterns with three environmental compartments (seawater, surface sediment, and SPMs; as abiotic) to evaluate the status of fecal contamination and possible risk in the Iranian coastal waters of the Caspian Sea. To the best of our knowledge, this is the first report assessing the occurrence, uptake, distribution, and sources of sterols in Miankaleh Wildlife/Gorgan Bay, Iran.

2. MATERIALS AND METHODS

2.1. Sampling and Field Analysis. Ten Caspian seals *P. caspica* (males, females, and pups) killed or severely injured in fishing nets were anesthetized and captured from the coast of Miankaleh Peninsula (southeast Caspian Sea, 36°52′11″ N − 53°13′08″ E, Iran) during the feeding and fatting season in October−December 2017. From each individual, blubber, fur, brain, liver, kidney, heart, spleen, blood, muscle, and intestine were grossly dissected and then frozen at −20 °C for chemical analysis. Surface sediments (0−5 cm, n = 8 per site), seawater (0−40 cm, n = 8 per site) and SPM (4 L from each seawater sample; n = 8 per site) samples were gathered at five locations (ST) along the coastal waters of Gorgan Bay, Iran (Supporting Information (SI) Figure S1), as described previously.[35,36] The water quality index (WQI) was also assessed. Additional details on field and experimental analyses are provided in the SI.

2.2. Chemical Analysis. Freeze-dried and sieved (<2 mm) samples (sediment, tissue, and SPMs), spiked with an internal standard (IS) solution containing coprostanol-d5, epicoprostanol-d5, cholesterol-d4, and cholestanol-d5, were subjected to Soxhlet extraction following previously published procedures.[37] Sterols analysis was carried out on an Agilent 7890A GC-coupled with Agilent Technologies 5975C quadrupole mass spectrometer and a fused silica capillary DB-5MS column. Helium (99.995% purity) was used as the carrier gas and the analysis was performed in a selective ion monitoring (SIM) mode. Only peaks with a purity >65% were considered for analysis. Quantification was according to the response factors of authentic standards relative to 5α-cholestane, applied as the IS. The analytical laboratory quality assurance/quality control (QA/QC) was performed.[36] The QA sample results met established laboratory limits of detection (MLODs) and limits of quantification (MLOQs).[38] See SI for more details.

2.3. Sterol Ratios. Twenty-three different sterol-based diagnostic ratios with the discriminative reference values used in the literature (SI Table S1) were herein calculated from the concentration of sterols identified in the biotic/abiotic samples. See SI for more details.

2.4. Statistical Analyses. Sterol concentrations, expressed as mean, were on a dry weight (dw) basis for all samples. One-way ANOVA (Levene's test for equality of variance) was applied to demonstrate the statistical significance ($p < 0.05$) in $\sum$25 sterols among different abiotic and biotic samples. To estimate significance, a post hoc was performed utilizing the least significant difference (LSD). To analyze data, multivariate analyses were applied, that is, Pearson correlation analysis (PA) to recognize the degree of interconnection between the sterol congeners in abiotic and biotic samples; Two-way hierarchical cluster (heatmap) to exhibit the relations among all samples using the Gplots package in R software; and Principal component analysis (PCA) to reveal the plausible sources of sterols in sediments, seawater and SPMs. Data analyses were carried out in OriginPro (Version: 2019b, 9.65), house developed R-software (R Development Core Team, Vienna, Austria), and CANOCO 4.5 (Microcomputer Power, Inc.). See SI for more details.

3. RESULTS AND DISCUSSION

3.1. Bulk Parameters of Surface Water. Water quality parameters[39] are presented in SI Table S2, and show slight variations of pH (6.95−7.17), dissolved oxygen (DO) (7.21 to 7.65 mg L^{-1}), electrical conductivity (EC) (2428.0−2497.5 μS/cm), and turbidity (133.8−154.2 NTU), among surface seawater from the sampling sites. Based on the sampling time, pH was neutral in all sites with small increases of about 0.2 units; EC was highest, due to the dominating soluble salts, mineralization and hardness in inflow and surface runoffs inlet to the Gorgan Bay in the wet season; and DO level tended to be higher (by up to 5 mg L^{-1} in the winter) and support the rich variety of life, whereas the differences for all tested parameters were not significant and small ($p > 0.05$) (SI Table S2). However, other factors such as biological oxygen demand (BOD), chemical oxygen demand (COD), nitrate (NO_3^{-1}), and phosphate (PO_4^{-3}) showed significantly lower levels at

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ST5 compared to ST1 (SI Table S2). Their concentrations (ST1→ ST5) were 83.04−69.42 mg/L, 103.80−86.77 mg/L, 6.95−4.74 mg/L, and 2.09−1.71 mg/L, respectively (SI Table S2), that may be attributable to the raise in population density in the eastern coastal area of Gorgan Bay (especially ST1, ST2, and ST3), increased soil erosion and discharge of sewage effluent and household wastewater into the bay.[27,29] This wastewater contains a high level of BOD (>100 ppm), COD, nitrate, and phosphate and may determine contamination by OM and nutrients that contribute to eutrophication and reduction in BOD and COD of Miankaleh wetland and Gorgan Bay aquatic ecosystem.[40] In addition, the N and P input could be attributed to the improved ion exchange[41] and even to sterol compounds sinking between the sediment environment and the adjacent water. However, this result suggests that the Gorgan Bay is contaminated by biodegradable OM, which level was higher than the surface water quality standards.[42,43] Of course, these parameters can be affected by the sampling time and the pattern of water circulation into the Gorgan Bay. Under the Ramsar wetland type classification,[44] Miankaleh wetland and Gorgan Bay are identified as A-permanent shallow marine waters with an average depth of 4 m at low tide and the highest rainfall in December (with a mean precipitation of 450−800 mm/year). Therefore, this indicates that the chemo-biological properties of seawaters and nutrients are directly controlled by the hydrography of the Gorgan Bay, which water masses circulation has a counterclockwise pattern due to the Coriolis effects.[45,46] Moreover, satellite imagery (2006−2016) also confirmed that land-use change and increased agricultural fields and settlements (up to 59%) happened in the Gorgan Bay watershed,[27] determining the discharge of agricultural waste in the area as probably the main cause of water quality decline. According to our results, the Department of Environment in Iran also reported that the aggregate ecoregions of PO_4^{-3} and NO_3^{-1} in the bay in 2016 were approximately higher than EPA criteria and the probability of a hypertrophic state was of 65%, raising serious concerns about Gorgan Bay.[44,47]

3.2. Sterol Composition, Quantification, and Distribution in Abiotic Matrices. Human/vertebrates and plant sterol compounds were detected in all sediments, seawater, and SPMs as markers for the assignment of sources and composition of OM from sewage in the Gorgan Bay (SI Figure S2). A total of 25 sterol compounds were detected with similar patterns in abundances in the three environmental compartments from all sites (SI Figure S2). Overall, a significant difference among stations was found in ∑25s ($p < 0.05$), with the maximum values in all the three groups observed at ST1 compared to the other STs in the range of 833.91 ng L^{-1} (seawater), 1667.81 ng g^{-1} dw (surface sediment), and 3469.05 ng g^{-1} dw (SPMs) (SI Figure S2). ST1 is near to the waterway between the Caspian Sea and Gorgan Bay and it has become the convergent points of various wastewaters containing sterols from the Caspian Sea receiving waters. Indeed, the average water discharge from the Caspian Sea at the bay entrance is 35 m^3/s and the rate of water and sediment discharge from the watershed systems to the bay are 500 m^3/s and 3.5 mt/y, respectively.[45] Also, ST1 is affected by the water counterclockwise rotation in the bay, resulting in the discharge and accumulation of sewage pollutants (including sterols/stanols) from all coastal waters around this region to the exit point of the bay. On the contrary, the minimum ∑25s level was found in seawater, sediment and SPMs of ST5 (near to Miankaleh wetlands) (SI Figure S2). When silt+clay% > 50%, the lowest concentration of sterols occurs,[48] as it is the case of ST5 with 52% of silt+clay.[49] In addition, the sedimentological features of Gorgan Bay and its adjacent areas indicate that the grain-size distribution of sediments in the bottom of the bay is associated with the sterol accumulations,[48] since it is in the mud (i.e., silt and clay, particle sizes <63 μm) to sand (a sieve size >63 μm) range, with sand content increasing eastward, and the mean value of 30% OM in sediments of Gorgan Bay that decreases toward the inlet of the bay.[50] In view of time, the high level of sterols observed herein during the wet season (December) indicates the influence of the seasonal movement of river runoff and agricultural/domestic wastewater (containing sterols) from residential/local areas into the bay watershed system.[12]

The most abundant sterols (as % of ∑25s), with a constant ratio in all samples from each environmental compartment per site, were the fecal sterol coprostanol (5.41%), followed by cholestanol (4.29%), cholesterol (4.23%), cholestanone (4.16%), stigmastanol and β-sitosterol (4.14%), and 24-ethylcoprostanol and 24-ethylepicoprostanol (4.09%) (SI Table S3). Also, coprostanol, cholestanol, fucosterol, stigmastanol, and β-sitosterol showed the highest concentrations in all seawater, sediment and SPMs samples/STs (peaks in SI Figure S2). In all abiotic matrices, the quantities of ∑25s and relative abundance of each sterol congeners as biomarkers of human feces (i.e., cop and ep-cop) and plant sterols (sito-a, β-sito and 24-e-cop) or ubiquitous sterols (chol-a), decreased from ST1 to ST5, likely due to the hydrographical features of the Gorgan Bay and wastewater circulation (SI Figure S2). This suggests that natural phytosterol/zoosterol (marine and terrestrial/riverine) sources and/or sewage appear to have been transported to the bay water column and currents prior to deposition in the sedimentary bed. Noteworthy, ST1 is close to the main sources of sewage discharge in the east of Gorgan Bay, such as aquaculture and seafood processing factories in Turkmen port. Due to the high consumption of nutrients and fertilizers, effluents containing large amounts of organic/inorganic compounds and minerals are combined with high levels of oxygen demand.[51] Indeed, these load of farming wastewater effluents, urban/agricultural wastewater, and sources of fermentation of wood and refining of paper pulp,[52] are the major contributors to a variety of steroid compounds, whereas other sites in the bay are low to moderately polluted by fecal material. Moreover, these biomarker profile results are consistent with a semiclosed area, dried up in Gorgan Bay by lowering sea level in the multiple past years, decreasing dredging and increasing dynamic reorganization of river basins, increased sewage pollution and household wastewater by advanced population density in coastal area (especially ST1, ST2, and ST3) via deforestation of Caspian Hyrcanian forests,[53] which provide large amounts of steroid−organic matter input to the Gorgan bay and create predominance of critical condition.

Spatial variation in the level of fecal sterols herein reported, especially coprostanol (seawater: 45.1−20.3 ng L^{-1}; surface sediment: 90.2−70.3 ng g^{-1} dw; SPMs: 187.7−157.6 ng g^{-1} dw) is comparable to literature data of other bay regions worldwide such as in the southwest Gulf of Mexico (100−168 ng g^{-1} dw),[54] Santos Estuary (42.8 ng g^{-1} dw) and the Sao Sebastiao Channel (6.9−21.7 ng g^{-1} dw) in Brazil,[55] and Macao Estuary, southern China (150−280 ng g^{-1} dw).[56] However, the mean concentration of coprostanol in Gorgan

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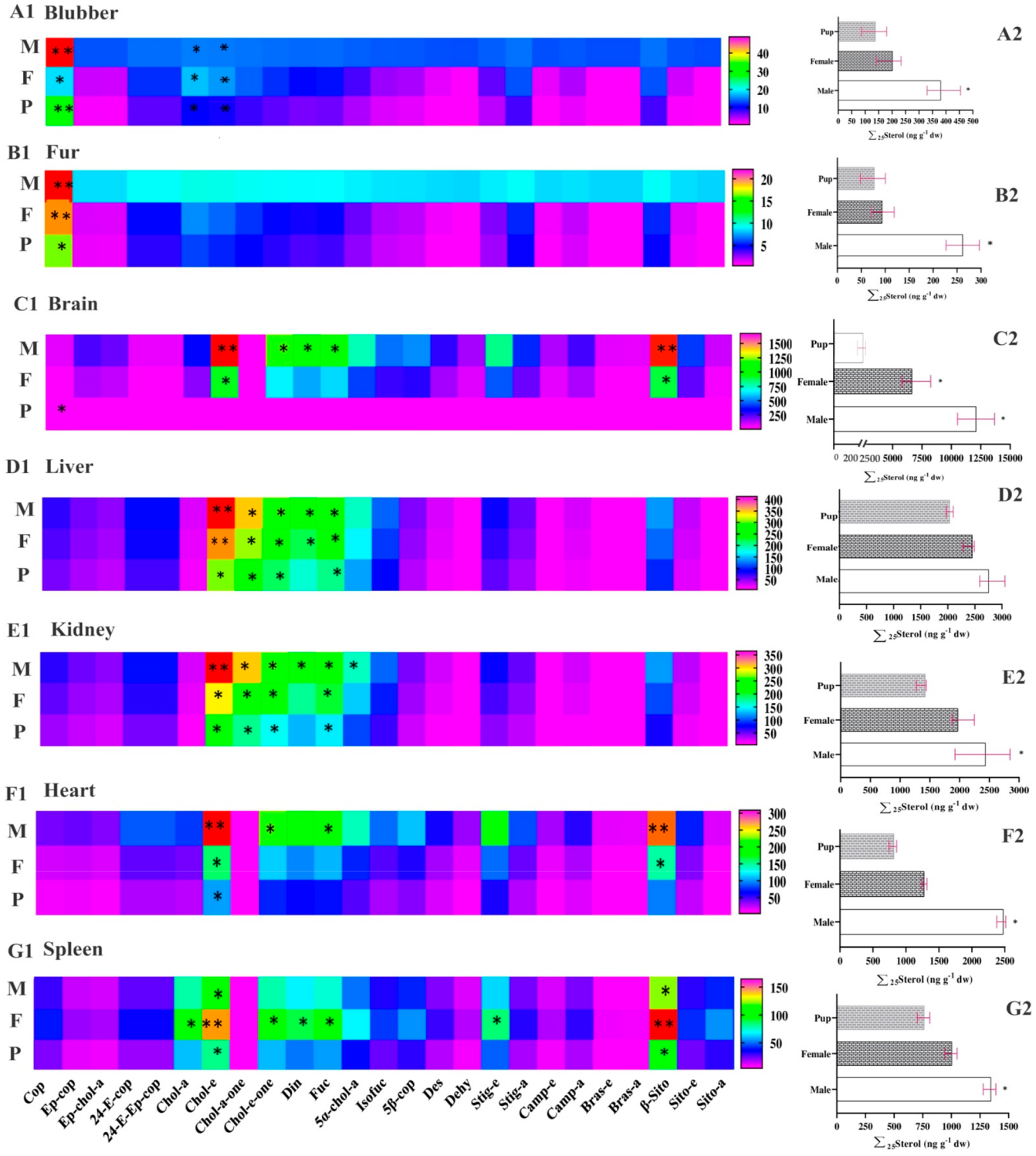

Figure 1. continued

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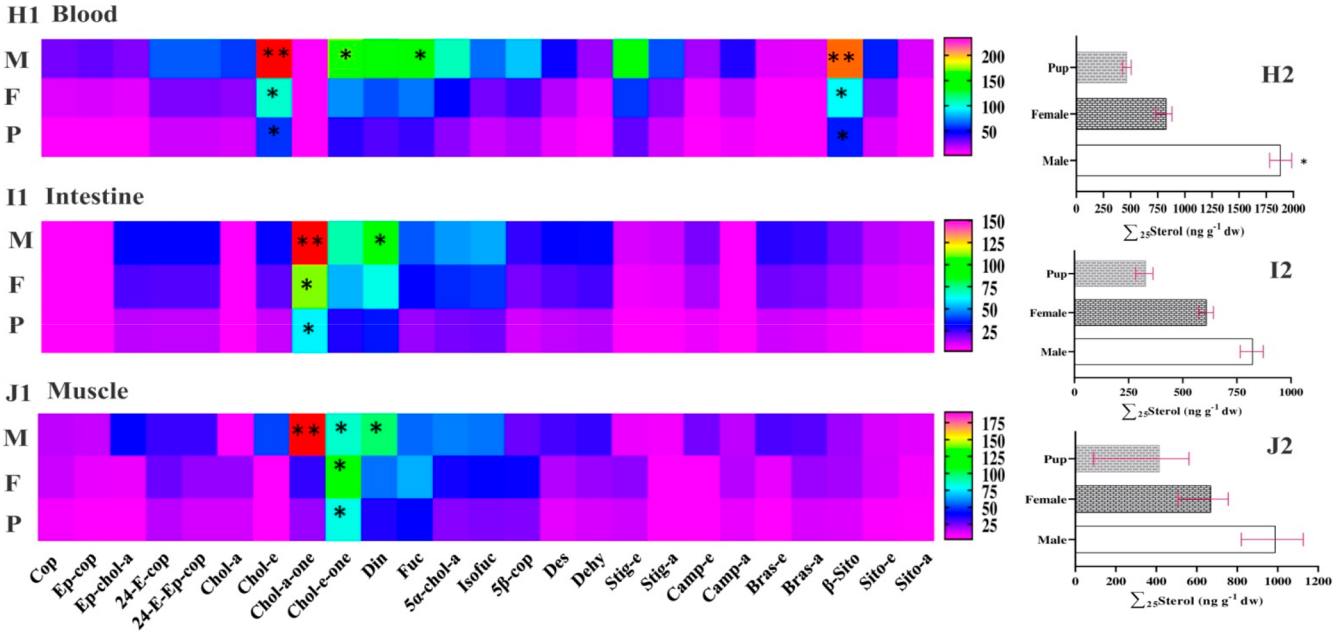

Figure 1. Quantification of 25 sterol congeners detected in various tissues (A1-J1) of *P. caspica* and their total sterols compounds (A2–J2), based on age-sex, male (M), female (F), and pup (P), from the southeast Caspian Sea environments. Concentration is the average pixel intensity. ∑25s data (A2-J2) are expressed mean ± SE (n = 10) *p < 0.05.

Bay is lower than that found in the Kuwait Bay (29–2420 ng g^{-1} dw),[57] Black Sea Ukraine (170–2600 ng g^{-1} dw) and Black Sea Russia (54–5400 ng g^{-1} dw),[58] Babitonga Bay, Brazil (30–6080 ng g^{-1} dw),[59] and Venice Lagoon, Italy (40–4410 ng g^{-1} dw).[60] Nonetheless, in many of these areas the high levels of coprostanol are affected by the sampling site, short/long-term or acute/chronic nature of outfall close to intertidal sediments, and distance from the main point-source of sewage outfalls.[57]

The highest level of coprostanol is expected as a proxy for human-feces (anthropogenic sources), microbial reduction synthesis from cholesterol, feces of marine mammals (whales and seals), bird guano, and other terrestrial mammalian animals (cats or livestock).[61,62] Although this mammalian metabolite of cholesterol (coprostanol) is one of the main components of omnivorous feces,[63] it is also present at trace amounts in marine plant materials.[64] From a diagnostic point of view, detectable concentrations of sewage contamination occur when the level of coprostanol is >100 ng g^{-1} dw, but other reports defined >500 ng g^{-1} dw,[65] and/or >700 ng g^{-1} dw,[66] the threshold of sewage contamination. However, there is no consensus with respect to coprostanol in the sediments that is indicative of sewage contamination, although it was suggested <10 ng g^{-1}.[67] Based on these threshold values, coprostanol levels in seawater, surface sediment, and SPMs from Gorgan Bay and Miankaleh wetlands were >10 ng g^{-1} and <500 ng g^{-1}, therefore indicating that Gorgan Bay regions are not significantly affected by sewage, yet.

Epicoprostanol, as one of the hydrophobic sterols,[67] was also detected in seawater (31.6–20.3 ng L^{-1}), surface sediment (63.3–49.4 ng g^{-1} dw), and SPMs (131.7–110.7 ng g^{-1} dw). It is a biomarker of the level of treated fecal material in sewage since in digested sludge samples it is commonly converted from coprostanol by intensive microbial communities or from cholesterol during anaerobic sewage treatment,[20] but it is not a major human fecal sterol.[68] Its low presence is consistent with the untreated/bulk wastewater found in the Gorgan Bay

Among dominant sterols, cholestanol (seawater: 35.8–16.1 ng L^{-1}; surface sediment: 71.6–55.8 ng g^{-1} dw; SPMs: 148.9–125.0 ng g^{-1} dw) was detected in sewage-contaminated samples of Gorgan Bay, and it is a biomarker of human sterols and plant material. This sterol was followed by the levels of terrestrial/marine plant/algae-derived sterols congeners,[69] such as fucosterol (seawater: 33.8–15.2 ng L^{-1}; surface sediment: 67.7–52.8 ng g^{-1} dw; SPMs: 140.9–118.4 ng g^{-1}), stigmastanol (seawater: 34.5–15.5 ng L^{-1}; surface sediment: 69.1–53.9 ng g^{-1} dw; SPMs: 136.9–115.0 ng g^{-1} dw), and β-sitosterol (seawater: 34.5–15.5 ng L^{-1}; surface sediment: 69.1–53.9 ng g^{-1} dw; SPMs: 143.7–120.7 ng g^{-1} dw). Additionally, municipal pollution associated with terrestrial OM can also be found as other plant sterols source in this area (SI Figure S2), revealing the possible input of river-derived OM to Gorgan Bay. However, accurate assignment of the source-specificity of these sterol compounds to their terrestrial/riverine-derived OM is still limited and requires the evaluation of ratios between selected sterols.

As shown in SI Figure S2, the levels of ∑25s and their hydrophobic congeners adsorbed onto SPMs were higher than the other two eco-compartments (surface sediment and seawater), but the composition of sterols is similar, maybe due to spatial and temporal variations effects. As SPMs in the Gorgan Bay and Miankaleh wetlands were detected in the rainy season, consistent with upstream river water and increase in coastal regime flow in the southeast of the bay, this could result in more degraded OM in the SPMs, leaching of subsurface watershed soils and remobilization of autochthonous production on the surface sediments,[70] with reduced sinking time for fecal particulate sterols due to the development of large particles. However, the solubility of steroids depends on the particle sizes distribution (percentage of fine particles), the presence of ionic suspending agent such as seasonally anoxic coastal salts (negative relationship between salinity and levels of sterols in SPMs),[71,72] surface charge and steric stabilization of the colloidal particles[73] and difference

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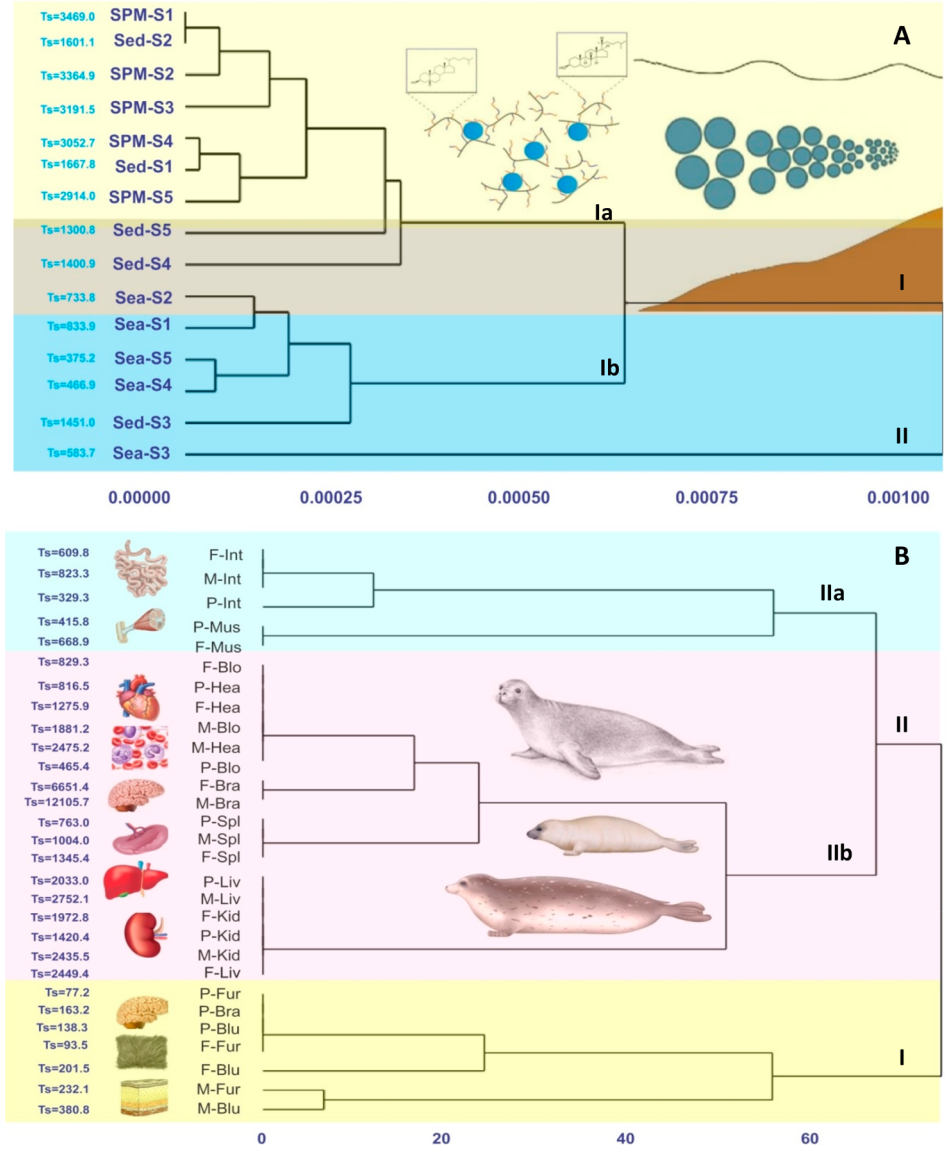

Figure 2. Dendrogram of hierarchical cluster analysis to distinguish the sources of the fecal sterols according to abiotic (A, seawater, surface sediment, and SPMs) and biotic (B, Caspian seal tissues) systems, in the southeast Caspian Sea, Iran.

between the proportion in situ conversion of Δ-stenols to 5α(H)-stanols in SPMs across the oxic−anoxic interface zone in the water column to sediment−water interface zone.[74] The SPMs during the wet season had significantly higher concentrations and lower assimilation efficiency and therefore, due to the steroid biochemistry, higher SPMs could dilute sterols and improved their hydrophobic relationship with the sewage particles, which is coincident with the preferentially SPMs associated with phytosterols overloaded.[54] However, a comparative monitoring of sterols as resistant biomarkers to ecological stress[75] in dry season is necessary for future.

3.3. Tissue-Specific Uptake and Bioaccumulation of Sterols in Caspian Seal As Biotic Matrices. Caspian seals as sea-dwelling top predators can be exposed to human-associated fecal contamination through trophic transfer in food webs.[76] Seals eat (2−3 kg/day) a varied diet consisting of crustaceans (as deposit feeder in contact with sewage) and fish, that is, tuna (as water-pollutants accumulators), and get water (containing fecal sterols) through drinks.[76,77] The diet sterol composition to identify fecal zoosterol and phytosterol-specific contamination in this species remains unknown. For this purpose, the capacity of Caspian seals to tissue-specific bioaccumulation of animal/plant sterols from sewage in the southeast Caspian Sea was herein investigated. The results of quantification in % of ∑25s in various tissues of *P. caspica* is plotted in SI Figure S3, which followed the order of brain (39.58%) > liver (15.13%) > kidney (12.19%) > heart (9.55%) > blood (6.64%) > spleen (6.51%) > muscle (4.33%) > intestine (3.68%) > blubber (1.50%) > fur (0.84%). However, when expressed as a concentration of total sterols depending on the age/sex (male (M), female (F), and pup (P)), it showed M-brain > F-brain > M-liver > M-heart > F-liver > M-kidney > P-liver > F-kidney > M-blood > P-kidney > F-spleen > F-heart > M-spleen > F-blood > M-intestine > P-heart > P-spleen > F-muscle > F-intestine > P-blood > P-muscle > M-blubber > M-fur > F-blubber > P-brain > P-blubber > F-fur > P-fur (Figure 1; Figure 2B). The concentrations and composition of 25 sterol congeners varied in different tissues of *P. caspica* (Figure 1). In both males and females, tissue levels of coprostanol (8.95−21.01% of ∑25s) were highest in the

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blubber, fur and P-brain, followed by cholesterol (6.88−14.96% of ∑25s) in brain, liver, kidney, heart, and blood, cholestanone (0.60−3.18% of ∑25s) in the intestine and muscle, and finally β-sitosterol (0.69% of ∑25s) in the spleen (SI Table S4), whereas plant sterols constituted 0.32−11.45% of ∑25s in various tissues (SI Table S4). This tissue-specific cholesterol signature is consistent with that of other aquatic organisms, that is, Atlantic salmon,[78] zebrafish,[79] rainbow trout,[80] herring,[81] oysters,[82] brown shrimp,[81] and some shellfish species.[83] However, sterols composition in tissues of marine species depends on the dietary sources, metabolism, season and time, geographical location, and depth of the sea,[84−87] and any change in their composition has marked effects on cell membranes.[88]

In seal diet, fish and crustaceans are the major sources of lipid compounds as cholesterol and desmosterol (C27 sterols), ecdysteroids, docosahexaenoic acid (DHA), and arachidonic acid (AA).[89−91] Cholesterol has an important role in lipid microdomain formation, stabilizing, permeability, and fluidity of membranes,[90] and its uptake in cells can be from exogenous (milieu) and endogenous (de novo synthesis) routes.[92] Cholesterol and its precursor desmosterol (des) are the main animal sterols, and in mammals des is ≤10% of cholesterol and total sterols,[91] as herein reported in Caspian seal tissues with des content of 2.03% of total sterols (SI Table S4). Because of the significant correlation between des and DHA and AA contents,[91] herein the animal and plant origins of sterols in Caspian seal tissues were evaluated. For the determination of animal sterol contents, R22 (chol-e/des) ratio was used (SI Table S1).[91,92] Pending on tissue/age/sex, R22 had the highest levels in liver and kidney ($M = F = P$; 14.42) > brain ($M = F > P$; 6.03) > blubber and fur ($F = P$; 5.73) > heart and blood ($M = F = P$; 5.21) > spleen ($M = F = P$; 4.89) (SI Table S4). Therefore, liver and kidney showed the highest levels of cholesterol, which may damage tissues because the excess of free cholesterol could be toxic to cells.[90]

In contrast, among individual phytosterols (i.e., β-sitosterol, sitostanol, campesterol, brassicasterol, stigmasterol, fucosterol), β-sitosterol was the most abundant in Caspian seal tissues. For the determination of plant sterol contents, R23 (chol-e/β-sito) ratio was applied (SI Table S1),[91] showing its highest levels in liver and kidney ($M = F = P$; 3.03) > muscle ($M > F = P$; 2.50) > intestine ($M = F = P$; 1.50) > blubber, and fur ($M = F = P$; 1.20) (SI Table S4). Overall, the plant sterol contents in seal tissues were lower than animal sterol contents. To the best of our knowledge, no data on the rate of plant/animal sterol intake in seals have been reported so far. However, the lipid composition of blood in Young elephant seals *Mirounga augustirostris* and harp seals *Pagophilus groenlandicus* showed that cholesterol esters, free cholesterol, and the plasma neutral lipids were the major components in blood of these mammals.[93] Thus, there is still debate about the effects of long-term variability in animal sterols intake upon the health and wellbeing of seals.

Some tissue-specific tendency can be observed in *P. caspica* to the fecal sterols profiles (Figure 2B), which may affect the regulation/deregulation of cholesterol homeostasis and a variety of bioactive sterols in seal tissues.[94,95] In the adipose tissue blubber, the concentration and percentage of each congener in the sterol profiles of male, female, and pup were similar, with the highest concentrations of cop > chol-a > chol-e > chol-a-one > stig-a ≈ β-sito. The most significant ($p < 0.05$) differences were found in coprostanol in male (max: 55.18 ng g^{-1}dw; 12.82% of ∑25s) and pup (max: 37.79 ng g^{-1}dw; 21.01% of ∑25s) seals, with the higher ∑25s recorded in male blubber (Figure 1A1, A2; Figure 2B; SI Table S4). Seals acquire organic pollutant, that is, fecal sterols through their environment/diet and accumulate them in lipid-rich tissues, that is, brain and fat/blubber, as a thick layer of fat under their skin. Here, levels of pollutant bioaccumulation vary across % tissue lipid content (blubber thickness variation), species of seals, age and sex.[96] Noteworthy, in Caspian seal adipose tissue the mean level of ∑25s as organic lipophilic pollutants was lower than in brain and liver (Figure 2B), and in blubber it was higher in pup than adult (Figure 1A2). The negative correlation between pollutant concentration and thickness of blubber in Caspian seals may be because during the postweaning fast (spring season), the blubber layer in the body of seals is thinner than after their breeding and moulting periods and, due to the magnification of pollutants in their bodies, seals may face higher health risk.[32]

In fur, the composition of sterol congeners depending on the age/sex was similar to the blubber, with the dominant sterols similar to what observed in the abiotic samples, likely due to the direct contact of skin and fur of the Caspian seals with their surrounding contaminated environment. The most significant ($p < 0.05$) difference in sterol congeners was found in coprostanol in male (max: 37.79 ng g^{-1}dw; 9.50% of ∑25s) and female (max: 24.96 ng g^{-1}dw; 21.01% of ∑25s) seals, with the higher ∑25s recorded in male fur (Figure 1B1, B2; Figure 2B; SI Table S3). Therefore, the high coprostanol levels, typical of fecal-pollution and recorded in all the abiotic matrices (SI Figure S2), were also found in seal fur and blubber indicating a first tissue protection of seals to exposure to Caspian sea fecal contamination. Similar results for human fingerprint were reported for coprostanol and 24-ethyl-coprostanol in oysters *C. gigas*.[82]

In brain, the sterols profiles of male and female were similar, with the dominant concentrations of chol-e > β-sito > chol-e-one > fuc > din > stig-e, whereas in pup brain the sterol composition was similar to that of fur and blubber. The most significant ($p < 0.05$) differences were found in cholesterol (male, max: 1889.36 ng g^{-1}dw, 13.81% of ∑25s; female, max: 1139.16 ng g^{-1}dw, 13.81% of ∑25s) and β-sitosterol (male, max: 1844.16 ng g^{-1}dw, 13.48% of ∑25s; female, max: 1111.91 ng g^{-1}dw, 13.48% of ∑25s) for male and female, and in coprostanol for pup (max: 37.03 ng g^{-1}dw; 21.01% of ∑25s), with the higher ∑25s recorded in male brain (Figure 1 C1, C2; Figure 2B; SI Table S3). For the high cholesterol content in brain, some regulatory mechanisms were hypothesized that reduce neuronal cholesterol biosynthesis by sirtuin 2 (SIRT2) deacetylase-dependent inhibition,[97] up-regulate target genes for cholesterol efflux, and secrete the excess oxidized cholesterol across the blood-brain barrier into the circulation.[98−100] This event might favor the accumulation of excess cholesterol into blood and it may explain the similarity in sterol composition of seal blood and brain. The high presence of plant sterols, that is, β-sito, fuc, din, and stig-e, indicated that unlike dietary cholesterol, phytosterols can accumulate in seal brain[101]

In liver, the composition of 25 sterols was similar in seal adult and pup, with the highest concentrations of chol-e > chol-a-one > chol-e-one > fuc > din >5α-chol-a. Cholesterol concentration was significantly ($p < 0.05$) highest in male (max: 457.24 ng g^{-1}dw; 14.96% of ∑25s), female (max: 368.16 ng g^{-1}dw; 14.96% of ∑25s) and pup (max: 306.93 ng

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g^{-1}dw; 14.96% of ∑25s), with the higher ∑25s recorded in male liver (Figure 1. D1, D2; Figure 2B; SI Table S3). Conversely to other tissues where cholesterol is mainly incorporated into membranes, in liver it is stored as exchangeable cholesteryl ester in the hepatic sterol ester pools to rapidly be transported into plasma at the desired time, and biliary excretion and the bile/liver ratios are the main factors affecting the deposition of sterols in liver.[102] Since zoosterol was more accumulated in liver than phytosterols, a large extent of plant sterols are likely excreted by seal biliary secretion, which is mediated by specific phytosterols transporters solely expressed in hepatocytes and enterocytes that suppress liver accumulation of plant sterols.[103] Because mammalian cells are not endogenous phytosterol synthesizers and can not regulate plant sterol homeostasis, phytosterol absorption is linked to cholesterol uptake regulated mechanisms and its excretion occurs through mechanisms that oxidize plant sterols.[104,105] Conversely, in liver liposomal membrane, electrostatic interaction between liver sterol carrier protein 2 (SCP2) and negatively charged membranes enhance cholesterol intermembrane transfer.[106] Nonetheless, liver is less important as a sterol biosynthesis site, and a large amount of sterols is locally synthesized within each extrahepatic tissue.

In kidney, the sterol profiles based on seal age/sex were similar to liver, with significantly ($p < 0.05$) highest concentrations of cholesterol in male (max: 426.51 ng g^{-1}dw, 14.96% of ∑25s) and female (max: 336.62 ng g^{-1}dw, 14.96% of ∑25s), and the higher ∑25s recorded in male kidney (Figure 1E1, E2; Figure 2B; SI Table S3). Accordingly, Pradas et al.[107] documented in rat that the cholesterol levels were higher in kidney, followed by liver, in respect to sterol/steroid metabolism, but the cholesterol precursors and derivatives were significantly higher in liver.

In heart, the composition of sterols depending on seal age/sex was similar to brain, with significantly ($p < 0.05$) high proportion of cholesterol (max: 309.49 ng g^{-1}dw; 12.44% of ∑25s) and β-sitosterol (max: 284.99 ng g^{-1}dw; 11.45% of ∑25s) in male, and with the higher ∑25s recorded in male heart (Figure 1F1, F2; Figure 2B; SI Table S3).

In spleen, the composition of sterols depending on seal age/sex was dominated by β-sito > chol-e > chol-a > chol-e-one > fuc > stig-e. The most significant ($p < 0.05$) differences in sterol congeners were in the levels of β-sitosterol (max: 165.74 ng g^{-1}dw; 12.18% of ∑25s) and cholesterol (max: 146.24 ng g^{-1}dw; 10.74% of ∑25s) in female, with the higher ∑25s recorded in male spleen (Figure 1G1, G2; Figure 2B; SI Table S3). β-sitosterol and cholesterol are the typical Δ5-sterols for plant material and animal tissues, respectively, in the environment.[108] Any dysfunction in the metabolism/transporters/carriers of cholesterol, oxysterols and sterol intermediates in vital organs could cause pathophysiological settings as cancers and diseases.[101,109] Therefore, pathological studies need to be performed in seals to provide more complete insights on these data and evaluate the risk levels of tissue sterol profiles.

Blood sterol profiles of seals by age/sex were similar to brain and heart, with the most significant ($p < 0.05$) differences in sterol congeners observed in cholesterol (max: 244.50 ng g^{-1} dw; 12.44% of ∑25s) and β-sitosterol (max: 225.14 ng g^{-1}dw; 11.45% of ∑25s) in male, with the higher ∑25s recorded in male blood (Figure 1H1, H2; Figure 2B; SI Table S3). Intimal sterol synthesis is the main source of plasma cholesterol accumulation in the arterial wall and the conversion of chol-e to chol-a is applied to the exchange of cholestanol between blood and tissues.[109] Plant sterols are cytotoxic to macrophages and may trigger potential risk in plasma. Increased levels of β-sitosterol induce the production of proinflammatory cytokines by macrophages, reduce the endotelium dependent vasodilation, and harden erythrocyte cell membranes,[110] which could be associated with shortened lifespan of seals.

In intestine, the sterol profiles based on seal age/sex were similar, with the dominant concentrations of chol-a-one > din > chol-e-one >5α-chol-a. The most significant ($p < 0.05$) differences were in male chol-a-one (max: 152.26 ng g^{-1}dw, 18.25% of ∑25s), with the higher ∑25s recorded in male intestine (Figure 1I1, I2; Figure 2B; SI Table S3). The higher intestinal absorption of animal sterols, such as cholestanol and other cholesterol epimers, compared to plant sterols suggests some selective factors attributable to the high solubility of cholesterol in micelles and incorporation into chylomicrons (lipoprotein particles), and then its lymphatic recovery,[111] as well as the biohydrogenation of sterols into stanols by intestinal microflora,[18] and/or lipid interactions functions triggerring signal transduction pathways to influx the sterols from mammalian enterocyte to bloodstream.[112−114] Accordingly, Sissener et al.[78] reported in Atlantic salmon a higher intestinal absorption of cholesterol compared to campesterol and brassicasterol, which are phytosterols.

In muscle, the seal sterol profiles by age/sex were similar to intestine. Cholestanone concentration was most significant ($p < 0.05$) in male (max: 216.60 ng g^{-1}dw, 19.22% of ∑25s), with the higher ∑25s recorded in male muscle (Figure 1J1, J2; Figure 2B; SI Table S3). Similar findings in sterol composition were also reported in muscle of fish and shrimp.[81,85] The higher amount of zoosterols, such as cholesterol epimers, in Caspian seal muscle may be a result of intestinal uptake, biliary excretion of phytosterols, lipoprotein metabolism, and/or changes in biosynthesis of cholesterol and sterols throughout the year.[85] Also, tissue distribution of mammalian dietary cholesterol is evolutionary conserved, and after 24 h most levels of exogenous cholesterol (45%) are uptaked from the gastrointestinal system and only 10% from the liver.[80,115]

Overall, in the distribution and composition of seal tissue sterols, no age/sex differentiation was observed except for brain tissue in pup, though the mean concentration of 25 sterol congeners was higher in male tissues than in females and pup. Indeed, for marine mammals (seals, sea lions, and walrus), based on the female capability to modulate their lactation periods (milk production) and pass their contaminants to offspring via placenta and milk, it is documented a gender-related difference in POP levels such as fecal sterols, with their levels often higher in males than females, with an age-dependent accumulation.[116] Therefore, the composition of sterols in the diet of Caspian seals and their metabolic effects in long-term feeding trial by fecal contaminated seafood should also be assessed in future studies.

3.4. Sterol Ratios. A set of 23 sterol ratios (R1−R23; SI Table S1) was herein applied to identify the level of human fecal contamination and differentiate between the sources of fecal matter (humans and animals) in the aquatic environment.[15] The ratios were classified into three categories: (1) human, (2) noncertain, and (3) no pollution (SI Table S1), and were applied to the abiotic (seawater, surface sediment, and SPMs) and biota (Caspian seals) matrices (SI Table S5).

The ratios R1−R7 were calculated to characterize human vs nonhuman sewage. Based on R1 (cop/chol-a) ratio, all samples

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were contaminated by sewage.[2,18] Similar results for R1 ratio were reported for oysters fecally contaminated with human wastewater after short-term exposure in seawater microcosms.[82] Moreover, according to the R2 (cop/chol-e) ratio, abiotic samples elucidated the positive human fecal contamination with a criterion of R2 > 1, showing strong differences between biogenic inputs and anthropogenic sources[2,117] The R3 (cop/cop+chol-a) ratio with a threshold value of 0.3−0.7 indicated the presence of noncertain sewage in the aquatic environment of the southeast Caspian Sea.[2,65] R4 (cop/ep-cop) ratio was used to differentiate the human vs other mammalian feces, and the criterion of R4 < 1.5 revealed high levels of other mammalian waste input in the southeast Caspian Sea.[2,62] To characterize any potential microbial treatment sewage by conversion of cop to ep-cop, R4 can be modified into R5 (cop+ep-cop/cop+ep-cop+chol-a) ratio.[2,22] Similar to R4, R5 < 0.7 showed that most of the Gorgan Bay were contaminated by untreated nonhuman wastewater input. In contrast to R4, R6 (ep-cop/cop) ratio is considered to be elucidative of the age of fecal matter and their treatment levels. R6 > 0.2 suggested that partially untreated nonhuman feces (such as herbivorous) are dominated at these regions.[2,118] Additionally, R7 (cop/(cop+24-e-cop)) ratio was applied to evaluate the influence of livestock wastes and, since it ranged from 0.4 (biota) to 0.5 (abiotic), it was associated with the dominant herbivore manure discharge in this ecosystem, which was in line with R4−R6.[119,61] Discharge of the total animal manure and cultivated land pollution load and/or substantial water runoff from the entire cities of the north of Iran may be the principal factors that affected the quality of rivers/groundwater in the Gorgan Bay basin.[120,121] The ratios of R7 > 0.06 suggested that all the abiotic/biotic samples had sterols derivated from anthropogenic sewage, and thus samples were probably close to the large effluent outlets.[2,122] However, for certain distinction of the human fecal contamination (with a high concentration of coprostanol), the value of 0.06 in alignment with the threshold levels of 10 ng g^{-1} (cop),[67] is too low. Therefore, to be indicative of human-sourced pollution, the index value of R7 should be >0.06 and it is consistent with a concentration of 500 ng g^{-1} (cop).[123]

In agreement with R7 and high levels of coprostanol detected, R8 (%cop) > 5−6% showed a human fecal signal in wastewater in all abiotic samples, but in biota is noncertain.[2,124] Similar to R8, R9 (%24-e-cop) was applied as an indicator of fecal sterol of ruminants (herbivore)[2,18] because 24-ethylcoprostanol is one of the sterols present in much lower levels in the digested sludge produced by anaerobic degradation of β-sitosterol.[125] Based on R9 < 5−6%, the presence of fecal sterols of domestic livestock, such as cow and sheep, was quantified in low concentrations in sludge input. In this line, to distinguish among different animal feces, and between herbivore, human, and mixed feces, R10 (cop+ep-cop +24-e-ep-cop/cop+chol-a) and R11 ((cop/cop+24-e-ep-cop) × 100) ratios could also be used, respectively.[2,23,125] This ratio could be explained to differentiate between animal (dominated by 24-e-ep-cop) and human-sourced manure (dominated by cop).[23,126] The ratio of R10 ≥ 1 is indicative of bovine-sourced feces,[2,22] and 38 < R11 < 73 can be attributed to the mixtures of the herbivore/human feces,[2,122] with the dominance of porcine-originated sterols (51 < R11 < 61).[2,126] Similarly, Harrault et al.[82] found the R11 ratio <0.73 for marine oysters *C. gigas*, indicative of human pollution. R12 (camp-e+sito-e/chol-e) ratio was applied to distinguish between pig (R12 > 1.5) and human (R12 < 1.0) feces contamination in soil or water sample,[2,68] and it supported the presence of pig slurry pollution in the southeast Caspian Sea environment. The sterol profiles of the manures of cow and poultry are dominated by campestanol and sitosterol, whereas the manures of pig by coprostanol. Since cholesterol is an ubiquitous steroid, to differentiate between the sources of bovine and porcine feces, R13 (cop+ep-cop/chol-e) ratio was used.[2,127] However, the 0.7 < R13 < 3.7 indicated that the certainty of the presence of porcine-originated sterols in waste discharge is less clear. As the identification of phytosterol sources through the degradation of cholesterol to stanol products, R14 (sito-a/cop) values in the range of 0.2 < R14 < 0.7 illustrated the proportion of porcine originated stanols in sewage and natural inputs.[2,126] Also, the choice of the R14 ratio has refined the differentiation between bovine (R14 > 1), and porcine manure (R14 < 1) and effluent samples (R14 < 1) based on the distribution of the steroid compounds.[126] However, the observed variation in the phytosterol biomarkers, without considering the degradation of OM, reflects a credible descriptor of shifting nutrient status from the Gorgan Bay by changes in phytoplankton productivity between sediment pore waters and the overlying water column.[128] According to the reference value of R15 (sito-a/sito-a+24-e-cop+24-e-ep-cop) and R16 (chol-a/chol-a+cop+ep-cop), all samples investigated herein did not receive the avian sources of fecal sterols.[2,129] Because sitosterol can be microbially degraded to stigmastanol at anoxia through both eutrophication and incorporation of excess nutrients from ornithogenic input (guano) into the bottom sediments.[130] Based on the high relative abundance of coprostanol in the feces of omnivores (humans and other mammals) compared to herbivores (livestock), which are dominated by plant sterols such as 24-ethylcoprostanol and 24-ethylepicoprostanol, R18 ((cop+ep-cop)/(24-e-cop+24-e-ep-cop)), R19 (ep-cop/(chol-a+cop)), and R20 ((24-e-ep-cop/24-e-cop)+(ep-cop/cop)) ratios were used for fingerprint diet or species distinction.[119,131] The R18 > 1 confirmed that the probability of fecal omnivores derivated sterols in abiotic samples were notably higher than that of the herbivores counterpart, but for biota samples are noncertain. R19 > 0.1 and R20 > 1.2 ratios gave similar omnivores fingerprints in the southeast Caspian Sea. Additionally, the R22 (chol-e/des) and R23 (chol-e/β-sito) ratios were evaluated in Caspian seal tissues for the determination of animal and plant origins of sterols, respectively, as discussed in the previous section.

Overall, some of the applied ratios gave positive point-source inputs of human fecal pollution but in general it was evident the combination of sterols originating from human and nonhuman sewage sources for all biotic/abiotic samples (SI Table S5). In the abiotic matrices, a similar value was calculated for the diagnostic ratios of sterols in all the three environmental compartments and their stations, which may be affected by the hydrography of the Gorgan Bay and uniform mixture of sterols originating from natural sources and sewage.[65,132] 3D scatter plot of the selected ratios R3, R6, and R2 (SI Figure S4A), and R4, R11, and R18 (SI Figure S4B), and their relationships, was applied to the space illustration of the results of the sterol source tracking in the study area. Regardless, to prevent the continuing trends, anthropogenic impacts from sewage discharge in this area require sustainability water quality monitoring programs. Based on the lack of a reliable quantitative criterion for sterol fecal fingerprinting in biota samples, in the current study numerous

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literature-based criteria were analyzed and suggested for biota samples (SI Table S5), even if so far these ratios have only been used for the environmental matrices (sediments or water). Despite the use of limited sterol ratios in previous reports,[37,119] there are still discrepancies and large gap in the values of sterol ratios (R1−R23; SI Table S1) between the biotic and abiotic samples, suggesting that the determination of conclusive threshold levels for fecal source tracking by these indexes in the living specimens requires an overall review and reconfirmation.

3.5. Multivariate Analysis (PCA, Correlation, and Cluster Analysis) Of All Abiotic and Biota Sterols Tested. For discrimination of sterols and their source tracking, PCA and heatmap of a correlation matrix was applied to the 25 sterol congeners detected in the three environmental compartments (SI Figure S5A and B). PC_1 showed positive correlations (r = 0.84−0.93 in surface sediments, r = 0.84−0.92 in seawater, and r = 0.83−0.89 in SPMs) among coprostanol, cholestanol, cholesterol, cholestanone, stigmastanol, β-sitosterol, 24-ethylcoprostanol, 24-ethylepicoprostanol, and dinosterol, which indicated human sewage pollution and distinguished between terrestrial and marine/microbial sources of sterols (SI Table S3; Figure S5B) PC_2 had a positive connection (r = 0.65−0.78 in surface sediments, r = 0.69−0.76 in seawater, and r = 0.70−0.79 in SPMs) to sitosterol, sitostanol, campesterol, desmosterol, brassicastanol, brassicasterol, isofucosterol, 5α-cholestanol, ehydrocholesterol, indicating low sewage with a biogenic contribution (plant sterol from terrestrial sources) (SI Table S3; Figure S5B). Factor 2 grouped sites with the higher fecal sterols and indicated sewage or biogenic contribution,[133] since cholesterol in Gorgan Bay sediments could originate from the biosynthesis by phyto- and zooplankton, from the diagenetic transformation of cop into chol-e through sewage by anaerobic microbial reduction/hydrogenation, and/or from terrestrial enrichment. Consequently, chol-e as an independent marker has some limitations, thus to estimate the decreases of redox potential in sediments, cholesterol can be applied in the form of a stanol/stenol ratios. Therefore, R21 (chol-a/chol-e) < 1.0 indicates anoxic conditions/microbial reduction, but values of R21 ≈ 1.0 indicate limited oxic and ideal conditions for biosynthesis of cholestanol, as revealed in this study for all samples (SI Table S5). Accordingly, PC_1 exhibited that chol-a comes from marine sources, because it has R21 = 1.01 and have close correlation with chol-e (SI Table S5; Figure S5).[133] PC_2 also indicated that phytosterols (stigmasterol and β-sitosterol) can be used as a sign of correlation between terrestrial OM (TOM) and marine originated sterols. Although it could be clearly determined by using campesterol/stigmasterol/β-sitosterol ratio,[133] the evidence of chol-a and chol-e in PC_1 confirmed a contribution in Caspian Sea sources of plants sterols in the Gorgan Bay and Miankaleh wetlands. Moreover, the terrigenous input of rivers from the vast and wide green belt of Caspian Hyrcanian forests seems more effective to change the sterol composition in this area to some of the sources of plant sterols.[53]

A correlation analysis of all the abiotic and biotic samples from Gorgan Bay was also performed (SI Figure S6). Based on the correlation matrix heatmap, a positive correlation between all the water quality parameters except DO and pH, and ST1 and ST2 was observed (SI Figure S6A). In contrast, a positive correlation between DO and pH, and ST2, ST3, and ST5 was also recorded. However, the variability in BOD, DO, and pH in the surface water could be explained by the change in the ratio of cop/ep-cop.[134] A strong positive relationship for the distribution of the 25 sterol congeners between abiotic and biotic systems was also observed in dendrograms that indicate clustering according to sterols concentrations and their similarities between variables (SI Figure S6B). The positive relationship was best explained by the significant variability in coprostanol levels as a common sterol bioindicator.[134]

Hierarchical clustering analysis (HCA) on the levels of total sterols was applied to assess similarities between environmentaland biotic variables (Figure 2A), and it split all samples into two main clusters that are in line with the sensitivity to coprostanol and cholestanol between abiotic sampling stations (Figure 2A and SI Figure S7A), and to coprostanol and cholesterol between various Caspian seal tissues (Figure 2B and SI Figure S7B). In Figure 2A, cluster I is composed of nine environmental samples (SPMs-S1−S5, and sed-S1,S2, S4,S5), where the amount of fecal sterols from terrigenous sources is significantly high (>75% for Ia (SPMs) and 50% for Ia (sediment)) and marine sources of sterols are slightly predominant at Euclidean distance. In this line, the variability of total sterols concentration in cluster Ia for sediment could best be explained by the ratio of cop/chol-a (126%), cop/chol-e+chol-a (63%), and % coprostanol (5.4%) (Figure 2A; SI Table S5),[134] whereas the variability and categorization of total sterols concentration in cluster Ia for SPMs may be explained by the close correlation/similarity with sterols ratios of cop/chol-e (100%) and cop/chol-e+chol-a (63%) (Figure 2A; SI Table S5). The other marine derivated fecal sterols are presented as cluster Ib, containing five environmental samples (sea-S1,S2, sea-S4,S5, and sed-S3), where the proportion of sterols is also high (>75% for Ib (seawater)) at Euclidean distance and terrestrial sources of sterols are rather low (Figure 2A).[133] Cluster II contained one sampling point (sea-S3) as a separate branch with less similarity with the other sites, where the quota of fecal sterols is intolerably low (<0.5% for II (seawater)), with slightly predominance of terrestrial originated sterols and a possible peak in concentration of plant sterols from low sewage with biogenic contribution, which caused the separation in cluster II. The HCA results are supported by the PCA results, as shown in Figure 2.

The results of creating a triple dendrogram of hierarchical tree and clustering of the concentration of total steroidal compounds to assessing similarities between selected tissues of the Caspian seals are drawn in Figure 2B. Among the two main branches I and II, a separate cluster I which built on the bioaccumulation of steroidal compounds in the lipophilic tissue, such as brain, blubber, and fur, was substantially depending on the seal age/sex (male (M), female (F), and pup (P)). Cluster I contained seven sampling tissues (M, F, P-blubber and fur, and P-brain) with ∑25s dominated in male seal blubber (380.8 ng g^{-1}dw; 0.79% of ∑25s) (Figure 2B). Cluster IIa contained five sampling tissues (M, F, P-intestine and F, P-muscle) with maximum similarity based on the total of sterols dominated in female seal muscle (668.9 ng g^{-1}dw; 1.39% of ∑25s) (Figure 2B). Cluster IIb contained 17 sampling tissues age- and sex-dependent, including metabolism-sensitive organs with the same clustering patterns and showing their close correlation in regulation/deregulation of cholesterol in seals. As expected, ∑25s dominated in male brain (12105.7 ng g^{-1}dw; 25.3% of ∑sterols), followed by other their male tissues in the order of liver, kidney, heart, blood, and spleen (Figure 2B). As the liver is a metabolic

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protein-rich tissue, cholesterol homeostasis is associated in general with a liver enzymatic system rather than lipids-rich blubber. This is consistent with findings on tissue distribution of perfluoroalkyl acids and their precursors in polar bears *Ursus maritimus* and their prey, the ringed seal *P. hispida*.[135]

In summary, endangered Caspian seals *P. caspica* were herein used effectively to study the effects of fecal pollution on the uptake and organ distribution of sterols in order to record a source-specific fingerprint and improve the toxic database for the Caspian Sea health. Findings from this study revealed that both the animal originated sterol content and the ratio of phytosterols affected the tissue sterol composition in the Caspian seals, with increased cholesterol and its precursor cholestanol content in most of the vital organs, that is, brain, liver, kidney, and heart. However, the identification of specific sources of sterol contamination in seal tissues and their discrimination from natural counterpart seemed limited. In the distribution and composition of seal tissue sterols, no age/sex differentiation was observed, though the mean concentration of 25 sterol congeners was higher in male tissues than in females and pup. Overall, by application of different diagnostic ratios, it was evident the combination of sterols originating from human and nonhuman sewage sources for all biotic/abiotic samples. Therefore, findings from this study pinpoint the urgent necessity to investigate on the ecotoxicity of fecal sterols in mammals, and on the consequent implications for human health. Also, it is of high importance to promote sustainable water quality monitoring programs to prevent the anthropogenic impact from sewage discharges in marine environments. Refs 28, 30, 31, 136, 137.

■ ASSOCIATED CONTENT

Supporting Information

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Vorosmarty, C. J.; McIntyre, P. B.; Gessner, M. O.; Dudgeon, D.; Prusevich, A.; Green, P.; Glidden, S.; Bunn, S. E.; Sullivan, C. A.; Liermann, C. R.; Davies, P. M. Global threats to human water security and river biodiversity. *Nature* **2010**, *467* (7315), 555−561.

(2) Derrien, M.; Yang, L.; Hur, J. Lipid biomarkers and spectroscopic indices for identifying organic matter sources in aquatic environments: A review. *Water Res.* **2017**, *112*, 58−71.

(3) Clarke, B. O.; Porter, N. A. Persistent organic pollutants in sewage sludge: Levels, sources, and trends. In *ACS Symp. Ser.*. ACS Symposium Series Vol. *1048*:137−171. **2010**;

(4) Farrington, J. W.; Takada, H. Persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), and plastics: Examples of the status, trend, and cycling of organic chemicals of environmental concern in the ocean. *Oceanography.* **2014**, *27* (1), 196−213.

(5) Field, K. G.; Samadpour, M. Fecal source tracking, the indicator paradigm, and managing water quality. *Water Res.* **2007**, *41*, 3517−3538.

(6) Korajkic, A.; Brownell, M. J.; Harwood, V. J. Investigation of human sewage pollution and pathogen analysis at Florida Gulf coast beaches. *J. Appl. Microbiol.* **2011**, *110* (1), 174−183.

(7) Lyons, B. P.; Devlin, M. J.; Abdul Hamid, S. A.; Al-Otiabi, A. F.; Al-Enezi, M.; Massoud, M. S.; Al-Zaidan, A. S.; Smith, A. J.; Morris, S.; Bersuder, P.; Barber, J. L.; Papachlimitzou, A.; Al-Sarawi, H. A. Microbial water quality and sedimentary faecal sterols as markers of sewage contamination in Kuwait. *Mar. Pollut. Bull.* **2015**, *100* (2), 689−98.

(8) USEPA, U.S. Environmental Protection Agency. *Method 1604. Total Coliforms and Escherichia coli in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium). Rept. Mo. EPA 821-R-02-024*; Environmental Protection Agency: Washington DC, 2002.

(9) Derrien, M.; Jardé, E.; Gruau, G.; Pourcher, A. M.; Gourmelon, M.; Jadas-Hecart, A.; Pierson Wickmann, A. C. Origin of fecal contamination in waters from contrasted areas: Stanols as microbial source tracking markers. *Water Res.* **2012**, *46* (13), 4009−4016.

(10) Harwood, J. J. Molecular markers for identifying municipal, domestic and agricultural sources of organic matter in natural waters. *Chemosphere* **2014**, *95*, 3−8.

(11) Hagedorn, C.; Weisberg, S. B. *Chemical-Based Fecal Source Tracking Methods. Microbial Source Tracking: Methods, Applications, And Case Studies*; Springer: New York, 2011, pp 189−206.

(12) Alsalahi, M. A.; Latif, M. T.; Ali, M. M.; Dominick, D.; Khan, M. F.; Mustaffa, N. I.; Nadzir, M. S.; Nasher, E.; Zakaria, M. P. Sterols as biomarkers in the surface microlayer of the estuarine areas. *Mar. Pollut. Bull.* **2015**, *93* (1−2), 278−83.

(13) Fahrenfeld, N. L.; Del Monaco, N.; Coates, J. T.; Elzerman, A. W. Fecal Sterol and Runoff Analysis for Nonpoint Source Tracking. *J. Environ. Qual.* **2016**, *45* (1), 315−22.

(14) Froehner, S.; Sanez, J. Evaluation of potential sewage contamination by fecal sterol biomarkers adsorbed in natural biofilms. *Environ. Sci. Process. Impacts* **2013**, *15*, 2080−2086.

(15) Furtula, V.; Liu, J.; Chambers, P.; Osachoff, H.; Kennedy, C.; Harkness, J. Sewage treatment plants Efficiencies in removal of sterols and sterol ratios as indicators of fecal contamination sources. *Water, Air, Soil Pollut.* **2012**, *223*, 1017−1031.

(16) Tanabe, S. Contamination and toxic effects of persistent endocrine disrupters in marine mammals and birds. *Mar. Pollut. Bull.* **2002**, *45* (1−12), 69−77.
(17) Antanasijević, D.; Bujagić, I. M.; Grujić, S.; MilaLaušević, M. Effect of compositional data in the multivariate analysis of sterol concentrations in river sediments. *Microchem. J.* **2018**, *139*, 188−195.
(18) Leeming, R.; Ball, A.; Ashbolt, N.; Nichols, P. Using fecal sterols from humans and animals to distinguish faecal pollution in receiving waters. *Water Res.* **1996**, *30*, 2893−2900.
(19) Volkman, J. K. A review of sterol markers for marine and terrigenous organic matter. *Org. Geochem.* **1986**, *9*, 83−99.
(20) McCalley, D. V.; Cooke, M.; Nickless, G. Effect of sewage treatment on fecal sterols. *Water Res.* **1981**, *15*, 1019−1025.
(21) Volkman, J. K. Sterols and others triterpenoids: source specificity and evolution of biosynthetic pathways. *Org. Geochem.* **2005**, *36* (2), 139−159.
(22) Bull, I. D.; Lockheart, M. J.; Elhmmali, M. M.; Roberts, D. J.; Evershed, R. P. The origin of faeces by means of biomarker detection. *Environ. Int.* **2002**, *27*, 647−654.
(23) Shah, V. G.; Dunstan, R. H.; Geary, P. M.; Coombes, P.; Roberts, T. K.; Nagy-Felsobuki, E. V. Evaluating potential applications of faecal sterols in distinguishing sources of faecal contamination from mixed faecal samples. *Water Res.* **2007**, *41* (16), 3961−3700.
(24) Bassi, N.; Kumar, M. D.; Sharma, A.; Pardha-Saradhi, P. Status of wetlands in India: A review of extent, ecosystem benefits, threats and management strategies. *J. Hydrol. Reg. Stud.* **2014**, *2*, 1−19.
(25) Stacke, T.; Hagemann, S. Development and evaluation of a global dynamical wetlands extent scheme. *Hydrol. Earth Syst. Sci.* **2012**, *16*, 2915−2933.
(26) Russi, D.; ten Brink, P.; Farmer, A.; Badura, T.; Coates, D.; Förster, J.; Kumar, R.; Davidson, N. *The Economics of Ecosystems and Biodiversity for Water and Wetlands*; IEEP, London and Brussels Ramsar Secretariat: Gland, 2013.
(27) Ranjbar, M. H.; Jandaghi Alaee, M.; Nazarali, M. A modeling study of the impact of increasing water exchange rate on water quality of a semi-enclosed bay. *Ecol. Eng.* **2019**, *136*, 177−184.
(28) Sinkakarimi, M. H.; Pourkhabbaz, A. R.; Hassanpour, M.; Levengood, J. M.; Ghasempouri, S. M. Potential human health risk assessment of heavy metals in the flesh of mallard and pochard in the South Eastern Caspian Sea region of Iran. *J. Adv. Environ. Health. Res.* **2015**, *3* (2), 139−45.
(29) Jafari, H. R.; Karimi, S.; Maddah, F. Analysis of pollutant's parameters of ″Miankaleh wetland″ using geographical information systems (GIS). *J. Environ. Stud.* **2008**, *33* (44), 55−64.
(30) Bastami, K. D.; Bagheri, H.; Kheirabadi, V.; Zaferani, G. G.; Teymori, M. B.; Hamzehpoor, A.; Soltani, F.; Haghparast, S.; Harami, S. R. M.; Ghorghani, N. F.; Ganji, S. Distribution and ecological risk assessment of heavy metals in surface sediments along southeast coast of the Caspian Sea. *Mar. Pollut. Bull.* **2014**, *81*, 262−267.
(31) Li, J. G.; Pu, L. J.; Zhu, M.; Zhang, J.; Li, P.; Dai, X. Q.; Xu, Y.; Liu, L. L. Evolution of soil properties following reclamation in coastal areas: a review. *Geoderma* **2014**, *226−227*, 130−139.
(32) Kajiwara, N.; Watanabe, M.; Wilson, S.; Eybatov, T.; Mitrofanov, I. V.; Aubrey, D. G.; Khuraskin, L. S.; Miyazaki, N.; Tanabe, S. Persistent organic pollutants (POPs) in Caspian seals of unusual mortality event during 2000 and 2001. *Environ. Pollut.* **2008**, *152* (2), 431−442.
(33) Robinson, K. J.; Hall, A. J.; Debier, C.; Eppe, G.; Thomé, J. P.; Bennett, K. A. Persistent organic pollutant burden, experimental POP exposure, and tissue properties affect metabolic profiles of blubber from gray seal pups. *Environ. Sci. Technol.* **2018**, *52* (22), 13523−13534.
(34) Numberger, D.; Herlemann, D. P. R.; Jürgens, K.; Dehnhardt, G.; Schulz-Vogt, H. Comparative analysis of the fecal bacterial community of five harbor seals (*Phoca vitulina*). *MicrobiologyOpen* **2016**, *5* (5), 782−792.
(35) Ranjbar Jafarabadi, A.; Riyahi Bakhtiari, A.; Mitra, S.; Maisano, M.; Cappello, T.; Jadot, C. First polychlorinated biphenyls (PCBs) monitoring in seawater, surface sediments and marine fish communities of the Persian Gulf: distribution, levels, congener profile and potential health risk assessment. *Environ. Pollut.* **2019**, *253*, 78−88.
(36) Ranjbar Jafarabadi, A.; Riyahi Bakhtiari, A.; Maisano, M.; Pereira, P.; Cappello, T. First record of bioaccumulation and bioconcentration of metals in Scleractinian corals and their algal symbionts from Kharg and Lark coral reefs (Persian Gulf, Iran). *Sci. Total Environ.* **2018**, *640−641*, 1500−1511.
(37) Derrien, M.; Jarde, E.; Gruau, G.; Pierson-Wickmann, A.-C. Extreme variability of steroid profiles in cow feces and pig slurries at the regional scale: implications for the use of steroids to specify fecal pollution sources in waters. *J. Agric. Food Chem.* **2011**, *59*, 7294−302.
(38) Vanden Berghe, M.; Weijs, L.; Habran, S.; Das, K.; Bugli, C.; Rees, J. F.; Pomeroy, P.; Covaci, A.; Debier, C. Selective transfer of persistent organic pollutants and their metabolites in grey seals during lactation. *Environ. Int.* **2012**, *46*, 6−15.
(39) APHA *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington DC, 2005.
(40) Liu, Y.; Du, J.; Hu, P.; Ma, M.; Hu, D. Microtopographic modification conserves urban wetland water quality by increasing the dissolved oxygen in the wet season. *J. Environ. Sci.* **2020**, *87*, 71−81.
(41) Saeed, T.; Sun, G. A review on nitrogen and organics removal mechanisms in subsurface flow constructed wet-lands: dependency on environmental parameters, operating conditions and supporting media. *J. Environ. Manage.* **2012**, *112*, 429−448.
(42) USEPA. U.S. Environmental Protection Agency. EPA, National Guidance Water Quality Standards for Wetlands. In *Water Quality Standards. Hand Book EPA 823-B-94-005a*, 2nd ed., August, 1994, pp 344.
(43) USEPA. U.S. Environmental Protection Agency. *Draft Guidance for Water Quality-Based Decisions: The TMDL Process (EPA 841-D-99-001)*, 2nd ed.; United States Environmental Protection Agency, Office of Water: Washington, DC, 1999.
(44) CBD SBSTTA (Convention on Biological Diversity, Subsidiary Body on Scientific, Technical and Technological Advice). Inland water ecosystems: review, further elaboration and refinement of the programme of work. Information document UNEP/CBD/SBSTTA/8/INF/4 prepared for the SBSTTA Eighth meeting, 10−14 March 2003, Montreal. 2003.
(45) Bashari, L.; Mahmudy Gharaie, M. H.; Moussavi Harami, R.; Alizadeh Lahijani, H. Hydrogeochemical study of the Gorgan Bay and factors controlling the water chemistry. *Oceanography* **2014**, *5* (20), 31−42. (In Persian).
(46) Ranjbar, M. H.; Hadjizadeh Zaker, N. Numerical modeling of general circulation, thermohaline structure, and residence time in Gorgan Bay, Iran. *Ocean.Dyn.* **2018**, *68* (1), 35−46.
(47) Saleh, A.; Hamzehpour, A.; Mehdinia, A.; Darvish Bastami, K.; Mazaheri, S. Hydrochemistry and nutrient distribution in southern deep-water basin of the Caspian Sea. *Mar. Pollut. Bull.* **2018**, *127*, 406−411.
(48) Martins, C. C.; Montone, R. C.; Gamba, R. C.; Pellizari, V. H. Sterols and fecal indicator microorganisms in sediments from Admiralty Bay, Antarctica. *Braz. J. Oceanogr.* **2005**, *53* (1−2), 1−12.
(49) Ghorbanzadeh Zaferani, S. G.; Machinchian Moradi, A.; Mousavi Nadushan, R.; Sari, A. R.; Fatemi, S. M. R. Spatial and temporal patterns of benthic macrofauna in Gorgan Bay, south Caspian Sea, Iran. *Iran. J. Fish. Sci.* **2017**, *16* (1), 252−274.
(50) Lahijani, H.; Haeri Ardakani, O.; Sharifi, A.; Naderi Beni, A. Sedimentological and geochemical characteristics of the Gorgan Bay sediments. *Oceanography* **2010**, *1* (1), 45−55. (In Persian).
(51) Omitoyin, B. O.; Ajani, E. K.; Okeleye, O. I.; Akpoilih, B. U.; Ogunjobi, A. A. Biological treatments of fish farm effluent and its reuse in the culture of Nile Tilapia (Oreochromis niloticus). *J. Aquacult. Res. Dev.* **2017**, *8*, 469.
(52) Kiaei, M.; Tajik, M.; Vaysi, R. Chemical and biometrical properties of plum wood and its application in pulp and paper production. *Maderas, Cienc. Tecnol.* **2014**, *16* (3), 313−322.

https://dx.doi.org/10.1021/acs.est.0c01479
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

(53) Poorzady, M.; Bakhtiari, F. Spatial and temporal changes of Hyrcanian forest in Iran. *iForest − Biogeosciences and Forestry* **2009**, *2*, 198−206.
(54) Carreón-Palau, L.; Parrish, C. C.; Pérez-España, H. Urban sewage lipids in the suspended particulate matter of a coral reef under river influence in the South West Gulf of Mexico. *Water Res.* **2017**, *123*, 192−205.
(55) Bataglion, G. A.; Meurer, E.; de Albergaria-Barbosa, A. C.; Bícego, M. C.; Weber, R. R.; Eberlin, M. N. Determination of geochemically important sterols and triterpenols in sediments using ultrahigh-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). *Anal. Chem.* **2015**, *87* (15), 7771−7778.
(56) Peng, X.; Zhang, G.; Mai, B.; Min, Y.; Wang, Z. Spatial and temporal trend of sewage pollution indicated by coprostanol in Macao Estuary, southern China. *Mar. Pollut. Bull.* **2002**, *45*, 295−299.
(57) Lyons, B. P.; Devlin, M. J.; Abdul Hamid, S. A.; Al-Otiabi, A. F.; Al-Enezi, M.; Massoud, M. S.; Al-Zaidan, A. S.; Smith, A. J.; Morris, S.; Bersuder, P.; Barber, J. L.; Papachlimitzou, A.; Al-Sarawi, H. A. Microbial water quality and sedimentary faecal sterols as markers of sewage contamination in Kuwait. *Mar. Pollut. Bull.* **2015**, *100* (2), 689−698.
(58) Readman, J. W.; Fillmann, G.; Tolosa, I.; Bartocci, J.; Mee, L. D. The use of steroid markers to assess sewage contamination of the Black Sea. *Mar. Pollut. Bull.* **2005**, *50* (3), 310−318.
(59) Martins, C. C.; Cabral, A. C.; Barbosa-Cintra, S. C.; Dauner, A. L.; Souza, F. M. An integrated evaluation of molecular marker indices and linear alkylbenzenes (LABs) to measure sewage input in a subtropical estuary (Babitonga Bay, Brazil). *Environ. Pollut.* **2014**, *188*, 71−80.
(60) Fattore, E.; Benfenati, E.; Mariani, G.; Cools, E.; Vezzoli, G.; Fanelli, R. Analysis of organic micropollutants in sediment samples of the Venice Lagoon, Italy. *Water, Air, Soil Pollut.* **1997**, *99* (1−4), 237−244.
(61) Leeming, R.; Latham, V.; Rayner, M.; Nichols, P. Detecting and distinguishing sources of sewage pollution in Australian inland and coastal waters and sediments. In *Molecular Markers in Environmental Geochemistry*; Eganhouse, R. P., Ed.; American Chemical Society: Washington, DC, 1997. pp 306−319.
(62) Venkatesan, M.; Santiago, C. A. Sterols in ocean sediments: novel tracers to examine the habitats of cetaceans, pinnipeds, penguins, and humans. *Mar. Biol.* **1989**, *102*, 431−437.
(63) Marvin, C.; Coakley, J.; Mayer, T.; Brown, M.; Thiessen, L. Application of faecal sterol ratios in sediments and effluents as source tracers. *Water Qual. Res. J. Can.* **2001**, *36* (4), 781−792.
(64) Martins, C. C.; Venkatesan, M. I.; Montone, R. C. Sterols and linear alkylbenzenes in marine sediments from Admiralty Bay, King George Island, South Shetland Islands. *Antarct. Sci.* **2002**, *14*, 244−252.
(65) Grimalt, J. O.; Fernandez, P.; Bayona, J. M.; Albaiges, J. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. *Environ. Sci. Technol.* **1990**, *24* (3), 357−363.
(66) Rada, J. P. A.; Duarte, A. C.; Pato, P.; Cachada, A.; Carreira, R. S. Sewage contamination of sediments from two Portuguese Atlantic coastal systems, revealed by fecal sterols. *Mar. Pollut. Bull.* **2016**, *103*, 319−324.
(67) Tolosaa, I.; Mesab, M.; Alonso-Hernandezb, C. M. Steroid markers to assess sewage and other sources of organic contaminants in surface sediments of Cienfuegos Bay, Cuba. *Mar. Pollut. Bull.* **2014**, *86* (1−2), 84−90.
(68) Jardé, E.; Gruau, G.; Mansuy-Huault, L.; Peu, P.; Martinez, J. Using sterols to detect pig slurry contribution to soil organic matter. *Water, Air, Soil Pollut.* **2007**, *178* (1), 169−178.
(69) Abdul, Q. A.; Choi, R. J.; Jung, H. A.; Choi, J. S. Health benefit of fucosterol from marine algae: A review. *J. Sci. Food Agric.* **2016**, *96* (6), 1856−1866.
(70) Meur, M. L.; Mansuy-Huault, L.; Lorgeoux, C.; Bauer, A.; Gley, R.; Vantelon, D.; Montargès-Pelletier, E. Spatial and temporal variations of particulate organic matter from Moselle River and tributaries: A multimolecular investigation. *Org. Geochem.* **2017**, *110*, 45−56.
(71) Benzon, H. T.; Chew, T. L.; McCarthy, R. J.; Benzon, H. A.; Walega, D. R. Comparison of the particle sizes of different steroids and the effect of dilution: A review of the relative neurotoxicities of the steroids. *Anesthesiology* **2007**, *106* (2), 331−338.
(72) Kim, M.; Jung, J. H.; Jin, Y.; Han, G. M.; Lee, T.; Hong, S. H.; Yim, U. H.; Shim, W. J.; Choi, D. L.; Kannan, N. Origins of suspended particulate matter based on sterol distribution in low salinity water mass observed in the offshore East China Sea. *Mar. Pollut. Bull.* **2016**, *108* (1−2), 281−288.
(73) Velikov, K. P.; van Ruijven, M.; Popp, A. K.; Patel, A. R.; Flendrig, L. M.; Melnikov, S. M. Colloidal particles for the delivery of steroid glycosides. *Food Funct.* **2018**, *9* (1), 485−490.
(74) Wakeham, S. G.; Canuel, E. A. Fatty acids and sterols of particulate matter in a brackish and seasonally anoxic coastal salt pond. *Org. Geochem.* **1990**, *16* (4−6), 703−713.
(75) Tyagi, P.; Edwards, D. R.; Coyne, S. M. Distinguishing between human and animal sources of fecal pollution in waters: A review. *Int. J. Water* **2009**, *5*, 15−34.
(76) Cappello, T.; Giannetto, A.; Parrino, V.; De Marco, G.; Mauceri, A.; Maisano, M. Food safety using NMR-based metabolomics: assessment of the Atlantic bluefin tuna, *Thunnus thynnus*, from the Mediterranean Sea. *Food Chem. Toxicol.* **2018**, *115*, 391−397.
(77) Khuraskin, L.; Pochotoyeva, N. Status of the Caspian seal population. *Caspian Environment Program* **1990**, 86−94.
(78) Sissener, N. H.; Rosenlund, G.; Stubhaug, I.; Liland, N. S. Tissue sterol composition in Atlantic salmon (*Salmo salar* L.) depends on the dietary cholesterol content and on the dietary phytosterol:cholesterol ratio, but not on the dietary phytosterol content. *Br. J. Nutr.* **2018**, *119*, 599−609.
(79) van Spankeren, M.; Sibinga, N.; Reinshol, O.; Torstensen, B. E.; Sæle, O.; Liland, N. S. A single meal containing phytosterols does not affect the uptake or tissue distribution of cholesterol in Zebrafish (*Danio rerio*). *Front. Mar. Sci.* **2019**, *6*, 92.
(80) Ural, M. S.; Çalta, M.; Parlak, A. E. The comparison of fatty acids, fat-soluble vitamins and cholesterol in the muscle of wild caught, cage and pond reared rainbow trout (*Oncorhynchus mykiss* W., 1792). *Iran. J. Fish. Sci.* **2017**, *16* (1), 431−440.
(81) Mika, A.; Gołębiowski, M.; Skorkowski, E. F.; Stepnowski, P. Composition of fatty acids and sterols composition in brown shrimp *Crangon crangon* and herring *Clupea harengus membras* from the Baltic Sea. *Oceanol. Hydrobiol. Stud.* **2012**, *41* (2), 57−64.
(82) Harrault, L.; Jardé, E.; Jeanneau, L.; Petitjean, P. Are fecal stanols suitable to record and identify a pulse of human fecal contamination in short-term exposed shellfish? A microcosm study. *Mar. Pollut. Bull.* **2014**, *89* (1−2), 40−48.
(83) Phillips, K. M.; Ruggio, D. M.; Exler, J.; Patterson, K. Y. Sterol composition of shellfish species commonly consumed in the United States. Citation. *Food Nutr. Res.* **2012**, *56*, 18931.
(84) Kanazawa, A. Sterols in marine invertebrates. *Fish. Sci.* **2001**, *67*, 997−07.
(85) Ozyurt, G.; Kuley, E.; Etyemez, M.; Ozogul, F. Comparative seasonal sterol profiles in edible parts of Mediterranean fish and shellfish species. *Int. J. Food Sci. Nutr.* **2013**, *64* (4), 476−83.
(86) Zmrzljak, U. P.; Rozman, D. Circadian regulation of the hepatic endobiotic and xenobitoic detoxification pathways: The time matters. *Chem. Res. Toxicol.* **2012**, *25* (4), 811−24.
(87) Jing, X.; Grebenok, R. J.; Behmer, S. T. Sterol/steroid metabolism and absorption in a generalist and specialist caterpillar: Effects of dietary sterol/steroid structure, mixture and ratio. *Insect Biochem. Mol. Biol.* **2013**, *43*, 580−587.
(88) Maxfield, F. R.; Menon, A. K. Intracellular sterol transport and distribution. *Curr. Opin. Cell Biol.* **2006**, *18*, 379−385.
(89) Souchet, N.; Laplante, S. Seasonal and geographical variations of sterol composition in snow crab hepatopancreas and pelagic fish viscera from Eastern Quebec. *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **2007**, *147*, 378−386.

https://dx.doi.org/10.1021/acs.est.0c01479
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

(90) Afonso, M. S.; Machado, R. M.; Lavrador, M. S.; Quintao, E. C. R.; Moore, K. J.; Lottenberg, A. M. Molecular pathways underlying cholesterol homeostasis. *Nutrients* **2018**, *10* (6), E760.
(91) Claumarchirant, L.; Matencio, E.; Sanchez-Siles, L. M.; Alegría, A.; Lagarda, M. J. Sterol Composition in Infant Formulas and Estimated Intake. *J. Agric. Food Chem.* **2015**, *63* (32), 7245−7251.
(92) Serra, M.; Matabosch, X.; Ying, L.; Watson, G.; Shackleton, C. Hair and skin sterols in normal mice and those with deficient dehydrosterol reductase (DHCR7), the enzyme associated with Smith-Lemli-Opitz syndrome. *J. Steroid Biochem. Mol. Biol.* **2010**, *122* (5), 318−25.
(93) Nelson, G. J. The lipid composition of the blood of marine mammals—I. Young elephant seals, *Mirounga augustirostris* and harp seals, *Pagophilus groenlandicus*. *Comp. Biochem. Physiol.* **1970**, *34* (1), 109−112.
(94) Trapani, L.; Segatto, M.; Pallottini, V. Regulation and deregulation of cholesterol homeostasis: The liver as a metabolic ″power station″. *World. J. Hepatol.* **2012**, *4* (6), 184−90.
(95) Mitsche, M. A.; McDonald, J. G.; Hobbs, H. H.; Cohen, J. C. Flux analysis of cholesterol biosynthesis in vivo reveals multiple tissue and cell-type specific pathways. *eLife*. **2015**4:e07999.
(96) Lopez, J.; Boyd, D.; Ylitalo, G. M.; Littnan, C.; Pearce, R. Persistent organic pollutants in the endangered Hawaiian monk seal (*Monachus schauinslandi*) from the main Hawaiian Islands. *Mar. Pollut. Bull.* **2012**, *64*, 2588−2598.
(97) Taylor, D. M.; Balabadra, U.; Xiang, Z.; Woodman, B.; Meade, S.; Amore, A.; Maxwell, M. M.; Reeves, S.; Bates, G. P.; Luthi-Carter, R.; Lowden, P. A.; Kazantsev, A. G. A brain-permeable small molecule reduces neuronal cholesterol by inhibiting activity of sirtuin 2 deacetylase. *ACS Chem. Biol.* **2011**, *6* (6), 540−6.
(98) Zhang, J.; Qiang, L. Cholesterol metabolism and homeostasis in the brain. *Protein Cell* **2015**, *6* (4), 254−264.
(99) Gamba, P.; Testa, G.; Gargiulo, S.; Staurenghi, E.; Poli, G.; Leonarduzzi, G. Oxidized cholesterol as the driving force behind the development of Alzheimer's disease. *Front. Aging Neurosci.* **2015**, *7*, 119.
(100) Vanmierlo, T.; Rutten, K.; van Vark-van der Zee, L. C.; Friedrichs, S.; Bloks, V. W.; Blokland, A.; Ramaekers, F. C.; Sijbrands, E.; Steinbusch, H.; Prickaerts, J.; Kuipers, F.; Lütjohann, D.; Mulder, M. Cerebral accumulation of dietary derivable plant sterols does not interfere with memory and anxiety related behavior in Abcg5−/− mice. *Plant Foods Hum. Nutr.* **2011**, *66* (2), 149−56.
(101) Yamauchi1, Y.; Rogers, M. A. Sterol metabolism and transport in atherosclerosis and cancer. *Front. Endocrinol. (Lausanne, Switz.)* **2018**, *9*, 509.
(102) Coy, D. J.; Wooton-Kee, C. R.; Yan, B.; Sabeva, N.; Su, K.; Graf, G.; Vore, M. ABCG5/ABCG8-independent biliary cholesterol excretion in lactating rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2010**, *299*, 228−235.
(103) Schott, H. F.; Baumgartner, S.; Husche, C.; Luister, A.; Friedrichs, S.; Miller, C. M.; McCarthy, F. O.; Plat, J.; Laufs, U.; Weingärtner, O.; Lütjohann, D. Oxidation of sitosterol and transport of its 7-oxygenated products from different tissues in humans and ApoE knockout mice. *J. Steroid Biochem. Mol. Biol.* **2017**, *169*, 145−51.
(104) Plat, J.; Baumgartner, S.; Vanmierlo, T.; Lütjohann, D.; Calkins, K. L.; Burrin, D. G.; Guthrie, G.; Thijs, C.; Te Velde, A. A.; Vreugdenhil, A. C. E.; Sverdlov, R.; Garssen, J.; Wouters, K.; Trautwein, E. A.; Wolfs, T. G.; van Gorp, C.; Mulder, M. T.; Riksen, N. P.; Groen, A. K.; Mensink, R. P. Plant-based sterols and stanols in health & disease: ″Consequences of human development in a plant-based environment?″. *Prog. Lipid Res.* **2019**, *74*, 87−102.
(105) Butko, P.; Hapala, I.; Scallen, T. J.; Schroeder, F. Acidic phospholipids strikingly potentiate sterol carrier protein 2 mediated intermembrane sterol transfer. *Biochemistry* **1990**, *29* (17), 4070−7.
(106) Spady, D. K.; Dietschy, J. M. Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat. *J. Lipid. Res.* **1983**, *24* (3), 303−315.
(107) Pradas, I.; Huynh, K.; Cabré, R.; Ayala, V.; Meikle, P. J.; Jové, M.; Pamplona, R. Lipidomics reveals a tissue-specific fingerprint. *Front. Physiol.* **2018**, *9*, 1165.
(108) Prost, K.; Birk, J. J.; Lehndorff, E.; Gerlach, R.; Amelung, W. Steroid biomarkers revisited − Improved source identification of faecal remains in archaeological soil material. *PLoS One* **2017**, *12* (1), e0164882.
(109) Aram V. Chobanian, A. V. Sterol synthesis in the human arterial intima. *J. Clin. Invest.* **1968**, *47* (3), 595−603.
(110) Vergès, B.; Fumeron, F. Potential risks associated with increased plasma plant-sterol levels. *Diabetes Metab.* **2015**, *41* (1), 76−81.
(111) Hamada, T.; Goto, H.; Yamahira, T.; Sugawara, T.; Imaizumi, K.; Ikeda, I. Solubility in and affinity for the bile salt micelle of plant sterols are important determinants of their intestinal absorption in rats. *Lipids* **2006**, *41*, 551−556.
(112) Jia, L.; Betters, J. L.; Yu, L. Niemann-Pick C1-Like 1 (NPC1L1) protein in intestinal and hepatic cholesterol transport. *Annu. Rev. Physiol.* **2011**, *73*, 239−259.
(113) Nguyen, T. M.; Sawyer, J. K.; Kelley, K. L.; Davis, M. A.; Rudel, L. L. Cholesterol esterification by ACAT2 is essential for efficient intestinal cholesterol absorption: evidence from thoracic lymph duct cannulation. *J. Lipid Res.* **2012**, *53* (1), 95−104.
(114) Zhao, G. J.; Yin, K.; Fu, Y. C.; Tang, C. K. The interaction of ApoA-I and ABCA1 triggers signal transduction pathways to mediate efflux of cellular lipids. *Mol. Med.* **2012**, *18*, 149−58.
(115) Borgström, B. Quantitative aspects of the intestinal absorption and metabolism of cholesterol and b-sitosterol in the rat. *J. Lipid Res.* **1968**, *9*, 473−481.
(116) Peterson, S. H.; Hassrick, J. L.; Lafontaine, A.; Thomé, J. P.; Crocker, D. E.; Debier, C.; Costa, D. P. Effects of age, adipose percent, and reproduction on PCB concentrations and profiles in an extreme fasting North Pacific marine mammal. *PLoS One* **2014**, *9* (4), e96191.
(117) Quemeneur, M.; Marty, Y. Fatty acids and sterols in domestic wastewaters. *Water Res.* **1994**, *28* (5), 1217−1226.
(118) Mudge, S. M.; Bebianno, M.J.A. F.; East, J. A.; Barriera, L. A. Sterols in the Ria Formosa Lagoon, Portugal. *Water Res.* **1999**, *33* (4), 1038−1048.
(119) Harrault, L.; Milek, K.; Jardé, E.; Jeanneau, L.; Derrien, M.; Anderson, D. G. Faecal biomarkers can distinguish specific mammalian species in modern and past environments. *PLoS One* **2019**, *14* (2), e0211119.
(120) Ehteshami, M.; Biglarijoo, N. Determination of nitrate concentration in groundwater in agricultural area in Babol county, Iran. *Iranian J.Health. Sci.* **2014**, *2* (4), 1−9.
(121) Hussain, M. A.; Ford, R.; Hill, J. Determination of fecal contamination indicator sterols in an Australian water supply system. *Environ. Monit. Assess.* **2010**, *165*, 147−157.
(122) Writer, J. H.; Leenheer, J. A.; Barber, L. B.; Amy, G. L.; Chapra, S. C. Sewage contamination in the upper Mississippi River as measured by the faecal sterol, coprostanol. *Water Res.* **1995**, *29* (6), 1427−1436.
(123) Gonzales-Oreja, J. A.; Saiz-Salinas, J. I. Short-term spatio-temporal changes in urban pollution by means of faecal sterols analysis. *Mar. Pollut. Bull.* **1998**, *36*, 868−875.
(124) Isobe, K. O.; Tarao, M.; Zakaria, M. P.; Chiem, N. H.; Minh, L. Y.; Takada, H. Quantitative application of fecal sterols using gas chromatography-mass spectrometry to investigate fecal pollution in tropical waters: western Malaysia and Mekong Delta, Vietnam. *Environ. Sci. Technol.* **2002**, *36* (21), 4497−4507.
(125) Lu, Y.; Philp, R. P.; Biache, C. Assessment of fecal contamination in Oklahoma water systems through the use of sterol fingerprints. *Environments* **2016**, *3* (4), 28.
(126) Gourmelon, M.; Caprais, M. P.; Mieskin, S.; Marti, R.; Wery, N.; Jardé, E.; Derrien, M.; Jadas-Hecart, A.; Communal, P. Y.; Jaffrezic, A.; Pourcher, A. M. Development of microbial and chemical MST tools to identify the origin of the faecal pollution in bathing and

https://dx.doi.org/10.1021/acs.est.0c01479
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

shellfish harvesting waters in France. *Water Res.* **2010**, *44* (16), 4812−4824.
(127) Jardé, E.; Gruau, G.; Mansuy-Huault, L. Detection of manure-derived organic compounds in rivers draining agricultural areas of intensive manure spreading. *Appl. Geochem.* **2007**, *22* (8), 1814−1824.
(128) Zhang, Y.; Su, Y.; Liu, Z.; Yu, J.; Jin, M. Experimental and geochemical evidence for Δ7/Δ5 sterol ratios as indicators of trophic status in Lake Fuxian, a great lake in YunnanProvince, SW China. *Limnologica* **2016**, *60*, 31−38.
(129) Devane, M. L.; Wood, D.; Chappell, A.; Robson, B.; Webster-Brown, J.; Gilpin, B. J. Identifying avian sources of faecal contamination using sterol analysis. *Environ. Monit. Assess.* **2015**, *187*, 625.
(130) Hargan, K. E.; Stewart, E. M.; Michelutti, N.; Grooms, C.; Kimpe, L. K.; Mallory, M. L.; Smol, J. P.; Blais, J. M. Sterols and stanols as novel tracers of waterbird population dynamics in freshwater ponds. *Proc. R. Soc. London, Ser. B* **2018**, *285*, 20180631.
(131) Prost, K.; Birk, J. J.; Lehndorff, E.; Gerlach, R.; Amelung, W. Steroid biomarkers revisited−improved source identification of faecal remains in archaeological soil material. Bicho, N., ed. *PLoS One.* **2017**, *12*, e0164882.
(132) Matić Bujagić, I.; Grujić, S.; Jauković, Z.; Laušević, M. Sterol ratios as a tool for sewage pollution assessment of river sediments in Serbia. *Environ. Pollut.* **2016**, *213*, 76−83.
(133) Martins, C. C.; Seyffert, B. H.; Braun, J. A. F.; Fillmann, G. Input of organic matter in a large South American Tropical Estuary (Paranagu_a Estuarine System, Brazil) indicated by sedimentary sterols and multivariate statistical approach. *J. Braz. Chem. Soc.* **2011**, *22*, 1585e1594.
(134) Reichwaldt, E. S.; Ho, W. Y.; Zhou, W.; Ghadouani, A. Sterols indicate water quality and wastewater treatment efficiency. *Water Res.* **2017**, *108*, 401−411.
(135) Boisvert, G.; Sonne, C.; Rigét, F. F.; Dietz, R.; Letcher, R. J. Bioaccumulation and biomagnification of perfluoroalkyl acids and precursors in East Greenland polar bears and their ringed seal prey. *Environ. Pollut.* **2019**, *252* (Pt B), 1335−1343.
(136) Shillito, L. M.; Bull, I. D.; Matthews, W.; Almond, M. J.; Williams, J. M.; Evershed, R. P. Biomolecular and micromorphological analysis of suspected faecal deposits at Neolithic Catalhoyuk, Turkey. *J. Archaeol. Sci.* **2011**, *38*, 1869−1877.
(137) Standley, L. J.; Kaplan, L. A.; Smith, D. Molecular tracers of organic matter sources to surface water resources. *Environ. Sci. Technol.* **2000**, *34*, 3124−3130.

https://dx.doi.org/10.1021/acs.est.0c01479
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