

1 **Supporting Information**

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3 **Analytical strategy for identification and quantification of thirteen steroids in sole**
 4 **(*Solea senegalensis*) tissues, eggs and larvae for application in aquaculture studies**
 5 **on reproduction**

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8 **Table S1.** Signal suppression/enhancement (SSE) for each steroid in the matrices studied
 9 (see 2. EXPERIMENTAL, 2.5. Matrix effect evaluation)

Compounds	Plasma	Ovaries	Muscle	Eggs	Larvae
	SSE (%)				
Progesterone (P)	-91	-88	-95	-90	-93
11-deoxycortisol (S)	-78	-65	-85	-95	-90
Cortisol (F)	-	-57	-82	-	-
17 α ,20 β dihydroxy- pregnenone (17 α ,20 β -P)	-69	-69	-83	-96	-98
17 α ,20 β , 21 trihydroxy- Pregnenone (20 β -S)	-68	-65	-85	-96	-96
4-androstenedione (A4)	-76	-71	-90	-98	-96
Testosterone (T)	-79	-83	-90	-97	-97
5 Androstanolone (Dihydrotestosterone, DHT)	-88	-82	-92	-	-96
11-hydroxy androstenedione (11 β OHA4)	-84	-54	-83	-97	-98
11-keto- androstenedione (11-KA)	-68	-37	-77	-97	-99
11-ketotestosterone (11-KT)	-74	-47	-82	-97	-98
Estrone (E1) ^b	-72	-76	-99	-95	-96
17 β Estradiol (E2) ^b	-73	-87	-91	-98	-98

10 *SSE: Signal Suppression Enhancement. According to the criteria established, positive values indicate
 11 signal enhancement while negative values correspond to signal. - suppression. not calculated

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13 **Table S2.** Limits of detection (LOD) of target compounds ions used for quantification
 14 and identification in studied matrices

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Compounds	Precursor Ion	Product Ion	Plasma	Ovaries	Muscle	Eggs	Larvae
	m/z	m/z	ng/mL		ng/g		
Progesterone (P)	315.1	97.0 ^b	0.03	0.05	0.03	-	0.05
		109.0	0.14	0.09	0.03	0.12	0.05
		123.0	0.39	0.34	0.18	0.26	0.87
11-deoxycortisol (S)	347.0	97.0 ^b	0.01	0.14	0.02	0.04	0.01
		109.1	0.01	0.08	0.04	0.14	0.06
		317.0	0.01	0.07	0.04	0.15	0.13
Cortisol (F)	363.0	121.0 ^b	-	-	-	0.01	0.01
		309.1	-	-	-	0.03	0.04
		267.0	-	-	-	0.07	0.07
17 α ,20 β dihydroxy- Pregnenone (17 α ,20 β -P)	333.2	97.2 ^b	0.01	0.13	0.04	0.20	0.06
		109.2	0.04	0.17	0.07	0.11	0.20
		253.2	0.03	0.38	0.08	0.04	0.13
17 α ,20 β ,21 - trihydroxy Pregnenone (20 β -S)	349.2	109.2 ^b	0.03	0.11	0.03	0.13	0.03
		97.0	0.02	0.18	0.10	0.03	0.06
		271.2	0.10	0.09	0.03	0.05	0.13
4-androstenedione (A4)	287.1	97.0 ^b	0.02	0.02	0.02	0.07	0.04
		109.0	0.02	0.04	0.02	0.05	0.10
		123.0	-	0.07	-	0.18	0.84
Testosterone (T)	289.1	97.0 ^b	0.01	0.08	0.01	0.05	0.08
		109.0	0.02	0.02	0.01	0.02	0.26
		123.0	-	0.16	-	0.03	0.25
5 Androstanolone (Dihydrotestosterone, DHT)	291.2	255.2 ^b	0.19	0.21	0.29	-	0.60
		159.2	0.42	0.74	0.84	-	0.27
		145.2	1.36	5.13	-	-	0.25
11-hydroxy androstenedione (11 β OHA4)	303.2	267.2 ^b	0.03	0.10	0.03	0.16	0.10
		121.2	0.04	0.08	0.02	0.54	0.18
		145.2	0.09	0.10	0.08	0.27	0.32
11-keto- androstenedione (11KA)	301.2	257.2 ^b	0.03	0.03	0.02	0.01	0.06
		242.2	0.03	0.03	0.01	0.04	0.09
		121.2	0.03	0.06	0.04	0.03	0.09
11-ketotestosterone (11KT)	303.2	259.1 ^b	0.03	0.02	0.02	0.02	0.04
		121.2	0.03	0.06	0.04	0.02	0.12
		241.2	0.06	0.23	0.08	0.01	0.47
Estrone (E1) ^a	269.2	145.2 ^b	0.02	0.01	0.01	0.05	0.02
		159.2	0.02	0.01	0.01	0.06	0.15
		183.2	0.07	0.12	0.18	0.14	0.74
17 β Estradiol (E2) ^a	271.2	145.2 ^b	0.02	0.01	0.03	0.17	0.15
		183.2	0.02	0.06	0.05	0.10	0.84
		239.2	0.02	0.01	0.01	0.03	0.13

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17 ^a negative mode, ^b quantification ion

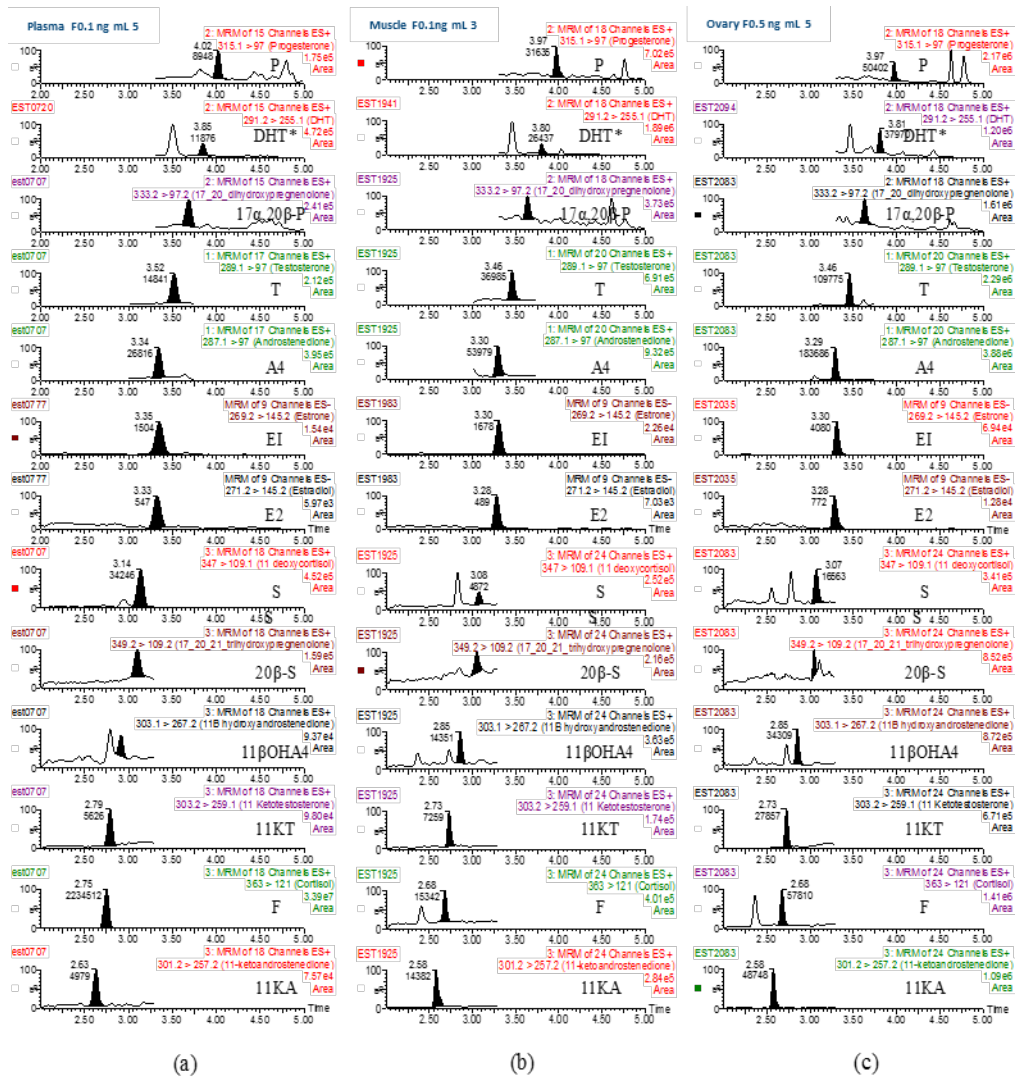
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25 **Fig. S1.** UHPLC-MS/MS chromatograms corresponding to the quantification SRM
26 transition for the studied steroid hormones at the lowest concentration validated in plasma
27 (0.1 ng/mL) (a), in muscle (0.1 ng/g) (b) and in ovaries (0.5 ng/g) (c).

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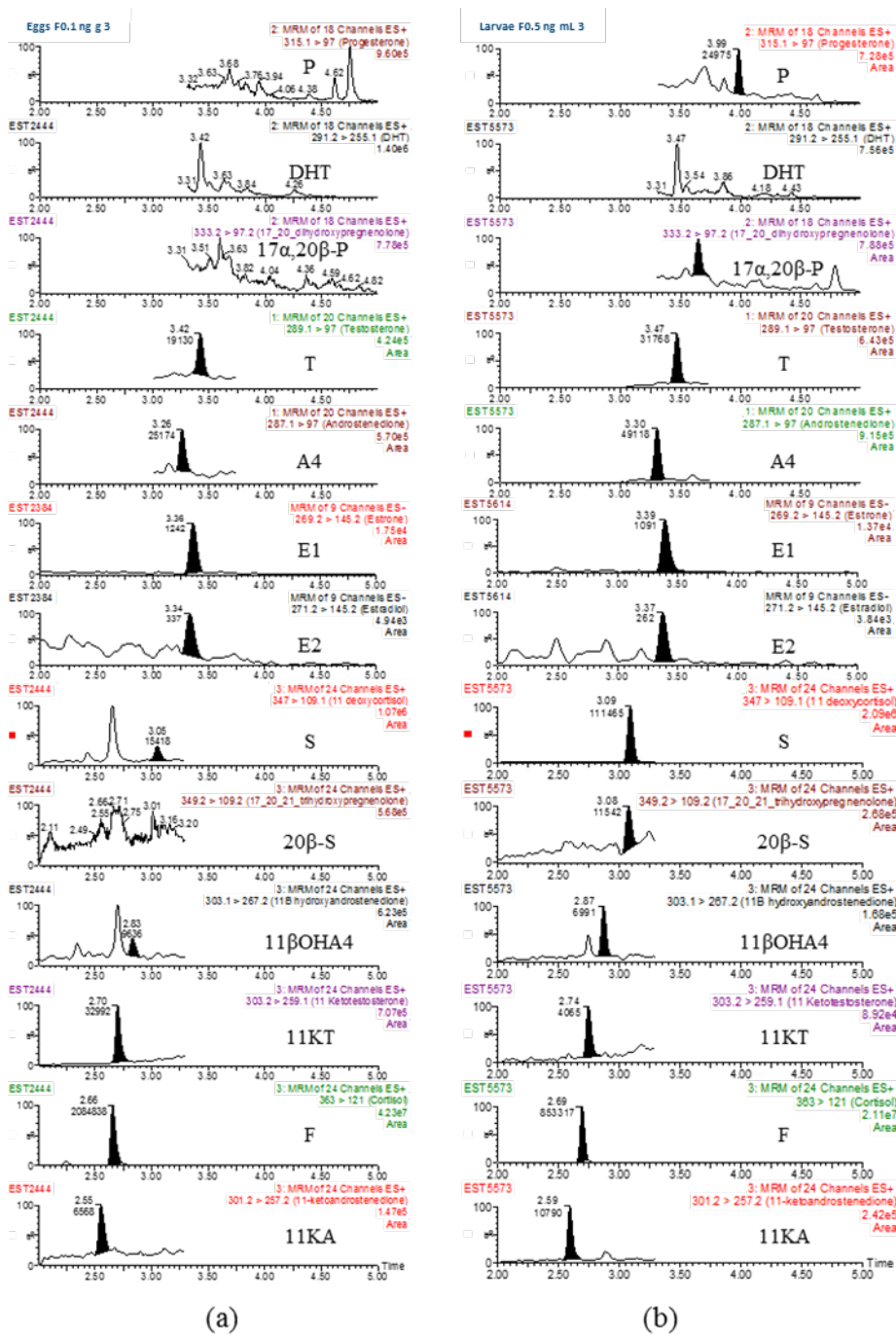
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37 **Fig. S2.** UHPLC-MS/MS chromatograms corresponding to the quantification SRM
38 transition for the studied steroid hormones at the lowest concentration validated in eggs
39 (0.1 ng/g) (a), and larvae (0.5 ng/g) (b).

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