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Spatiotemporal occurrence, distribution, and risk of steroid hormones along the coast of Guangdong, China

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Introduction: Steroid hormones are widely present in the environment and pose potential risks to organisms. Previous studies of steroid hormones have predominantly focused on terrestrial environments, with few studies conducted in marine environments.

Methods: In this study, we analyzed the occurrence of 44 steroid hormones in seawater, sediment, and marine organisms collected from the coast of Guangdong, China.

Results: Total concentration of steroid hormones ranged from 0.11 to 30.15 ng/L in seawater, ND to 8.58 ng/g (dw) in sediments, and ND to 80.52 ng/g (ww) in organisms. The highest average concentrations of steroid hormones detected in seawater, sediments, and marine organisms were progestins, estrogens, and glucocorticoids, respectively. Steroid hormone concentrations in seawater were significantly higher during the dry season than the rainy season. The concentrations of steroid hormones in Guanghai Bay, the Pearl River Estuary, Daya Bay, and Zhelin Bay were significantly higher than those in other bays.

Discussion: Negative correlations were observed between steroid hormones and salinity, indicating a potential continental input. Risk assessment results indicated that 17 α -ethinylestradiol in Zhelin Bay posed high risks. Nevertheless, the consumption of seafood does not pose a significant health risk to humans. To the best of our knowledge, this is the first study to concurrently analyze androgens, glucocorticoids, progestins, and estrogens in water, sediment, and organisms from diverse marine environments.

KEYWORDS

steroid hormones, risk assessment, spatial distribution, bioaccumulation, coastal areas

1 Introduction

Steroid hormones, including estrogens, androgens, progestogens, and glucocorticoids, are ubiquitous environmental contaminants with potential adverse effects on humans, animals, and plants (Fent, 2015). They are extensively used in medicine to treat or prevent diseases in both humans and animals and as growth promoters in animal husbandry (Streck, 2009). Although the majority of steroid hormones undergo degradation in wastewater treatment plants (WWTPs), their complete removal may not be achieved. Consequently, effluents from livestock breeding, aquaculture, industry, and domestic sewage sources, which potentially contain substantial quantities of hormones and their metabolites, may be discharged into the natural environment (Zhong et al., 2021). Besides, minor amounts of steroid hormones are introduced into natural aquatic environments via direct discharge (Yazdan et al., 2022).

Many studies have reported that steroid hormones are widely detected in various environmental media worldwide. Steroid hormones have been detected in surface water, groundwater, seawater, animal farm effluent, and WWTPs effluent, with concentrations up to 1440, 390, 831, 1720, and 4650 ng/L, respectively (Zhang et al., 2018; Lu et al., 2020; Ojoghoru et al., 2021; Zhong et al., 2021). Steroid hormones have also been detected in soil, sediment, and feces, with concentrations up to 135, 4800, and 7000 ng/g, respectively (Aris et al., 2014; Adeel et al., 2017; Huanyu et al., 2022). Several studies have also detected steroid hormones in aquatic organisms from both wild and marine aquaculture farms (Ojoghoru et al., 2021).

Environmental steroid hormones may pose a risk to wildlife because they are known for their potent endocrine-disrupting effects, which can have negative ecological and human health impacts (Ismanto et al., 2022). Several studies have demonstrated that steroid hormone exposure can feminize male fish (DeQuattro et al., 2015) alter anal fin development (Frankel et al., 2016), damage reproductive organs and reproductive behavior (Dong et al., 2022), and reduce larval survival and growth in aquatic animals (Islam et al., 2020).

Previous studies on steroid hormones have predominantly focused on terrestrial environments, with comparatively few studies in marine environments, with the exception of the Yellow, Yangtze, and Pearl River Estuaries. Therefore, it is necessary to study the occurrence, source, and risk of steroid hormones in various marine environments.

The coast of Guangdong is a highly developed region with approximately 130 million people residing in Guangdong Province, China. Guangdong's coastline features various functional regions, including areas for marine farming, fishing harbors, tourist destinations, farmlands, and multipurpose zones. The concentration of steroids may vary across these distinct functional zones, making the coastal area of Guangdong an ideal case study for obtaining comprehensive insights into steroid contamination in diverse marine environments.

This study analyzed the levels of steroid hormones in seawater, sediment, and marine organisms from the Guangdong coastline. The ultimate objective was to provide novel and comprehensive insights into the occurrence, risk, and sources of steroid hormones in diverse marine environments. This information is essential for effective management of steroid hormone pollution in coastal regions.

2 Materials and methods

2.1 Chemicals

Forty-four steroid hormones were selected as target compounds, comprising 14 androgens, 5 glucocorticoids, 21 progestins, and 4 estrogens. Basic chemical information for the target compounds is provided in [Supplementary Table S1](#) of the Supporting Information (SI). The internal standards (ISTDs) used included testosterone-16,16,17-d₃, stanozolol-d₃, cortisol-d₂, mifepristone-d₂, melengestrol acetate-d₃, 19-nortestosterone-d₆, progesterone-d₉, estrone-d₄, and 17 β -estradiol-d₄.

HPLC-grade organic solvents were purchased from Merck (Germany). Ultrapure water was obtained using a Milli-Q ultrapure system (Millipore, USA). All stock solutions were prepared in methanol, stored at -18 °C, and kept away from light to prevent degradation until use.

2.2 Sample collection

Samples were collected from ten bays along the coast of Guangdong, including Zhenhai Bay (ZHW), Guanghai Bay (GHW), the Pearl River Estuary (ZJK), Dapeng Bay (DPW), Daya Bay (DYW), Honghai Bay (HHW), Shenquan Bay (SQW), Guangao Bay (GAW), Shantou Port (STG), and Zhelin Bay (ZLW). Detailed information regarding the sampling sites is provided in [Figure 1](#) and [Supplementary Table S2](#). A total of 68 water samples (two replicates each), 68 sediment samples, and 29 marine organism samples were collected in August 2021, during the rainy season. To investigate seasonal variations, additional 68 water samples (two replicates each) were collected in March 2022 during the dry season.

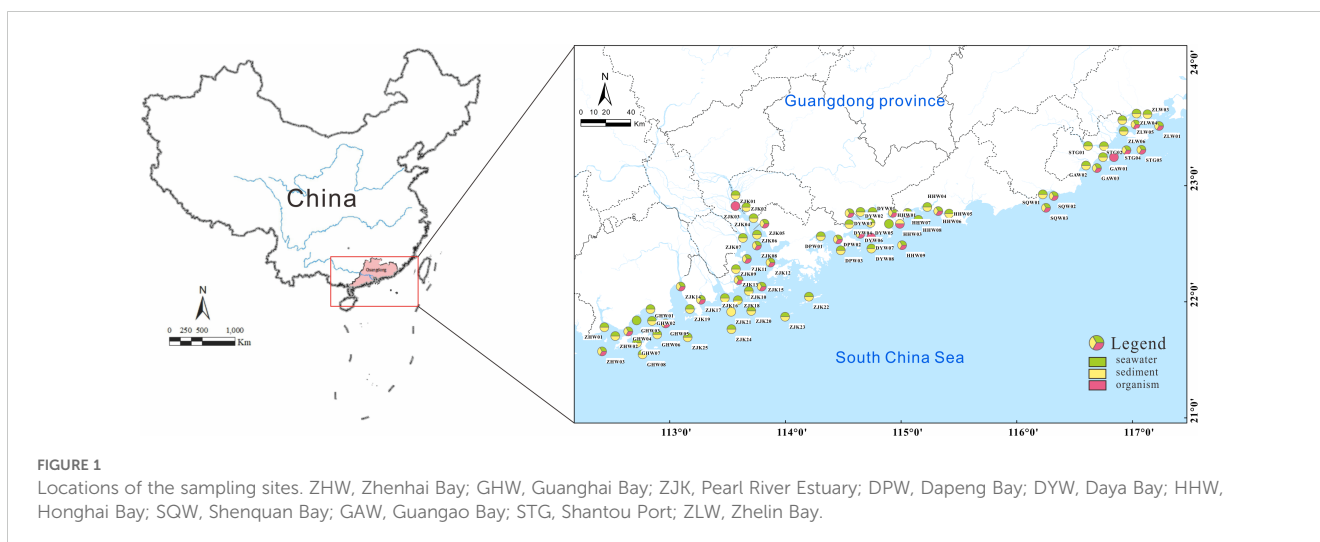
Two liters of water were collected in stainless steel buckets and then filled into two separate 1 L brown glass bottles that had been rinsed with tap water, ultrapure water, and methanol prior to use. Immediately after collection, the pH of the surface water samples was adjusted to 3.0 using 4 M H₂SO₄, and 50 mL of methanol was added to prevent microbial activity. Samples were stored at 4°C and transported to the laboratory.

Surface sediment sample (0-4 cm in depth) was collected using a grab sampler and placed in a 100 mL brown wide-mouth glass bottle.

Marine organism samples were collected by bottom trawl and stored at -18°C after collection. Detailed information regarding the marine organism samples is provided in [Supplementary Table S3](#).

2.3 Sample preparation and extraction

All water samples were filtered through 0.7 μ m GF/C Whatman filters prior to solid-phase extraction (SPE). The samples were spiked with 50 μ L 1 mg/L of isotope-labeled ISTDs. Each water sample was then loaded onto a HLB cartridge (500 mg, 6 mL, Waters) at a flow rate of 5-10 mL/min. The sample washing step was performed using 5 mL methanol, 4 mL ethyl acetate, and 3 mL



dichloromethane. Subsequently, the samples were evaporated to dryness under a stream of nitrogen.

The freeze-dried sediment samples were ground, passed through a 0.3 mm sieve, and fully homogenized. A 2.00 g aliquot of the pretreated sediment was weighed into a 50 mL centrifuge tube. The sample was spiked with 50 μ L 1 mg/L of ISTDs and allowed to stand overnight at 4°C in the dark. Before extraction, 5 mL of 0.1 M EDTA–McIlvaine buffer solution was added to the samples, followed by vortexing. Subsequently, 10 mL formic acid/acetonitrile (1/50, v/v) and a ceramic homogenizer were added to the mixture and vortexed thoroughly for 30 s. Subsequently, the QuEChERS extraction salts (4 g Na_2SO_4 and 1 g NaCl) were added to the tube, shaken immediately, and centrifuged at 6000 rpm for 5 min. The supernatant was then transferred to a QuEChERS dSPE tube, vortexed for 1 min, and centrifuged at 5000 rpm for 5 min. The supernatant was then transferred to a 10 mL glass test tube and dried under a stream of nitrogen.

Before pretreatment, the weight and length of each marine organism were recorded. A 2 g (wet weight) portion of homogenized muscle tissue was accurately weighed into a 50 mL centrifuge tube and mixed with 50 μ L of 1 mg/L ISTDs. Prior to extraction, 5 mL of 0.1 M EDTA–McIlvaine buffer solution and two ceramic homogenizers were added to the samples and vortexed for 3 min. Then, 100 μ L digestive enzyme was added to the samples, which were subsequently kept at 37°C in the dark overnight. Then, 8 mL of formic acid/acetonitrile (1/50, v/v) was added to the centrifuge tubes, shaken immediately, and centrifuged at 6000 rpm for 5 min. The supernatant was then transferred to Captiva EMR-Lipid cartridges for defatting and purification. The filtrate was then collected in a 10 mL glass test tube and dried under a stream of nitrogen.

Finally, each sample was redissolved in 500 μ L of methanol, passed through a 0.22 μ m filter, and transferred to a brown glass sample vial for analysis.

2.4 Instrumental methods

High-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS, Agilent 1260 Infinity-

AB SCIEX 4000 Qtrap) was used to analyze androgens, progestogens, and glucocorticoids. The chromatographic column was an Agilent Poroshell 120 EC-C18 column (2.1 mm \times 100 mm, 2.7 μ m). The column oven temperature was maintained at 25°C in the positive ion mode (ESI +), and the injection volume was 5 μ L. The mobile phase consisted of (A) methanol and (B) ultrapure aqueous solution containing 0.05% formic acid (v/v) at a flow rate of 0.25 mL/min. The gradient program involved a stepwise increase in the concentration of phase A over 14 min as follows: 60% A at 0 min, held for 5 min, 100% A from 6 to 8 min, and 60% A from 8.5 to 14 min. Nitrogen was used as collision gas.

Ultra-performance liquid chromatography coupled with a Xevo TQ-S triple quadrupole mass spectrometer (Waters Co., Milford, MA, USA) was used to analyze estrogens. In negative ion mode (ESI-), chromatographic separation was performed on an ACQUITY UPLC BEH C18 column (2.1 \times 50 mm, 1.7 μ m). The column temperature was maintained at 40°C and the injection volume was 5 μ L. The mobile phase consisted of 0.05% (v/v) ammonium acetate in Milli-Q water (A) and methanol (B), at a flow rate of 0.4 mL/min. The total analysis time for each sample was 5.2 mins. The gradient elution program was as follows: 40% B at 0 min, increased to 98% B at 3 min, held at 98% B from 3.5 to 3.8 min, returned to 40% B from 3.8 to 4.0 min, and holding for 0.7 min.

2.5 Quality assurance and quality control

All experimental data was subjected to strict quality assurance and quality control processes. Two field blanks and two laboratory blanks were prepared for each batch to assess potential contamination during the experimental procedure. None of the target compounds were detected in the blank samples. Seven standard solution concentrations (0.5, 2, 8, 30, 100, 200, and 500 μ g/L) were used to calculate calibration curves ($R^2 > 0.995$). The limit of detection (LOD) and limit of quantitation (LOQ) were defined as the sample concentrations at signal-to-noise ratios (S/N) of three and ten times, respectively. The recoveries of the target steroid hormones ranged from 63% to 117% in water samples, 56%

to 122% in sediment samples, and 64% to 111% in organism samples (Supplementary Table S5). The relative standard deviations (RSDs) of field parallel samples were all below 15%.

2.6 Data analysis

The bioconcentration factor (BCF, L/kg) was used to describe the uptake and enrichment of substances from the surrounding water by aquatic organisms.

$$BCF = \frac{C_b}{C_w} \times 1000$$

where C_b (ng/g ww) is the concentration of steroid hormones in the marine organism samples and C_w (ng/L) is the concentration of steroid hormones in the seawater samples.

The ecological risks associated with the levels of steroid hormones in aquatic environments were assessed using risk quotients (RQ):

$$RQ = \frac{MEC_{water}}{PNEC_{water}}$$

where MEC is the measured environmental concentration (ng/L) and PNEC is the predicted no-effect concentration (ng/L). The latter was calculated by dividing the quotient of the median effective concentration (EC50) or lethal concentration (LC50) by an assessment factor (AF) for acute toxicity associated with water. Estimated daily intake (EDI) and hazard quotient (HQ) were calculated using the following formulae:

$$EDI = \frac{C_s \times M_s}{W_p}$$

$$HQ = \frac{EDI}{RfD/ADI}$$

where C_s (ng/g ww) represents the concentration of steroid hormones in the organism samples; M_s (g/(d-person)) is the consumption rate of seafood per person per day; W_p (kg bw/person) represents the average body weight of each age group. RfD is the reference dose for daily intake. ADI is the average daily intake. EDI is the ratio of the estimated daily intake.

The Mann-Whitney test and Pearson's correlation coefficients were conducted using SPSS (IBM SPSS Statistics) 22.0. Redundancy analysis was performed using Canoco5.0. CorelDRAW 2024, ArcGIS 10.8, and Origin 2021 were used to visualize the results.

3 Results

3.1 Occurrence of steroid hormones in seawater

Among the 44 steroid hormones analyzed, 16 were detected in seawater, with concentrations ranging from 0.01 ng/L (medroxyprogesterone) to 28.32 ng/L (5 α -dihydroprogesterone)

(Figure 2). The average concentrations of androgens, glucocorticoids, progestins and estrogens in seawater were 0.03, 0.01, 0.09, and 0.04 ng/L, respectively (Table 1). The concentrations of androgens, glucocorticoids, and progestins in seawater in this study are higher than those in the Pearl River Estuary (Chen et al., 2022; Xu et al., 2024), Beihai Bay of China (Ren et al., 2022), and the coast of Spain (Méndez-Catalán et al., 2024), but lower than those in the South China Sea (Liu et al., 2015). The concentrations of estrogens in seawater in this study are comparable to those observed in Halifax Harbour in Canada (Robinson et al., 2009), the coast of South Florida (Singh et al., 2009), and Chesapeake Bay in the USA (He et al., 2019), but significantly lower than those recorded in the central Venice Lagoon in Italy (Pojana et al., 2007) and Marmara Sea in Turkey (Korkmaz et al., 2022). Detailed comparisons of contaminant levels in various media from this study with those from previous studies are shown in Supplementary Table S4–S6.

During the rainy and dry seasons, 11 and 14 steroid hormones were detected, respectively, with average concentrations of 0.02 and 0.09 ng/L, respectively. The detection ranges were 0.01 to 8.34 ng/L and 0.02 to 56.6 ng/L, respectively. 5 α -dihydroprogesterone showed the highest concentrations in both seasons, although the concentration in the dry season was 6.8 times than that in the rainy season (Figure 3).

The concentrations of some other steroid hormones in seawater during the dry season were also significantly higher than those during the rainy season, including 4-androstene-3,17-dione, androsterone, 5 α -dihydroprogesterone, and 17 α -hydroxyprogesterone acetate. The detection rate of 4-androstene-3,17-dione was 95.0% in the dry season, but only 19.1% in the rainy season. This discrepancy is likely due to the higher precipitation and the resulting dilution effects during the rainy season. The average precipitation in Guangdong is 264 mm during the rainy season and 128 mm during the dry season (<http://slt.gd.gov.cn/>).

3.2 Occurrence of steroid hormones in sediment

Among the 44 steroid hormones analyzed, 12 were detected in the sediment, among which progesterone and 4-androstene-3,17-dione showed the highest detection rates of approximately 73.5% and 51.5%, respectively. Concentration of each steroid hormone ranged from 0.03 ng/g (4-androstene-3,17-dione) to 4.56 ng/g (5 α -dihydroprogesterone) (dw) (Figure 2). The average concentrations of androgens, progestins and estrogens in sediment were 0.01, 0.04, and 0.05 ng/g, respectively. Glucocorticoids were not detected in the present study. The mean concentrations followed the order of estrogens > progestins > androgens > glucocorticoids. These results are similar to those of a previous study that explored the fishing port environment along the southeastern coast of China (Liu et al., 2022). This pattern may be attributed to the fact that progestogens are expected to absorb more readily onto sediments than estrogens, androgens, and glucocorticoids, due to their significantly higher log Kow values ranging from 3.1 to 5.4 (Fent, 2015). The androgen levels are comparable to those reported in the

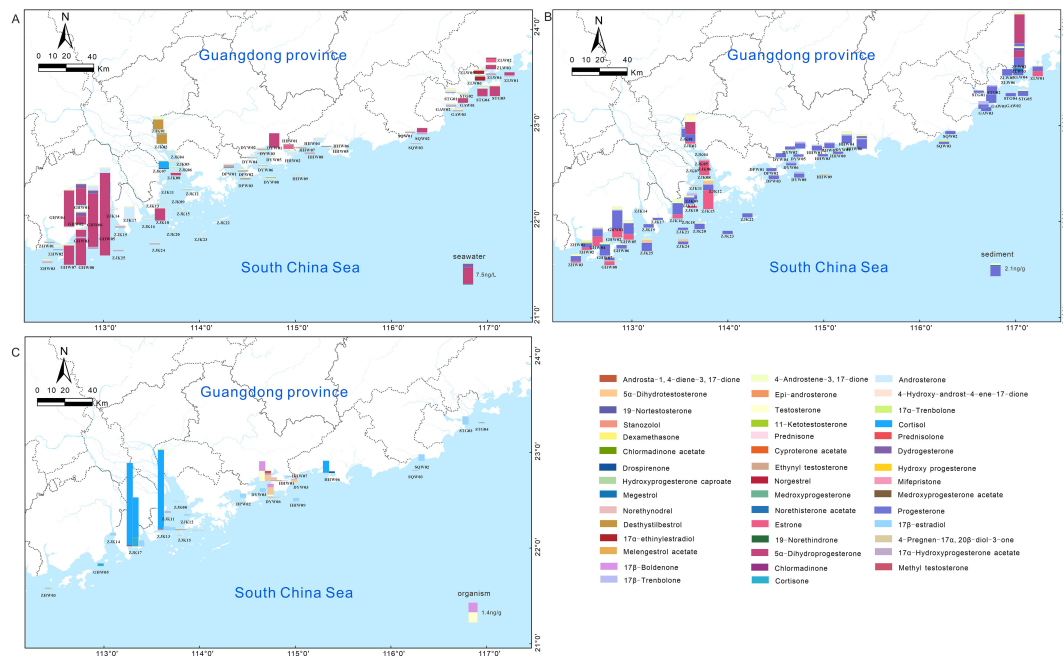


FIGURE 2 Spatial distribution of steroid hormones in seawater (A), sediment (B), and marine organisms (C) along the coast of Guangdong. The mean concentration of each steroid hormone during the dry and rainy seasons was used to determine its spatial distribution in seawater. Different columns at the same sampling site were used to represent the concentrations of different types of marine organisms. ZHW, Zhenhai Bay; GHW, Guanghai Bay; ZJK, Pearl River Estuary; DPW, Dapeng Bay; DYW, Daya Bay; HHW, Honghai Bay; SQW, Shenquan Bay; GAW, Guangao Bay; STG, Shantou Port; ZLW, Zhelin Bay.

TABLE 1 Concentration of steroid hormones along the coast of Guangdong.

Analytes	Seawater(ng/L)				Sediment (ng/g, dw ^c)				Organism (ng/g, ww ^d)			
	range	mean	median	DR ^a (%)	range	mean	median	DR (%)	range	mean	median	DR (%)
Steroid hormones	0.11~30.15	2.54	0.89	100	ND ^b ~8.58	1.19	0.7	82	ND~80.52	5.34	0.44	64
Androgens	ND~4.15	0.44	0.27	94	ND~1.27	0.2	0.1	62	ND~1.27	0.2	0.1	62
Glucocorticoids	ND~2.22	0.06	ND	13	ND	ND	ND	0	ND~79.33	3.57	ND	10
Progestins	ND~28.32	1.87	0.35	82	ND~7.19	0.79	0.6	76	ND~1.91	0.15	ND	26
Estrogens	ND~3.25	0.17	ND	9	ND~2.98	0.2	ND	19	ND~7.82	0.76	ND	26

^aDR, detection rate (%).

^bND, not detected.

^cdw, dry weight.

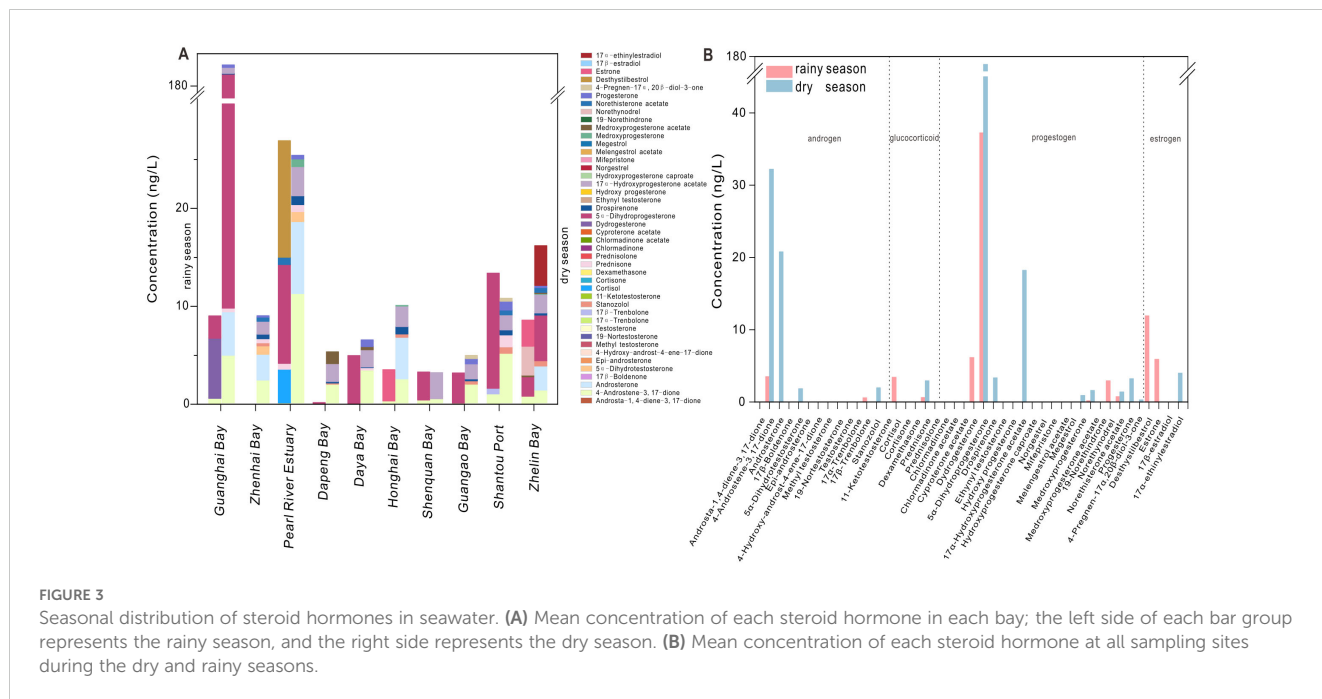
^dww, wet weight.

Pearl River Estuary (Chen et al., 2022; Liu et al., 2023), Beihai Bay in China (Ren et al., 2022), and Hailing Bay in the South China Sea (Liu et al., 2015), but lower than those in the Marmara Sea in Turkey (Aysel and Yurdun, 2023). The levels of progestogens are higher than those reported in Pulau Kukup Johor, Malaysia (Ismail et al., 2020) but are two orders of magnitude lower than the levels in the Marmara Sea in Turkey (Aysel and Yurdun, 2023). The concentrations of estrogens are comparable to those in the Halifax harbor in Canada (Robinson et al., 2009) and Xiamen Bay in China (Zhang et al., 2009), but are lower than those in the Santos and São Vicente estuaries in Brazil (Puscetdu et al., 2019),

Kuwait's coastal areas (Saeed et al., 2017), and the Venice lagoon in Italy (Pojana et al., 2007). Overall, steroid hormone concentrations in sediments from the coast of Guangdong are relatively low.

3.3 Occurrence of steroid hormones in marine organisms

Among the 44 hormones analyzed, 27 were detected in marine organisms, with concentrations ranging from 0.01 ng/g (4-pregnene-17 α ,20 β -diol-3-one) to 79.33 ng/g (cortisol) (ww)



(Figure 2). The average concentrations of androgens, glucocorticoids, progestins and estrogens in marine organisms were 0.06, 0.71, 0.01, and 0.19 ng/g, respectively. The levels of androgens are slightly higher than those reported in the Pearl River Delta in China (Chen et al., 2022) and the Gulf of Gdańsk in Poland (Zabrzńska et al., 2015). The levels of glucocorticoids are significantly higher than those in the Klang River Estuary in Malaysia (Omar et al., 2019), primarily because of the high concentrations of cortisol. A previous study showed that cortisol levels in fish increase when they are exposed to environmental contaminants (Rohoczy et al., 2021), which may partly explain the high level of cortisol in the organisms in this study. The concentrations of progestogens are relatively high compared with those in the Po Delta in Italy and the Ebro Delta in Spain (Álvarez-Muñoz et al., 2015), but are lower than the concentrations measured in the Pearl River Delta, China (Chen et al., 2022). The concentrations of estrogens are higher than those reported in Malaysia's Klang River estuary (Omar et al., 2019) and Portugal's Tagus Estuary (Álvarez-Muñoz et al., 2015), but lower than those observed in Chesapeake Bay, USA (Álvarez-Muñoz et al., 2015), mariculture areas in the Pearl River Delta in China (Chen et al., 2022), and the Venice lagoon in Italy (Pojana et al., 2007).

The bioconcentration factors (BCFs) for 5 α -dihydroprogesterone, 17 α -hydroxyprogesterone acetate, and estrone were lower than 2000 L/kg (Figure 4), suggesting their low bioaccumulation potential in marine organisms. Additionally, desthystilbestrol is potentially bioaccumulative in marine organisms. The BCFs for 5 α -dihydrotestosterone, cortisol, drospirenone, medroxyprogesterone, medroxyprogesterone acetate, and 4-pregnen-17 α ,20 β -diol-3-one were higher than 5000 L/kg in most marine organisms, indicating that these compounds exhibit strong bioaccumulative potential. Among all species, *Caranx formosanus*, *Sardinella melanura* and *Clupanodon thrissa* exhibited stronger accumulation of steroid hormones. The bioaccumulation of steroid

hormones in marine organisms can be attributed to various factors, including living habit, nutrient level, lipid content, metabolic capacity, sex, and life stage of the species, and lipophilicity and environmental concentration of steroid hormones (Ho et al., 2023; Liao et al., 2024).

4 Discussion

4.1 Risk assessment of steroid hormones

The ecological risks posed by steroid hormones in the seawater along the coast of Guangdong are shown in Figure 5. 4-Androstene-3,17-dione, 5 α -dihydrotestosterone, 4-hydroxyandrost-4-ene-17-dione, 17 α -trenbolone, 17 β -trenbolone, testosterone, dexamethasone, prednisolone, melengestrol acetate, 19-norethindrone, and 17 β -estradiol posed no risk to aquatic life (RQ < 0.01). Progesterone posed low or insignificant risks (0.01 < RQ < 0.1). Cortisol presented medium risks (0.1 < RQ < 1). However, 17 α -ethinylestradiol in Zhelin Bay indicated high risk (RQ > 1). Considering the detected concentrations, detection rates, and risk assessment results, progestins in the marine environment deserves greater attention in the future.

The estimated daily intakes (EDIs) of steroid hormones in different populations are summarized in Supplementary Table S8. For male in the age groups of children, youths, and adults, the EDIs through fish consumption are 0.04 to 61.08, 0.06 to 79.51, and 0.02 to 30.93 ng/ (kg bw-d), respectively. For females in the same age groups, the EDIs are 0.03 to 38.37, 0.04 to 52.41, and 0.02 to 33.62 ng/ (kg bw-d), respectively. The acceptable daily intake (ADI) or reference dose (RfD) of only nine contaminants are available and used to calculate the hazard quotient (HQ) values (Supplementary Table S9). All HQ values are less than 1, indicating that there is no risk to human health from consuming these aquatic products from the coast of Guangdong.

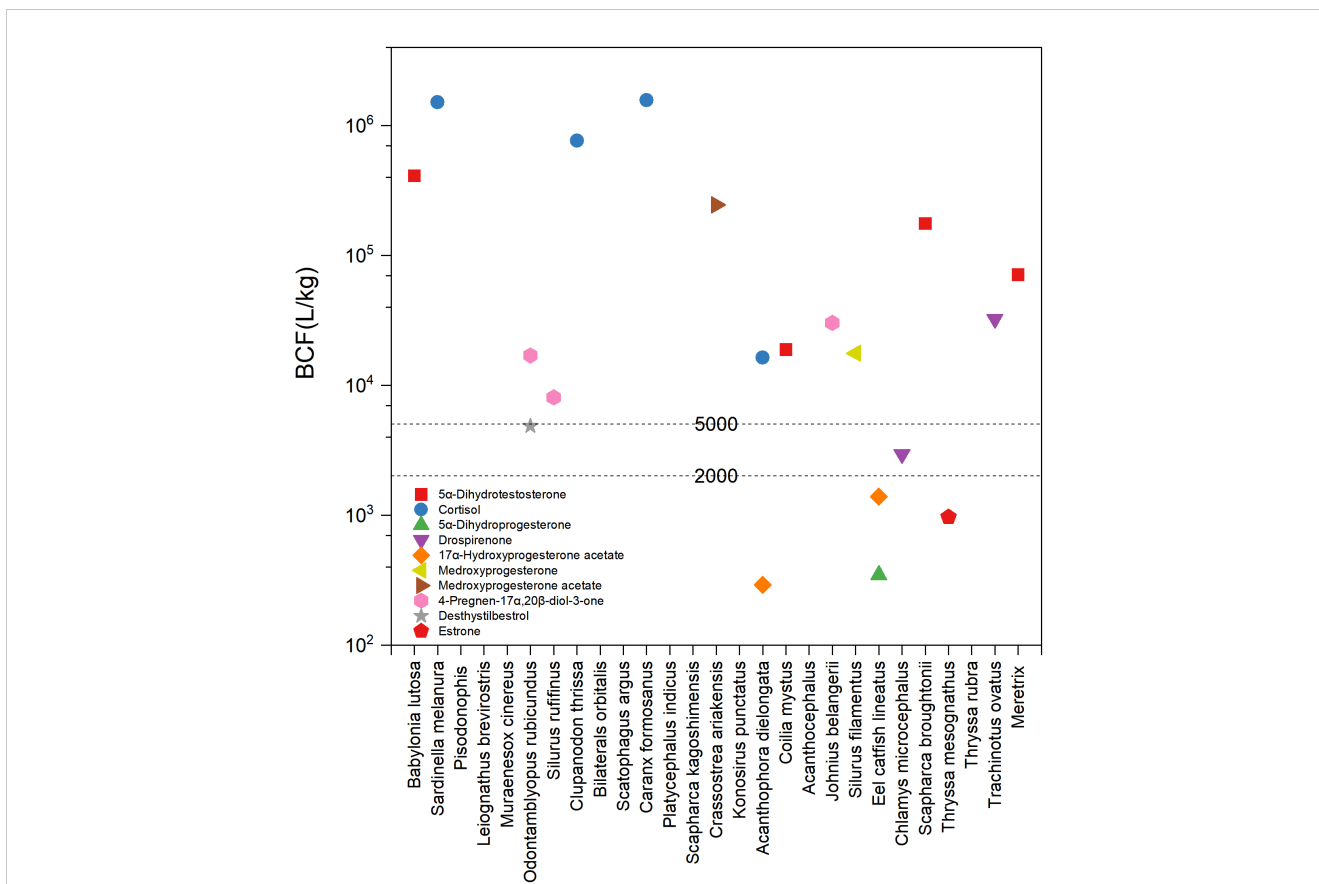


FIGURE 4 Bioconcentration factor of steroid hormones in marine organisms along the coast of Guangdong. BCFs above 5000 mean significant bioaccumulation of the compound within the organism. BCFs between 2000 and 5000 suggest potential bioaccumulation. BCFs below 2000 indicate negligible bioaccumulation. The BCFs were calculated only for substances detected in both seawater and organisms.

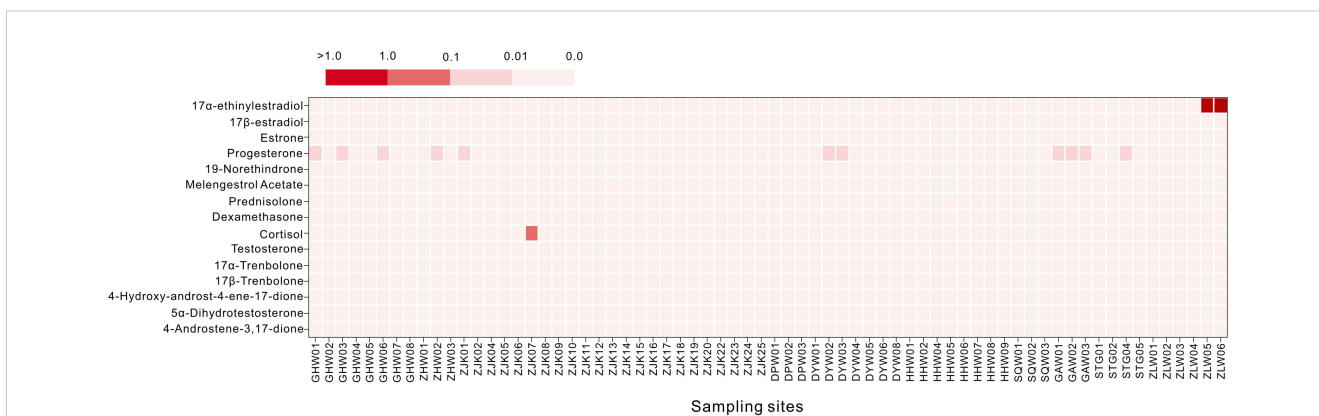


FIGURE 5 Ecological risks of steroid hormones in seawater along the coast of Guangdong. Darker colors indicate higher risk. The ecological risk assessment was conducted only for substances with available toxicological data. The PNECs used are presented in [Supplementary Table S7](#).

4.2 Spatial distribution of steroid hormones

As shown in [Figure 2](#), the concentrations of steroid hormones in the seawater of Guanghai Bay were far greater than those in the other bays, followed by those in the Pearl River Estuary. In the sediment, the

highest concentration of steroid hormones was detected in Zhelin Bay, followed by Guanghai Bay and the Pearl River Estuary. Among the marine organisms, the highest concentration of steroid hormones was detected in the Pearl River Estuary, followed by Daya Bay. In addition, an ecological risk assessment showed that 17 α -ethinylestradiol in

Zhelin Bay indicated high risk ($RQ > 1$). In summary, the concentrations of steroid hormones in Guanghai Bay, the Pearl River Estuary, Daya Bay, and Zhelin Bay were significantly higher than those in other bays.

The most polluted sites in the Pearl River Estuary were ZJK01 and ZJK02, which may be attributed to riverine input from the Pearl River. Data suggest that the Pearl River drains approximately 320 billion m³ per year of surface runoff into the Pearl River Estuary (Zhao et al., 2019). Steroid hormones have been detected in surface waters, sediment, and suspended particulate matter from the Pearl River system up to 78.7 ng/L, 38.0 ng/g, and 33.1 ng/g, respectively (Zhao et al., 2011; Gong et al., 2023). Furthermore, higher concentrations of steroid hormones were observed at ZJK13, ZJK15, ZJK17, and ZJK18, which are located near WWTPs and densely populated areas of Zhuhai. This observation is in agreement with the results of previous study (Xu et al., 2024).

Guanghai Bay experiences substantial discharge pressure from the Pearl River (Gu et al., 2024). Seawater samples collected in Guanghai Bay at sites GHW05 and GHW06 exhibited higher steroid hormone concentrations, which may be due to their location at the mouth of the bay where the Pearl River Estuary tidal current enters Guanghai Bay. The high concentration detected in seawater is due to wave action and increased water flow, which causes pollutants adsorbed in sediments to be released back into seawater (Wang et al., 2022; Gu et al., 2024).

Sampling site DYW01 is located near aquaculture and agricultural areas. Studies have shown that the feces of livestock and poultry are commonly used for agricultural fertilization (Lin et al., 2024), and artificial addition of steroid hormones also occurs in aquaculture (Liu et al., 2015, 2024). Thus, agricultural runoff and aquaculture breeding wastes are important contributors to the elevated concentrations of steroid hormones in Daya Bay (Lin et al., 2024).

Zhelin Bay is the largest marine cage culture base in China, and has become one of the most densely populated areas for marine aquaculture (Xia et al., 2017), which may account for the high concentrations of steroid hormones (Li et al., 2021). The occurrence of steroid hormones in sediments is associated with direct dosing of steroid hormones and feeding with medicated feed (Xie et al., 2019). Studies have shown that the concentration of steroid hormones detected in sediments of marine aquaculture areas is significantly higher than other areas (Lu et al., 2020; Chen et al., 2022).

4.3 Correlations between steroid hormones and environmental parameters

The influence of human and environmental variables on steroid distribution was analyzed using redundancy analysis (RDA) (Supplementary Table S10). The results indicated that domestic water consumption of urban residents was the most significant anthropogenic factor affecting steroid levels.

In order to investigate the potential sources of steroid hormones identified in the coastal areas of Guangdong, the correlations between steroid hormone concentrations and environmental parameters were analyzed (Figure 6, Supplementary Table S11-S12). Pearson

correlation analysis demonstrated that many steroid hormones were significantly correlated with aqueous physicochemical parameters, such as salinity, chemical oxygen demand (COD), dissolved oxygen (DO), total nitrogen (TN), total phosphorus (TP), inorganic nitrogen (IN), suspended solid (SS), and chlorophyll a (Chl a) (Figure 6A). A negative correlation was observed between 4-androstene-3,17-dione, cortisol, prednisone, norethisterone acetate, diethylstilbestrol, and medroxyprogesterone concentrations and salinity ($p < 0.05$), indicating a potential continental input. Estrone was positively correlated with Chl a, which is consistent with the findings of a previous study on the Pearl River Delta (Deich et al., 2021). These correlations suggest common sources of steroid hormones and nitrogen and phosphorus, such as wastewater discharge, agriculture, and aquaculture industry (Yi et al., 2019; Li et al., 2021). Dydrogesterone exhibited a positive and statistically significant correlation with 5 α -dihydroprogesterone and progesterone, indicating a potential common origin for these compounds. Dydrogesterone, 5 α -dihydroprogesterone, and progesterone have been detected in swine wastewater, swine feces, and effluents from WWTPs (Liu et al., 2020). 5 α -dihydroprogesterone and progesterone have been detected in feed and sediment samples from freshwater aquaculture ponds (Liu et al., 2024). Therefore, steroid hormones in seawater may originate from aquaculture waste, wastewater discharge, livestock production, and agricultural surface runoff. These results suggest a diversity of steroid hormone sources along the coast of Guangdong.

Pearson correlation analysis showed significant correlations between many steroid hormones and particle size, salinity, oil pollutants, heavy metals, and total organic carbon (TOC) (Figure 6B). Salinity was negatively correlated with testosterone in the sediment in the present study, suggesting that terrestrial input may be an important source. TOC was positively correlated with testosterone in the sediment, as a higher organic matter content has been reported to have a higher sorption affinity (Qi and Zhang, 2016). Particle size exhibited significant positive relationships with 5 α -dihydroprogesterone, progesterone, and 17 β -estradiol, which agrees with a previous study (Sangster et al., 2015), and may be due to the combined effects of cation exchange capacity, percentage of organic carbon, and surface area. Oil pollutants positively correlated with 19-nortestosterone, testosterone, and progesterone. Progesterone positively correlated with 4-androstene-3,17-dione, 19-nortestosterone, 5 α -dihydroprogesterone, estrone, and 17 β -estradiol, suggesting common sources. In addition, Pb was positively correlated with testosterone in the sediment.

5 Conclusion

This study measured the levels of 44 steroid hormones in seawater, sediments, and organisms from the coast of Guangdong. Total hormone concentrations ranged from 0.11 to 30.15 ng/L in seawater, ND to 8.58 ng/g (dw) in sediments, and ND to 80.52 ng/g (ww) in organisms. Compared to other coastal areas, the coastal region of Guangdong showed lower concentrations of steroid hormones in seawater and sediment, while higher levels in marine organisms. The concentrations of steroid hormones in Guanghai Bay, the Pearl River

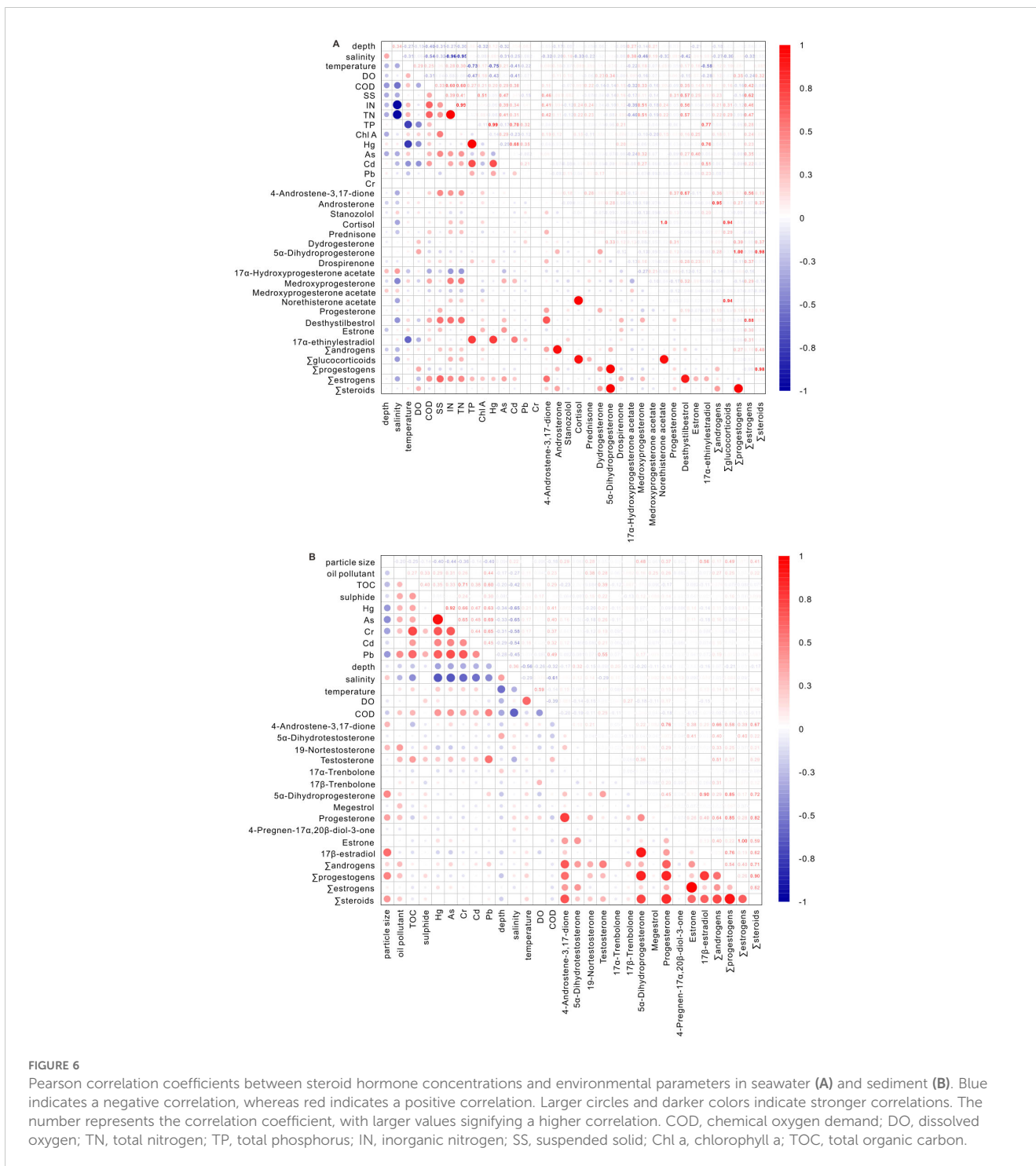


FIGURE 6

Pearson correlation coefficients between steroid hormone concentrations and environmental parameters in seawater (A) and sediment (B). Blue indicates a negative correlation, whereas red indicates a positive correlation. Larger circles and darker colors indicate stronger correlations. The number represents the correlation coefficient, with larger values signifying a higher correlation. COD, chemical oxygen demand; DO, dissolved oxygen; TN, total nitrogen; TP, total phosphorus; IN, inorganic nitrogen; SS, suspended solid; Chl a, chlorophyll a; TOC, total organic carbon.

Estuary, Daya Bay, and Zhelin Bay were significantly higher than those in other bays. Steroid hormone concentrations in seawater were higher during the dry season than the rainy season. Ecological risk assessment results indicated that 17 α -ethinylestradiol in Zhelin Bay posed high risks. However, no health risks were associated with the consumption of seafood from the coast of the Guangdong. Overall, our study revealed the occurrence of steroid hormones in the marine

environment, which is essential for management of steroid hormone pollution.

However, the ecological risk assessment results in this study cannot fully reflect the risk posed by steroid hormones on Guangdong's coastline, as toxicological data for some compounds are absent. Further research is warranted to evaluate the risks associated with steroid hormones in nearshore marine environments.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

XZ: Data curation, Investigation, Visualization, Writing – original draft. YLJ: Investigation, Writing – original draft, Methodology. HD: Investigation, Writing – original draft. YL: Investigation, Writing – original draft. SL: Supervision, Writing – review & editing. YXJ: Supervision, Writing – review & editing, Funding acquisition, Project administration. KS: Supervision, Writing – review & editing.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2025.1546186/full#supplementary-material>

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