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Steroids in marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioconcentration, and human dietary exposure

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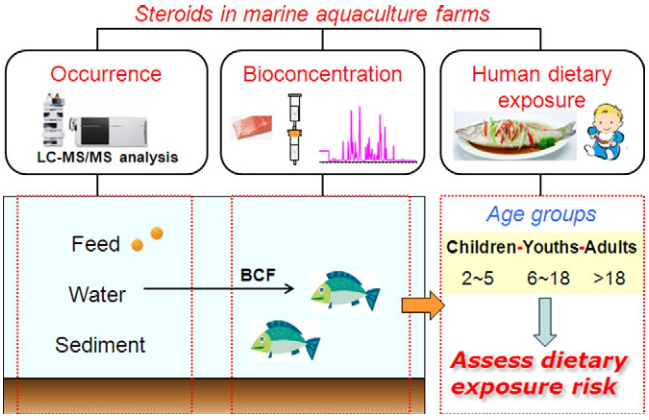
HIGHLIGHTS

- The occurrence of 24 steroids in typical aquaculture farms was investigated.
- The illegal use of synthetic steroids in the aquaculture feed was detected.
- The selected aquaculture farms have been polluted by various steroids.
- Bioconcentration of steroids occurred in the biota, especially for mollusks.
- Individual steroid might not post significant risk to human from dietary exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

The occurrence, bioconcentration, and human dietary exposure via seafood consumption of 24 steroids were investigated by rapid resolution liquid chromatography–tandem mass spectrometry (RRLC–MS/MS) in six typical marine aquaculture farms surrounding Hailing Island, South China. Ten, 9, 10, 15 of 24 steroids were detected at concentrations ranging from <0.1 (testosterone) to 40 ng/L (prednisolone), from 0.1 (4-androstene-3,17-dione) to 2.4 ng/g (progesterone), from 0.3 ng/g (testosterone) to 21.4 ng/g (epi-androsterone), and from <0.1 (testosterone) to 560 ng/g (cortisol) (wet weight) in the water, sediment, feed and biota samples, respectively. Synthetic steroids (androsta-1,4-diene-3,17-dione, 17α-boldenone, 17β-boldenone, 17β-trenbolone, prednisolone, norgestrel) were detected in the feed samples, clearly demonstrating the illegal use of steroids in the feed. The field bioconcentration factors (BCFs) of steroids calculated in different aquatic organisms ranged from 93.8 to 4000. The estimated daily intakes (EDIs) of androgens, glucocorticoids, and progestagens via consumption of seafood (i.e., shrimps, crabs, mollusks, and fish) for different age groups were in the range of 33.4–134, 2061–8566, and 40.4–155 ng/d for children (2–5 years), youth (6–18 years), and adults (>18 years), respectively. Even though no significant risk from dietary exposure arises from individual steroid, elevated risk to humans can

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result from the occurrence of multiple steroids in the seafood raised in the aquaculture farms, especially for the sensitive populations, such as pregnant women and children.

1. Introduction

Steroids in the aquatic environment have become a public concern in recent years (Ying et al., 2002) because of their potential endocrine-disrupting effects, such as decreased fertility, feminization, and hermaphroditism on aquatic organisms, even at very low concentrations of ~1 ng/L (Fick et al., 2010; Mills and Chichester, 2005; Orlando et al., 2004; Zeilinger et al., 2009). Natural and synthetic steroids have been widely detected in various environmental matrices, including surface water, groundwater, soil and sediments (Bradley et al., 2009; Casey et al., 2004; Chang et al., 2009; Liu et al., 2012a). Natural steroids originate mainly from the excretion (feces and urine) of human, livestock and aquatic organisms (Hanselman et al., 2003; Kolodziej et al., 2004; Liu et al., 2012b, 2012c). Some natural and synthetic steroids have also been used by humans for many purposes, such as contraception and therapy, and in livestock and aquaculture production to prevent and treat disease, promote growth, improve productivity, produce meat that is more appealing to consumers, and develop single-sex populations of fish in aquaculture (Beardmore et al., 2001).

Most studies have focused on the fate and occurrence of steroids in municipal wastewater treatment plants (WWTPs) and the receiving environment (Chang et al., 2011; Fan et al., 2011; Liu et al., 2012b). Intensive animal agriculture operations including livestock and aquaculture also represent important sources of steroids in the environment (Liu et al., 2012a, 2012c; Scott and Sorensen, 1994; Sorensen et al., 2005). In our previous study (Liu et al., 2012a), the total masses of steroids released from livestock farms in China were found on the same order magnitude as or exceeding the amounts by human excretion. Only few studies have investigated steroids in aquaculture, focusing mainly on the accumulation of individual steroid in fish under laboratory conditions (Fick et al., 2010; Nallani et al., 2012; Steele et al., 2013) or the occurrence and bioconcentration of several estrogens in the tissue of fish exposed to steroids in polluted waters (Liu et al., 2011a, 2011b, 2012a, 2012b, 2012c, 2012d).

China is the largest aquatic product farming country in the world. The output of aquatic products was 43.0 million tons in 2012, which accounted for 65.7% of the total output in the world (Seafood Information of China, 2012). The output of aquatic products via marine culture in China was 23.0 million tons in 2012, which accounted for 80% of the total output via marine culture in the world (Seafood Information of China, 2012). Most marine aquaculture farms are on a small scale and do not have wastewater treatment facilities. To the best of our knowledge, no previous studies have reported the occurrence, bioconcentration, and human dietary exposure of androgens, glucocorticoids and progestagens in the aquaculture farms.

The objectives of the present study were to (i) investigate the occurrence and fate of androgens, glucocorticoids and progestagens in different environmental matrix (surface water and sediment) in six typical marine aquaculture farms in South China, (ii) investigate the use patterns of steroids in different aquaculture farms, (iii) calculate the bioconcentration factors (BCFs) of steroids for different biota, (iv) and estimate human dietary exposure of steroids via consumption of seafood raised in the aquaculture farms.

2. Materials and methods

2.1. Chemicals and sample collection

High purity standards of 24 natural and synthetic steroids, including 14 androgens, 5 glucocorticoids and 5 progestagens, 4 internal standards, namely, testosterone-16, 16, 17-d3 (T-d3), stanozolol-d3 (S-d3), progesterone-d9 (P-d9), cortisol-d2 (CRL-d2), were purchased from different chemical suppliers (Table S1). The detailed information on chemicals and materials is summarized in the Supplementary Information.

Hailing Island is the most important marine culture zone in Yangjiang, Guangdong Province, and also a well-known marine culture base along the southern coast of China. Pond, cage, and oyster pile cultures are the most common marine aquaculture models in this area. Pond culture is mainly used for shrimps and some aquatic animals at their young stage. Cage culture is the most common coastal aquaculture model to grow aquatic animals from young to adult stage. Oyster pile culture is a famous form of fish farming surrounding Hailing Island. In the present study, six typical marine aquaculture farms including three pond culture farms (A1, A2, and A3), two cage culture farms (A4 and A5), and one oyster pile culture farm (A6) surrounding Hailing Island were selected as the study sites, as shown in Fig. 1. All samples (seafood, water, sediment and feed) were collected from the six farms in September 2013, with the relevant sampling information given in Tables S2 and S3. Seafood samples, including fish (LR, *Lutjanus russelli*, LE, *Lutjanus erythopterus*, and TO, *Trachinotus ovatus*), mollusks (APL, *Atrina pectinate Linnaeus*, ML, *Meretrix lusoria*, TK, *Trisidos kiyoni*, and CRG, *Crassostrea rivularis Gould*), crabs (CP, *Calappa philargius*), and shrimps (FP, *Fenneropenaeus penicillatus*), which represent the most common marine products in South China, were sampled in these farms. The collected seafood samples were frozen and transferred to the laboratory. After measurement of weight and length of each sample, muscle was dissected. About 2 g of muscle samples (wet weight) was prepared for each extraction. For water samples, two parallel samples were collected at each sampling site. About 5% (v/v) of methanol was added to each water sample (2.5 L) and the sample pH value was adjusted to 3 using 4 M H_2SO_4 in the field. Waterproof membrane was used in pond culture farms A1 and A2 to prevent the material exchange between pond water and sediment, thus no sediment samples could be obtained. Sediment samples were collected from the rest of the sampling sites. Surface sediments (0–30 cm) were collected using Peterson grab sampler and one gram of sodium azide was added to each liter of solid sample to suppress microbial activity. All samples were transported back in coolers to laboratory. The collected water samples were then processed within 48 h. The sediment samples were freeze dried, ground and homogenized. Dried sediment samples (0.5 g) were prepared for each extraction.

2.2. Sample extraction and instrumental analysis

Sample extraction and instrumental analysis were carried out following our previously established analytical method (Liu et al., 2011b), with the detailed method information given in the Supplementary information (Tables S4). Briefly, the biota samples were extracted with methanol/water—0.1 M acetic acid (50:50, v/v) by ultrasonication. The strong anion exchange/primary–secondary amine (SAX/PSA) cartridges (6 mL, 500 mg) and HLB cartridges (6 mL, 200 mg) were set up in tandem for the cleanup and enrichment of the aqueous solutions in biota sample extracts. Water samples were extracted by solid-phase extraction using Waters Oasis HLB cartridges (500 mg, 6 mL), while the solid samples were extracted with ethyl acetate by ultrasonication. The extracts of sediment and feed samples were further purified by silica gel columns before being analyzed by rapid resolution liquid chromatography–tandem mass spectrometry (RRLC–MS/MS). An Agilent 1200 LC-Agilent 6460 QQQ (RRLC–MS/MS) with an electrospray ionization (ESI) source was applied for the

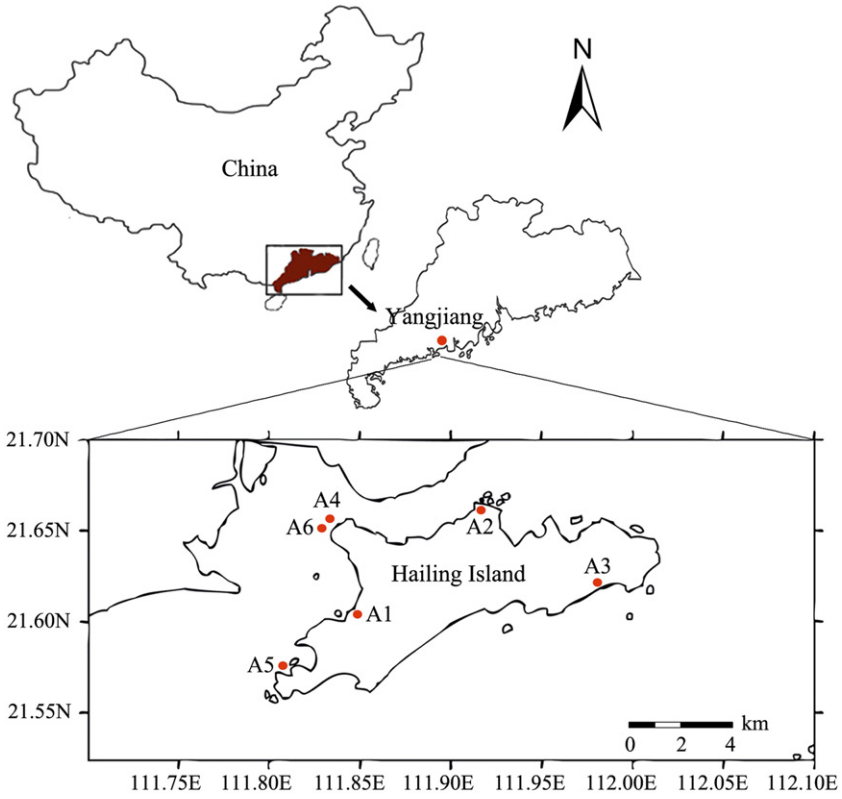

Fig. 1. Map of sampling locations surrounding Hailing Island, South China.

determination of the target steroid compounds. Quantitative analysis of the target compounds was carried out in multiple reaction monitoring (MRM) mode using isotope-labeled compounds as the internal standards. More information about sample extraction and instrumental analysis can be found in the Supplementary information.

2.3. Calculation of BCF

Bioconcentration of steroids in the marine organisms can be quantitatively described by the BCF, which is defined as the ratio of the chemical concentration in an organism or biota (C_{biota}) to the concentration in water (C_w). Here, the weight of the biota is a wet weight (ww) and the unit of the BCF is expressed as L/kg.

2.4. Calculation of EDI

Seafood consumption is an important source of exposure to steroids for residents in the coastal areas of South China. The estimated daily intake (EDI) of a selected steroid via seafood consumption for a specific age group of local residents was calculated according to the following equation:

$$EDI = C_{biota} \times M_{biota}$$

where EDI (ng/d/person) is the estimated daily intake of a selected steroid via seafood consumption; C_{biota} (ng/g) stands for the average concentration of the target steroid in adult biota sample (wet weight); M_{biota} (g/d/person) represents the daily consumption amount of selected seafood (shrimps, crabs, mollusks or fish) for a specific age group. The daily seafood consumption amounts in the present study were obtained from a questionnaire-based dietary survey in the coastal areas of South China (Guo et al., 2010).

3. Results and discussion

3.1. Free steroids in the water samples

Ten of 24 steroids were detected in the water samples of aquaculture (A1–A6) with concentrations ranging from <0.1 (testosterone) to 40 ng/L (prednisolone) (Tables 1 and 2), which are similar to the levels detected in the effluents from WWTPs or surface water (Chang et al., 2011; Liu et al., 2011b). Among the 10 steroids detected, three (androsta-1,4-diene-3,17-dione, norgestrel and progesterone) were found in all water samples. Steroids detected in the water from pond culture farms (Farms A1–A3), which are closed system, were apparently different from those detected in the other two culture models, especially for the synthetic ones. Prednisolone, a synthetic glucocorticoid, that occurred at concentrations of up to 40 ng/L, far exceeded the typical levels in the influents of WWTPs (1.5–7.5 ng/L) (Chang et al., 2007), indicating significant contribution from the farming activities. The types and concentrations of steroids detected were similar between farms A4 and A6. The steroids detected in these farms originated mainly from the aquaculture zone since the sampling sites were located far away from the residential district. Farm A5 is located near the wharf, which has a domestic sewage discharge site nearby. The total concentrations of steroids detected in surface water in farm A5 were about fourfold of those detected in farms A4 and A6, which probably resulted from the significant steroid inputs of the sewage discharge.

Synthetic progestagen norgestrel was detected in all water samples of the aquaculture farms (Tables 1 and 2). Norgestrel is used as an oral contraceptive at low doses either alone or combined with estrogens such as 17β-ethynylestradiol (EE2). According to clinical studies, the progestagenic potency of norgestrel is approximately 1000 times higher than that of progesterone (King and Whitehead, 1986; Stanczyk, 2003). In addition, synthetic progestagens may also have other hormonal activities, such as estrogenic, antiandrogenic, and androgenic activities (Rozenbaum, 2001; Schindler et al., 2003). A previous study indicated that synthetic progestagens are capable of inhibiting reproduction in

Table 1
Summary of concentrations of steroids detected in various samples of aquaculture farms A1–A4.

Compound	A1 (pond culture)			A2 (pond culture)			A3 (pond culture)				A4 (cage culture)			
	Feed (ng/g dw)[a]	Water (ng/L)	Young LR (ng/g ww)	Feed (ng/g dw)	Water (ng/L)	Young FP (ng/g ww)	Feed (ng/g dw)	Water (ng/L)	Sand (ng/g dw)	Adult FP (ng/g, ww)	Feed (ng/g dw)	Water (ng/L)	Sediment (ng/g dw)	Adult TO (ng/g ww)
Androsta-1,4-diene-3,17-dione	N.D.	0.9[b]	N.D.	0.8	1.8	0.5	N.D.	1.2	0.2	N.D.	N.D.	0.6	0.2	0.4
4-Androstene-3,17-dione	N.D.	0.7	N.D.	0.3	N.D.	0.2	N.D.	1.6	0.1	N.D.	N.D.	0.6	0.2	0.3
17α-Boldenone	N.D.	1.2	0.5 (N.D.–0.9)[c]	N.D.	1.3	N.D.	1.0	1.7	N.D.	N.D.	N.D.	N.D.	N.D.	0.8
17β-Boldenone	N.D.	N.D.	N.D.	2.3	N.D.	N.D.	N.D.	0.4	N.D.	N.D.	N.D.	N.D.	0.2	0.4
Epi-androsterone	N.D.	N.D.	N.D.	21.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	14.9	N.D.	0.8	N.D.
4-Hydroxy-androst-4-ene-17-dione	N.D.	N.D.	N.D.	N.D.	3.2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Methyl testosterone	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.4
19-Nortestoserone	N.D.	N.D.	N.D.	N.D.	N.D.	<0.2[d]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Testosterone	N.D.	0.1	N.D.	0.3	N.D.	N.D.	N.D.	0.4	N.D.	N.D.	N.D.	N.D.	0.2	<0.1
17β-Trenbolone	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.8	N.D.	N.D.	N.D.	N.D.	N.D.	0.1	<0.2
Sum of androgens	**N.D.**	**2.9**	**0.5 (N.D.[e]–0.9)**	**25.1**	**6.3**	**0.7**	**1.8**	**5.3**	**0.3**	N.D.	**14.9**	**1.2**	**1.7**	**2.3**
Cortisol	N.D.	N.D.	51.0 (25.4–109)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	560
Cortisone	N.D.	N.D.	2.7 (1.5–6.2)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	75.9
Prednisolone	19.6	N.D.	N.D.	4.5	N.D.	N.D.	6.7	40.0	<2.6	N.D.	7.2	N.D.	N.D.	N.D.
Sum of glucocorticoids	**19.6**	**N.D.**	**53.7 (27.5–115)**	**4.5**	**N.D.**	**N.D.**	**6.7**	**40.0**	**N.D.**	**N.D.**	**7.2**	**N.D.**	**N.D.**	**636**
Medroxyprogesterone	N.D.	N.D.	0.6 (0.5–0.8)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.6
Norgestrel	5.2	2.8	0.6 (N.D.–0.9)	1.6	3.8	0.7	N.D.	3.8	N.D.	1.3 (1.2–1.3)	1.5	1.4	N.D.	<0.5
Progesterone	0.7	0.5	0.4 (N.D.–0.5)	1.4	0.6	0.5	0.5	0.9	0.2	1.0 (1.0–1.0)	0.7	0.4	0.6	0.6
Sum of progestagens	**5.9**	**3.3**	**1.6 (0.8–2.0)**	**3.0**	**4.4**	**1.2**	**0.5**	**4.7**	**0.2**	**2.3 (2.2–2.4)**	**2.2**	**1.8**	**0.6**	**1.7**
Sum of steroids	**25.5**	**6.2**	**55.8 (29.5–117)**	**32.6**	**10.7**	**1.9**	**9.0**	**50.0**	**0.5**	**2.3 (2.2–2.4)**	**24.3**	**3.0**	**2.3**	**640**

[a] dw, dry weight, ww, wet weight.
[b] Mean (n = 2, replicate samples at the same time).
[c] Mean (mix–max).
[d] Below limit of detection.
[e] Not detected.

Table 2
Summary of concentrations of steroids detected in various samples of aquaculture farms A5 and A6.

Compound	A5 (cage culture)								A6 (oyster pile culture)		
	Water (ng/L)	Sediment (ng/g dw)[a]	APL (ng/g ww)	ML (ng/g ww)	TK (ng/g ww)	CP (ng/g ww)	Adult LR (ng/g ww)	Adult LE (ng/g ww)	Water (ng/L)	Sediment (ng/g dw)	ORG (ng/g ww)
Androsta-1,4-diene-3, 17-dione	1.6[b]	0.2	0.4 (0.3–0.5)[c]	0.9 (N.D. [e]–2.8)	N.D.	N.D.	N.D.	N.D.	0.5	0.5	N.D.
4-Androstene-3,17-dione	3.2	0.8	0.3 (0.2–0.4)	0.7 (0.3–2.2)	0.5 (0.3–0.6)	0.2 (0.2–0.2)	N.D.	0.3 (0.2–0.7)	0.6	0.6	0.3 (0.2–0.7)
17α-Boldenone	N.D.	N.D.	N.D.	1.4 (0.9–2.5)	N.D.	N.D.	0.2 (N.D.–0.7)	0.4 (N.D.–0.7)	N.D.	N.D.	0.4 (N.D.–0.7)
17β-Boldenone	0.3	0.2	0.5 (0.4–0.6)	0.9 (0.5–0.7)	1.0 (0.6–1.4)	N.D.	N.D.	N.D.	N.D.	0.4	N.D.
Epi-androsterone	N.D.	1.2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Methyl testosterone	N.D.	N.D.	N.D.	<0.2 (N.D.–0.9)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
19-Nortestoserone	N.D.	0.3	N.D.	0.3 (N.D.–1.5)	N.D.	<0.2[d]	0.3 (<0.2–0.7)	<0.2	N.D.	N.D.	<0.2 (N.D.–0.6)
Testosterone	0.1	0.3	0.2 (0.1–0.5)	0.4 (0.1–1.1)	(N.D.–< 0.1)	N.D.	N.D.	N.D.	<0.1	0.5	N.D.
17α-Trenbolone	N.D.	N.D.	N.D.	<0.1 (N.D.–0.6)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
17β-Trenbolone	N.D.	0.2	N.D.	(N.D.–< 0.2)	<0.2	N.D.	N.D.	N.D.	N.D.	0.3	N.D.
Sum of androgens	**5.2**	**3.2**	**1.4 (1.0–2.0)**	**4.6 (2.3–14.0)**	**1.5 (0.9–2.2)**	**0.2 (0.2–0.2)**	**0.5 (0.1–0.8)**	**0.7 (0.2–1.1)**	**1.1**	**2.3**	**0.7 (0.3–1.3)**
Cortisol	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	23.3 (17.7–33.1)	17.4 (8.7–23.4)	N.D.	N.D.	N.D.
Cortisone	3.5	N.D.	N.D.	N.D.	N.D.	7.1 (1.4–11.1)	1.7 (0.8–2.7)	1.4 (N.D.–2.4)	N.D.	N.D.	N.D.
Sum of glucocorticoids	**3.5**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**7.1 (1.4–11.1)**	**25.1 (20.3–33.9)**	**18.8 (9.7–24.5)**	**N.D.**	**N.D.**	**N.D.**
Ethynyl testosterone	N.D.	N.D.	N.D.	0.2 (N.D.–1.1)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Medroxyprogesterone	N.D.	N.D.	N.D.	0.2 (N.D.–0.8)	0.6 (N.D.–1.1)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Norgestrel	2.8	N.D.	0.7 (<0.5–0.8)	1.5 (0.8–3.3)	1.6 (1.0–2.0)	<0.5	0.8 (0.6–1.2)	1.3 (N.D.–5.4)	2.0	N.D.	0.7 (N.D.–1.3)
Progesterone	0.5	2.1	0.5 (0.4–0.7)	1.1 (0.5–2.5)	0.7 (N.D.–1.1)	0.4 (0.4–0.4)	0.6 (0.4–1.0)	1.1 (0.4–2.0)	0.4	2.4	0.5 (0.4–1.0)
Sum of progestagens	**3.3**	**2.1**	**1.2 (0.9–1.4)**	**2.9 (1.3–6.9)**	**2.9 (1.5–4.1)**	**0.9 (0.9–1.0)**	**1.4 (1.1–2.2)**	**2.4 (1.0–6.6)**	**2.4**	**2.4**	**1.2 (0.5–2.3)**
Sum of steroids	**12.0**	**5.3**	**2.6 (1.9–3.4)**	**7.5 (3.6–20.9)**	**4.4 (2.4–6.3)**	**8.2 (2.5–15.4)**	**27.0 (22.1–35.1)**	**21.9 (10.9–32.1)**	**3.5**	**4.7**	**1.9 (1.4–3.6)**

[a] dw, dry weight, ww, wet weight.
[b] Mean (n = 2, replicate samples at the same time).
[c] Mean (mix–max).
[d] Below limit of detection.
[e] Not detected.

fathead minnows at concentrations as low as 0.8 ng/L (Zeilinger et al., 2009). Based on the concentration levels (1.4–3.8 ng/L) of norgestrel detected in the farming water, it is expected that norgestrel is likely to cause adverse effects on aquatic organisms raised in the marine aquaculture farms surrounding Hailing Island.

3.2. Steroids in the sediment samples

Nine steroids were detected in the sediment samples with concentrations ranging from 0.1 (4-androstene-3,17-dione) to 2.4 ng/g (progesterone) (Tables 1 and 2), which were similar to those detected in typical marine sediments (N.D. to 3.6 ng/g) (Isobe et al., 2006), but lower than those detected in river sediments (N.D. to 17.3 ng/g) (Gong et al., 2011; Liu et al., 2012a, 2012c). Although the concentrations of steroids in the sediment samples were low, they were predicted by BIOWIN to resist biodegradation (Musson et al., 2010), especially under anaerobic conditions (Ying and Kookana, 2003; Zheng et al., 2012). Synthetic steroids were also found to have much slower degradation rates than the natural ones under the same conditions (Liu et al., 2013). Since very limited numbers of studies have been conducted on the persistence of steroids in the aquaculture sediment, their potential risks to the sediment environment remain to be evaluated.

3.3. Steroids in feeds

Both endogenous steroids, including estradiol, testosterone, and progesterone, as parent compounds or esters, and synthetic ones, such as ethynylestradiol, nortestosterone, stanozolol, trenbolone acetate, and melengestrol acetate are widely used as veterinary drugs or growth promoters in animal farming (Fragkaki et al., 2009; Kaklamanos et al., 2009; Khan et al., 2008, 2009; Lange et al., 2002). For the health and safety of consumers, the use of these compounds as growth promoters in animal breeding has been banned in many countries, including China. Ten steroids, including six synthetic steroids (androsta-1,4-diene-3,17-dione, 17α-boldenone, 17β-boldenone, 17β-trenbolone, prednisolone, norgestrel), were detected in the four feed samples obtained in this study, with concentrations ranging from 0.3 ng/g (testosterone) to 21.4 ng/g (epi-androsterone) (Table 1). These findings demonstrated that synthetic steroids are still being used illegally in marine culture in China.

3.4. Steroids in biota samples

Fifteen steroids were detected in the biota samples with concentrations ranging from <0.1 (testosterone) to 560 ng/g (cortisol) (wet weight) (Tables 1 and 2), which were higher than those reported in aquaculture products (0.44–0.57 ng/g) from local supermarkets in Changchun, China (Wang et al., 2012). Norgestrel and progesterone were the most frequently detectable steroids in the biota samples. Apparently, the types of steroids detected in crustaceans (shrimps and crabs) were less than those detected in other biota samples (Tables 1 and 2), which were in agreement with previous studies (Wang et al., 2012; Yang et al., 2009). Yang et al. (2009) measured 50 anabolic steroids in pork, liver, milk, beef and shrimp, and found only one cortisol in shrimps with concentrations ranging from 0.51 ng/g to 6.78 ng/g. Two steroids 17α-estradiol and 19-nortestosterone were detected in the shrimp with concentration of 0.39 and 0.30 ng/g, respectively (Wang et al., 2012). Thirteen steroids were detected in mollusks (shellfishes and oysters) with concentrations ranging from <0.1 to 3.3 ng/g, similar to those reported in the literature (Negrato et al., 2008; Reishenriques et al., 1990; Zhou et al., 2010). Up to now, there is no conclusive evidence for biosynthesis of vertebrate steroids by mollusks, while mollusks are able to absorb vertebrate steroids from the environment and store some of them for weeks to months (Scott, 2012). Compared with the types of steroids detected in the water with those detected in mollusks, only some of the steroids were detected in mollusks (Table 2). This might be due to the low concentrations of steroids in the water, which were close to or below the limit of detection leading to be undetectable in the aquatic phase, as well as the different bioconcentration behaviors of the sterioids. Among all species of mollusks, the types of steroids detected in *M. lusoria* (ML) were much more than others, indicating that it could potentially be a good biological indicator for steroid-polluted environment.

Some natural steroids were also detected in fish muscle, which probably originated from circulating endogenous sex steroids present naturally in fish (Liu et al., 2012a). For example, two natural glucocorticoids, cortisol and cortisone, both occurred in fish muscle at concentrations and detection frequencies much higher than in muscles of other marine organisms. Furthermore, cortisol was not detected in the environmental samples or feed samples, but occurred in all fish muscle samples with concentrations up to 560 ng/g (Tables 1 and 2), supporting its endogenous origin.

Two species of shrimp and fish were chosen to further study the impact of different growth stage on the residual concentrations of steroids. Large differences were found for some steroids between the young and adult stages. For shrimps, two androgens and two progestagens were detected in the young *F. penicillatus* (FP), while only two progestagens were detected in the adult FP. The concentrations of progestagens detected in the adult FP were about twice-fold of those detected in the young FP (Tables 1 and 2). For fish, the total concentrations of androgens and progestagens in young and adult *L. russelli* (LR) were similar, while the total concentrations of glucocorticoids in young LR were two-fold of those in adult LR (Tables 1 and 2).

3.5. Bioconcentration of steroids in biota

Previous studies mainly focused on bioconcentration of single steroid in aquatic biota under laboratory conditions (Contardo-Jara et al., 2011; Steele et al., 2013) or bioconcentration of multiple estrogens in wild aquatic biota (Huang et al., 2013; Liu et al., 2011a). To the best of our knowledge, this is the first study that characterized the bioconcentration of 24 steroids in the typical seafood raised in aquaculture farms. Based on the concentrations of steroids detected in the surface water of the aquaculture farms and those measured in biota muscle samples (Tables 1 and 2), the field BCFs of steroids were calculated for different aquatic organisms (Table 3). Some natural steroids are likely

Table 3
Bioconcentration factors (L/kg) of selected steroids in aquatic biota calculated from the monitoring results of this study.

Compound	Shrimp		Mollusca				Fish			
	Young FP	Adult FP	Adult APL	Adult ML	Adult TK	Adult ORG	Adult TO	Young LR	Adult LR	Adult LE
Androsta-1,4-diene-3,17-dione	278		250	563			667			
4-Androstene-3,17-dione			93.8	219	156	500				
17α-Boldenone								417		
17β-Boldenone			1667	3000	3333					
Testosterone			2000	4000						
Norgestrel	184	342	250	536	571	350		214	286	464
Progesterone			1000	2200	1400	1250				

Crab was not included because of no synthetic steroids were detected both in crab muscle and aquatic phase.

to be endogenous in crustaceans and vertebrates (Liu et al., 2012a; Ye et al., 2010), and BCFs were calculated only for those detected synthetic steroids. For mollusks, they could not produce endogenous steroids (Scott, 2012), and BCFs were calculated for both the natural and synthetic steroids. The values of BCFs in all biota samples ranged from 93.8 to 4000, similar to the values calculated in wild environment or at low concentration exposure (~ng/L) (Contardo-Jara et al., 2011; Länge et al., 2001), but higher than some results obtained under laboratory conditions (~μg/L) (Nallani et al., 2012; Steele et al., 2013). When the fathead minnow was exposed to 47 ng/L of 17α-ethynyl estradiol, the estimated BCF was 660 (Länge et al., 2001). Contardo-Jara et al. (2011) reported that the BCF of levonorgestrel (0.312–6.24 μg/L) in mollusk *Dreissena polymorpha* was in the range of 30–208. The concentrations (always in the range of μg/L) of target compounds tested under laboratory conditions were much higher than those of environmental exposure, which could decrease the BCF values from hundreds to tens (Nallani et al., 2012; Steele et al., 2013). In addition, factors species, gender, growth stage, and body size can all affect the BCF value of steroids in aquatic biota.

3.6. Estimated daily intakes of steroids via seafood consumption

The daily intake of steroids from consumption of four seafood groups (i.e. shrimps, crabs, mollusks, and fish) were evaluated (Table 4). The EDIs of androgens, glucocorticoids, and progestagens via seafood consumption ranged from 33.4–134, 2061–8566, and 40.4–155 ng/d for different age groups, respectively (Table 4), which are similar to estimations reported in the literature (Hartmann et al., 1998). The dietary intakes of androgens, glucocorticoids, and progestagens for male were slightly greater than those of female in all the age groups. The EDIs of children were much less than the other groups, primarily due to the low seafood consumption of this group (Guo et al., 2010). The relative contributions of mollusks and fish to the total EDIs of steroids were much higher than those of shrimps and crabs (Table 4). For androgens and progestagens, mollusks and fish contributed the most to the EDI (>99.5%), while fish alone contributed to >99.7% of the EDI for glucocorticoids. The significantly higher contributions to the total EDI of steroids from mollusks and fish resulted from their relatively high consumption rates, as well as the high levels and detection frequencies of androgens and progestagens in their bodies.

The EDIs of androgens, glucocorticoids, and progestagens via seafood consumption for adults (>18 years) were about thousands fold lower than that of normal human excretion (Liu et al., 2012c) or the acceptable daily intakes (ADIs) (Table S6). For example, the ADIs for the testosterone and progesterone were 0–2 and 0–30 μg/kg bw/d, respectively (Table S6). For an adult with body weight of 60 kg, these ADIs translate to allowable intakes of 120 and 1800 μg/d for testosterone and progesterone, respectively, far exceeding their EDIs through seafood consumption (Table S5). As a result, no hormonal effect is expected for testosterone and progesterone detected in the seafood in this study. Only some of the steroids measured in this study are regulated by the guidelines of Ministry of Agriculture of the People's Republic of China (2002) and the Maximum Residue Limits (MRLs) of the EU (Table S6) The MRL for prednisolone is 4 ng/g in animal muscle, thus the corresponding ADI is 254 ng/d based on 63.6 g of daily fish consumption by an adult male (Table S5). Prednisone was not detected in any of the seafood samples in this study. However, other glucocorticoids such as cortisol and cortisone were detected and the estimated ΣGlucocorticoids in fish (8552 ng/d) far exceeded the ADI of prednisone (254 ng/d). The potential health risk of steroids from seafood consumption is difficult to estimate due to limited studies and exposure standards. Nonetheless, the intake of multiple steroids from seafood consumption might pose a potential health risk to humans, especially for the sensitive populations, such as pregnant women and children.

4. Conclusion

This work reports the occurrence, bioconcentration, and human dietary exposure via seafood consumption of 24 steroids in different farming models (pond, cage and oyster pile cultures), different biota (fish, shrimps, crabs, mollusks), different growth stages (young and adult), different feeds, and different environmental matrixes (water and sediment) in the six typical marine aquaculture farms, South China. Six synthetic steroids were detected in the feed samples, clearly demonstrating the illegal use of steroids in the aquaculture farms. Given their high capacity of bioconcentration, mollusks, especially *M. lusoria* (ML), could be used as an effective biomarker for steroids in polluted environment with low concentrations. Even though no elevated health risk was found from individual steroids present in the seafood, the presence of multiple steroids might pose a potential risk to humans,

Table 4
Estimated daily intakes (ng/d) of steroids via seafood consumption and the relative contribution (%) of each seafood group to the total EDIs of androgens, glucocorticoids, and progestagens.

	Children (2–5 years)		Youth (6–18 years)		Adult (>18 years)	
Gender	Male	Female	Male	Female	Male	Female
ΣAndrogens						
Shrimp	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Crab	0.1 (0.2)	0.1 (0.2)	0.4 (0.3)	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)
Mollusca	29.3 (64.0)	17.6 (52.8)	94.4 (70.6)	58.4 (60.9)	58.4 (47.1)	55.3 (49.4)
Fish	16.4 (35.8)	15.7 (47.0)	38.9 (29.1)	37.2 (38.8)	65.2 (52.7)	56.4 (50.4)
Total	45.8 (100)	33.4 (100)	134 (100)	95.8 (100)	124 (100)	112 (100)
ΣGlucocorticoids						
Shrimp	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Crab	7.2 (0.3)	4.4 (0.2)	22.7 (0.4)	13.8 (0.3)	13.8 (0.2)	12.7 (0.2)
Mollusca	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Fish	2152 (99.7)	2057 (99.8)	5096 (99.6)	4881 (99.7)	8552 (99.8)	7396 (99.8)
Total	2159 (100)	2061 (100)	5119 (100)	4895 (100)	8566 (100)	7409 (100)
ΣProgestagens						
Shrimp	0.2 (0.3)	0.1 (0.2)	0.5 (0.4)	0.3 (0.3)	0.3 (0.2)	0.3 (0.2)
Crab	0.03 (0.1)	0.02 (0.04)	0.1 (0.1)	0.1 (0.05)	0.1 (0.03)	0.05 (0.04)
Mollusca	25.3 (48.9)	15.2 (37.7)	81.3 (56.5)	50.3 (45.7)	50.3 (32.5)	47.6 (34.5)
Fish	26.2 (50.7)	25.0 (62.1)	62.0 (43.1)	59.4 (54.0)	104 (67.3)	90.0 (65.2)
Total	51.6 (100)	40.4 (100)	144 (100)	110 (100)	155 (100)	138 (100)

especially for the sensitive populations, such as pregnant women and children.

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Appendix A. Supplementary data

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