Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Optimization of mass spectrometry settings for steroidomic analysis in young and old killifish

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Table S1 Optimization of spray voltage

Spray voltage	1 kV		2 kV		3 kV		4 kV		5 kV		6 kV	
Compound	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)
Squalene	2.02E+05	4.37	7.89E+05	6.91	1.96E+06	6.69	2.76E+06	7.30	2.94E+06	4.39	2.48E+06	5.77
Lanosterol	3.42E+05	2.68	5.54E+05	4.43	7.23E+05	1.92	2.23E+06	8.63	5.73E+06	2.12	6.63E+06	9.04
7 Dehydrocholesterol	3.60E+05	3.41	9.41E+05	4.35	2.38E+06	10.98	2.83E+06	12.58	1.68E+06	5.65	1.27E+06	5.79
24 Hydroxycholesterol	1.62E+04	5.57	3.02E+04	8.67	1.20E+05	2.44	1.66E+05	11.73	1.50E+05	2.27	1.74E+05	5.97
25 Hydroxycholesterol	3.80E+03	4.29	3.62E+03	6.68	1.20E+04	4.96	2.20E+04	9.68	4.19E+04	5.32	5.67E+04	7.48
27 Hydroxycholesterol	7.67E+03	1.92	8.61E+03	5.48	1.65E+04	6.06	2.39E+04	5.39	3.07E+04	11.74	3.78E+04	4.50
Progesterone	7.28E+05	9.16	2.55E+06	9.44	3.08E+06	10.86	2.49E+06	13.67	1.08E+06	10.68	7.25E+05	5.66
Corticosterone	5.83E+05	5.33	1.75E+06	7.86	4.54E+06	8.77	5.85E+06	4.21	5.55E+06	1.06	4.38E+06	3.32
Testosterone	1.41E+06	10.77	4.01E+06	5.75	9.78E+06	3.56	1.56E+07	8.52	1.20E+07	4.10	9.18E+06	10.69

Table S2 Optimization of resolution

Resolution	17,500		30,000		70,000		140,000		280,000	
Compound	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)
Squalene	2.15E+06	8.77	2.82E+06	3.25	2.76E+06	7.30	2.31E+06	1.39	2.04E+06	9.80
Lanosterol	2.30E+06	6.63	1.69E+06	9.49	2.23E+06	8.63	1.58E+06	12.80	1.63E+06	5.08
7 Dehydrocholesterol	0.00E+00	0.00	0.00E+00	7.74	2.83E+06	12.58	0.00E+00	0.00	0.00E+00	0.00
24 Hydroxycholesterol	1.68E+05	7.21	2.19E+05	5.88	1.66E+05	11.73	2.20E+05	11.10	1.58E+05	7.72
25 Hydroxycholesterol	4.10E+04	15.63	2.76E+04	7.99	2.20E+04	9.68	2.07E+04	9.81	1.35E+04	9.01
27 Hydroxycholesterol	4.84E+04	8.34	3.02E+04	5.84	2.39E+04	5.39	2.20E+04	1.75	1.72E+04	7.14
Progesterone	4.75E+06	6.25	4.41E+06	10.97	2.49E+06	13.67	3.20E+06	9.62	2.49E+06	0.28
Corticosterone	5.97E+06	5.20	3.10E+06	3.88	5.85E+06	4.21	6.20E+06	1.74	4.86E+06	5.10
Testosterone	1.09E+07	1.06	1.15E+07	1.82	1.56E+07	8.52	1.51E+07	7.37	1.21E+07	2.34

Table S3-1 AGC Parameter optimization

AGC target	2e4		5e4		1e5		2e5		5e5	
Compound	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)
Squalene	4.08E+06	8.68	5.44E+06	3.79	8.85E+06	13.17	5.44E+06	6.99	1.94E+06	8.73
Lanosterol	1.02E+07	8.27	4.95E+06	14.27	7.13E+06	4.77	2.48E+06	3.14	9.39E+05	11.23
7 Dehydrocholesterol	3.29E+06	8.78	3.59E+06	13.36	4.96E+06	5.42	4.82E+06	4.07	2.24E+06	4.15
24 Hydroxycholesterol	0.00E+00	0.00	0.00E+00	0.00	0.00E+00	0.00	6.18E+05	6.10	5.09E+04	9.63
25 Hydroxycholesterol	0.00E+00	0.00	0.00E+00	0.00	0.00E+00	0.00	0.00E+00	0.00	0.00E+00	0.00
27 Hydroxycholesterol	0.00E+00	0.00	0.00E+00	0.00	0.00E+00	0.00	0.00E+00	0.00	0.00E+00	0.00
Progesterone	2.27E+07	9.00	2.57E+07	10.70	2.40E+07	12.69	1.81E+07	14.41	1.30E+07	6.11
Corticosterone	1.13E+07	5.33	1.22E+07	7.42	1.12E+07	0.51	1.08E+07	2.99	8.37E+06	7.91
Testosterone	2.38E+07	2.57	2.36E+07	4.66	3.23E+07	6.83	2.92E+07	2.25	1.54E+07	3.25

Table S3-2 AGC Parameter optimization

AGC target	1e6		3e6		5e6	
Compound	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)
Squalene	1.27E+06	6.76	2.76E+06	7.30	7.65E+05	8.49
Lanosterol	7.40E+05	9.51	2.23E+06	8.63	6.15E+05	3.73
7 Dehydrocholesterol	1.76E+06	12.38	2.83E+06	12.58	1.25E+06	3.96
24 Hydroxycholesterol	3.41E+04	6.80	1.66E+05	11.73	2.16E+04	10.03
25 Hydroxycholesterol	0.00E+00	0.00	2.20E+04	9.68	8.39E+03	9.16
27 Hydroxycholesterol	0.00E+00	0.00	2.39E+04	5.39	9.18E+03	7.77
Progesterone	8.91E+06	3.66	2.49E+06	13.67	5.24E+06	12.82
Corticosterone	6.76E+06	1.66	5.85E+06	4.21	4.15E+06	3.98
Testosterone	1.20E+07	0.48	1.56E+07	8.52	6.52E+06	3.17

Table S4 Injection time optimization

Injection time	100 ms		150 ms		200 ms		250 ms	
Compound	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)
Squalene	1.16E+06	0.00	3.05E+05	7.46	8.46E+06	5.61	1.19E+06	7.54
Lanosterol	1.08E+06	4.12	9.88E+05	4.88	7.13E+06	2.34	9.93E+05	8.39
7 Dehydrocholesterol	1.45E+05	10.48	1.20E+05	2.70	4.96E+06	7.57	1.20E+05	6.08
24 Hydroxycholesterol	7.90E+03	7.53	1.21E+04	9.06	1.66E+05	5.10	8.10E+03	8.07
25 Hydroxycholesterol	6.31E+03	7.38	7.90E+03	10.02	2.20E+04	1.38	6.23E+03	8.55
27 Hydroxycholesterol	2.14E+04	9.81	2.08E+04	9.21	2.39E+04	2.33	2.08E+04	1.73
Progesterone	1.18E+05	8.20	1.17E+05	4.41	2.40E+07	1.44	0.00E+00	8.21
Corticosterone	4.88E+05	4.58	5.45E+05	5.77	1.12E+07	2.50	4.75E+05	11.29
Testosterone	2.87E+05	9.82	3.19E+05	2.40	3.23E+07	6.32	2.85E+05	7.01

Table S5 Flowrate optimization

Flowrate	50 ul/min		100 ul/min		150 ul/min		200 ul/min	
Compound	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)
Squalene	0.00E+00	0.00	5.21E+06	2.39	4.31E+05	8.94	1.06E+05	3.21
Lanosterol	5.70E+05	3.21	7.13E+06	3.64	6.76E+05	4.13	0.00E+00	0.00
7 Dehydrocholesterol	0.00E+00	0.00	4.96E+06	4.52	1.05E+08	2.42	1.77E+07	13.75
24 Hydroxycholesterol	7.40E+04	6.61	1.15E+04	2.80	5.57E+04	1.18	1.01E+04	11.30
25 Hydroxycholesterol	1.22E+05	5.02	1.22E+04	9.23	6.04E+03	7.01	1.26E+04	12.34
27 Hydroxycholesterol	2.06E+05	7.99	2.21E+04	7.37	3.48E+04	9.72	3.11E+04	8.30
Progesterone	0.00E+00	0.00	2.40E+07	4.50	2.65E+07	8.33	2.56E+07	13.02
Corticosterone	0.00E+00	0.00	1.12E+04	8.32	9.93E+06	4.82	7.95E+05	10.52
Testosterone	0.00E+00	0.00	3.23E+07	1.23	2.07E+07	4.56	1.55E+07	4.16

Table S6 Needle position optimization

Needle position	A		B		C		D	
Compound	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)
Squalene	3.58E+06	7.66	5.48E+06	2.37	4.31E+05	4.29	1.72E+06	9.71
Lanosterol	3.02E+06	1.02	2.47E+06	4.58	6.76E+05	7.37	5.11E+06	8.66
7 Dehydrocholesterol	5.52E+07	12.09	4.89E+07	8.66	1.05E+08	4.14	1.41E+08	11.26
24 Hydroxycholesterol	3.13E+04	9.79	6.91E+04	4.50	5.57E+04	7.58	1.14E+04	13.25
25 Hydroxycholesterol	1.20E+04	4.98	1.09E+04	7.32	6.04E+03	13.70	1.65E+04	8.18
27 Hydroxycholesterol	2.49E+04	10.90	3.09E+04	8.11	3.48E+04	13.89	5.27E+04	5.42
Progesterone	7.00E+06	3.16	1.50E+07	9.23	2.65E+07	8.67	4.39E+07	4.55
Corticosterone	1.45E+07	11.67	1.09E+07	2.42	9.93E+06	6.91	1.73E+07	3.62
Testosterone	3.01E+07	2.09	2.10E+07	7.51	2.07E+07	6.64	2.77E+07	3.28

Table S7 Extraction method optimization

Methods	Bligh and Dyer		MeOH		SPE	
Compound	mean	RSD (%)	mean	RSD (%)	mean	RSD (%)
Squalene	9.29E+05	4.20	2.22E+06	4.68	1.45E+06	2.36
Lanosterol	2.66E+06	1.12	2.66E+05	7.95	4.10E+05	10.30
7 Dehydrocholesterol	9.84E+04	2.35	1.68E+04	5.48	2.69E+05	7.36
24 Hydroxycholesterol	9.30E+03	4.65	1.68E+04	9.85	3.22E+04	8.35
25 Hydroxycholesterol	1.87E+04	7.63	6.39E+03	5.95	3.22E+04	2.56
27 Hydroxycholesterol	1.38E+04	8.87	1.87E+04	6.34	3.22E+04	6.35
Progesterone	4.27E+04	4.36	5.49E+04	2.35	8.02E+04	9.23
Corticosterone	4.30E+05	4.28	5.74E+05	1.93	2.35E+05	7.33
Testosterone	2.68E+05	1.35	2.02E+05	1.80	6.54E+06	4.89

Table S8 Accuracy and estimated concentration of steroids in killifish tissues

Compound	elemental composition	Δppm	Estimated concentration in tissues young/old (pg/mL)			
			gut	liver	brain	gonad
Squalene	$C_{30}H_{50}$	-1.407	≈0.36-0.33	≈0.35-0.17	≈0.34-0.15	≈0.9-0.45
Lanosterol	$C_{30}H_{50}O$	-2.322	≈21-22	≈21-12	≈6.3-1.6	≈56-32
7 Dehydrocholesterol	$C_{27}H_{44}O$	-0.781	≈20-21	≈12-6	≈25-23	≈42-49
24 Hydroxycholesterol	$C_{27}H_{46}O_2$	-3.938	≈10-9	≈12-10	≈10-5	≈10-13
25 Hydroxycholesterol	$C_{27}H_{46}O_2$	-2.198	≈4-5	≈5-10	≈10-20	≈5-37
27 Hydroxycholesterol	$C_{27}H_{46}O_2$	-2.274	≈2-4	≈0.2-2	≈2-10	≈10-41
Progesterone	$C_{21}H_{30}O_2$	1.634	≈172-50	≈44-40	≈50-23	≈181-40
Corticosterone	$C_{21}H_{30}O_4$	2.648	≈39-44	≈20-25	≈20-25	≈81-62
Testosterone	$C_{19}H_{28}O_2$	-2.919	≈670-698	≈374-113	≈117-112	≈3240-328

Table S9 LC-Gradient

Time (min)	Eluent %B
0.3	10
8	99
10	99
11	10
12	10

Figure S1
Gut Tissue

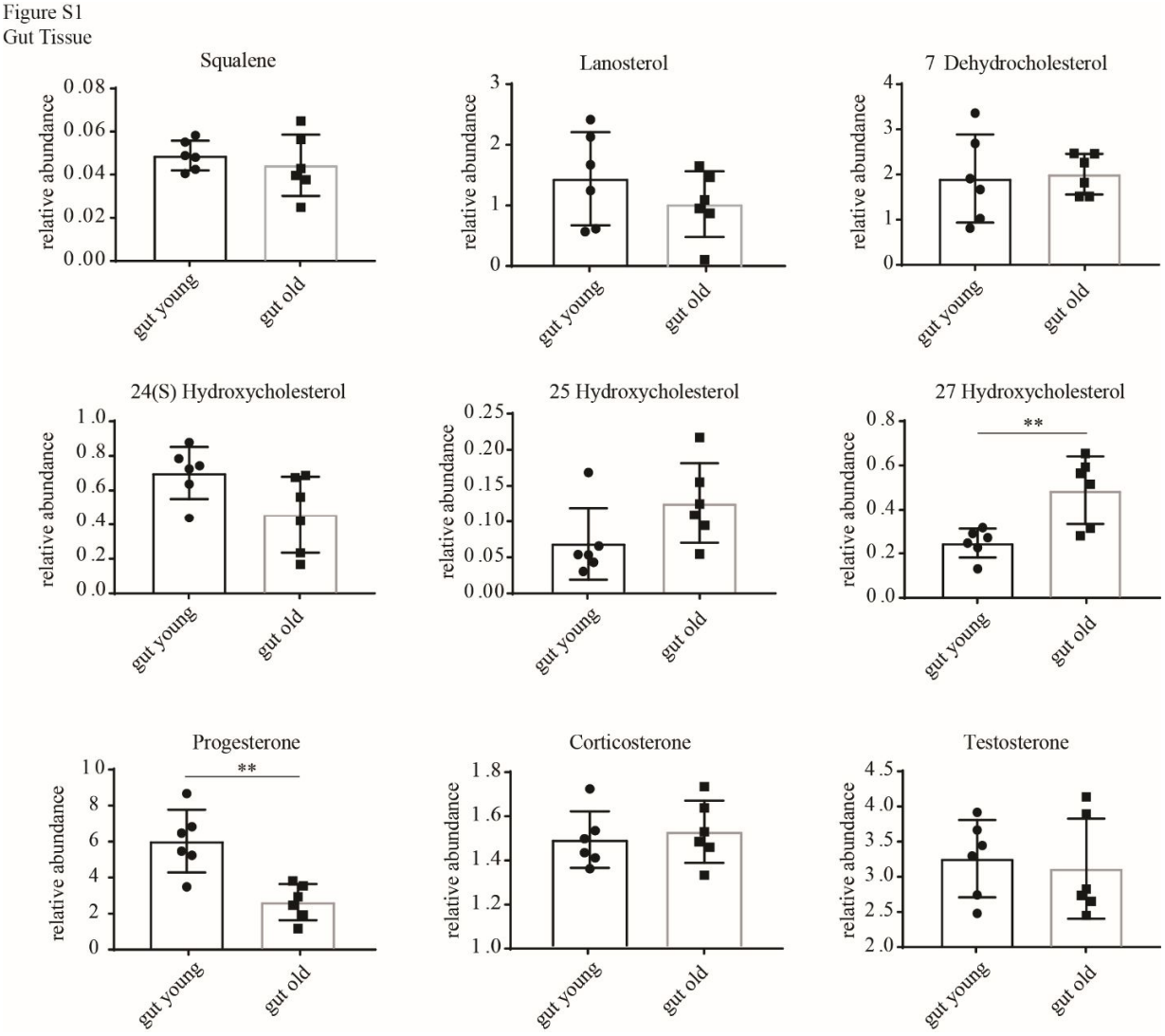

Fig. S1 Quantification of steroid in gut tissue of young and old killifish. Statistical test were performed using t-test. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Figure S2
Liver Tissue

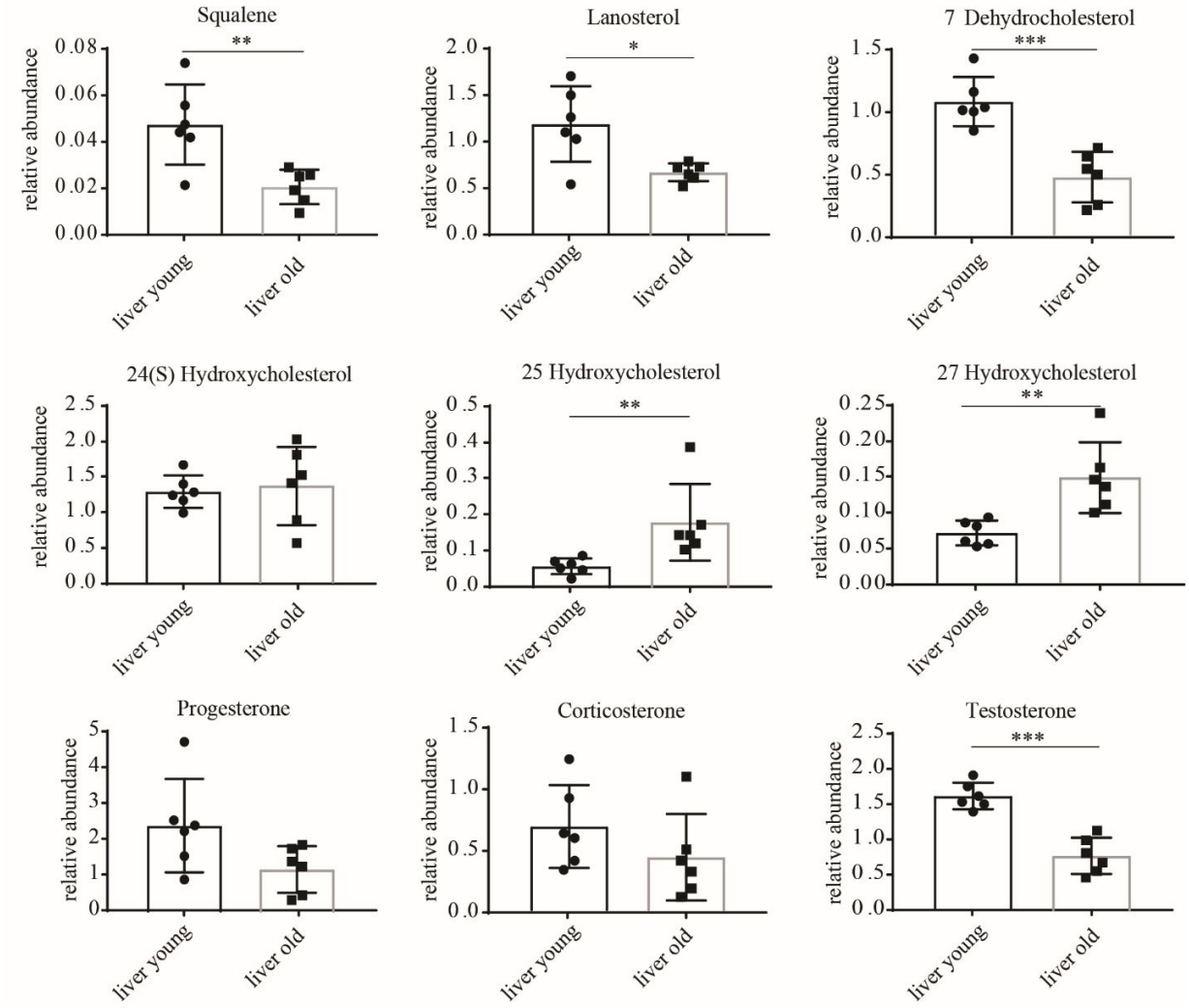

Fig. S2 Quantification of steroid in liver tissue of young and old killifish. Statistical test were performed using t-test. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Figure S3
Brain Tissue

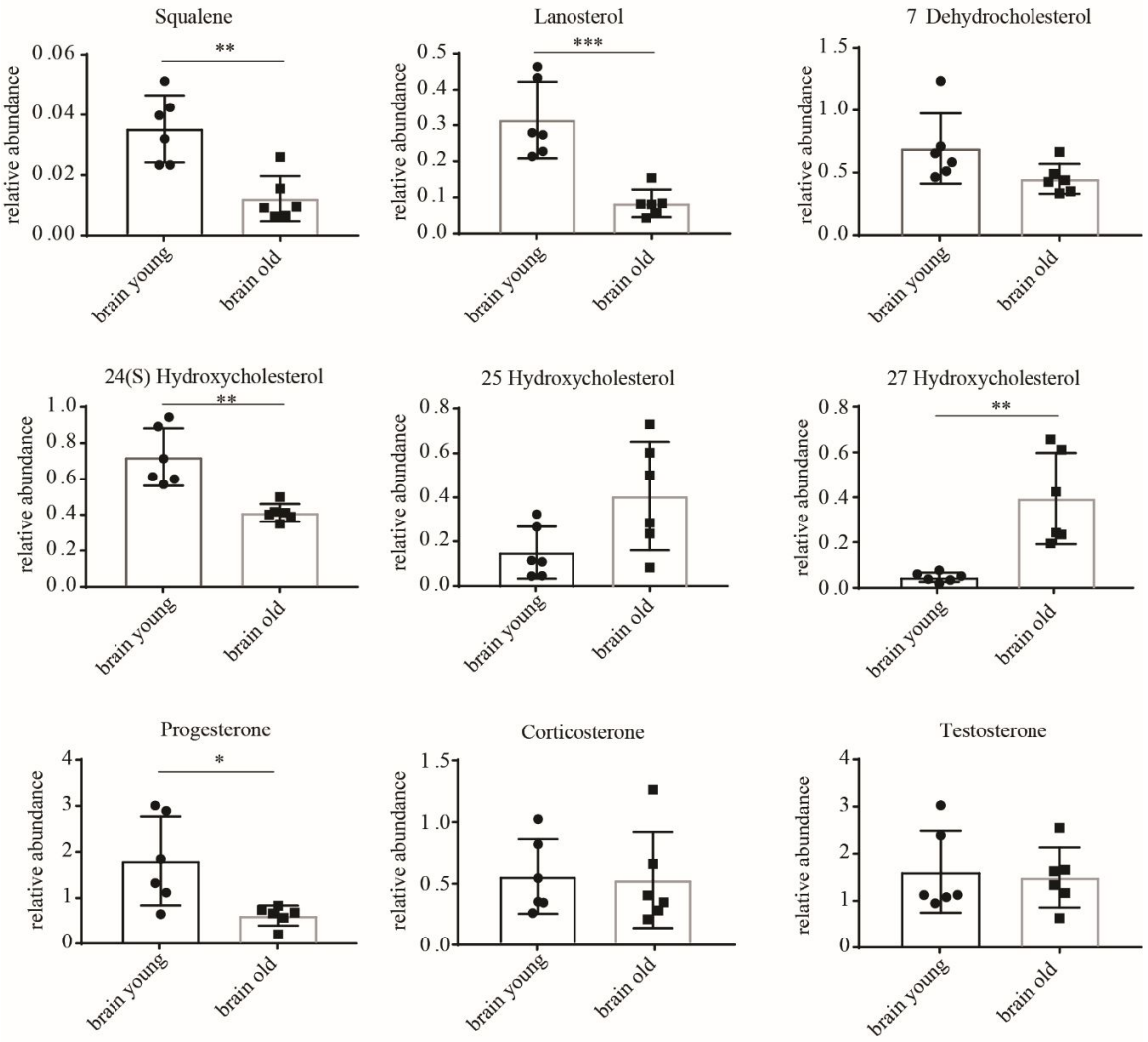

Fig. S3 Quantification of steroid in brain tissue of young and old killifish. Statistical test were performed using t-test. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Figure S4
Gonad Tissue

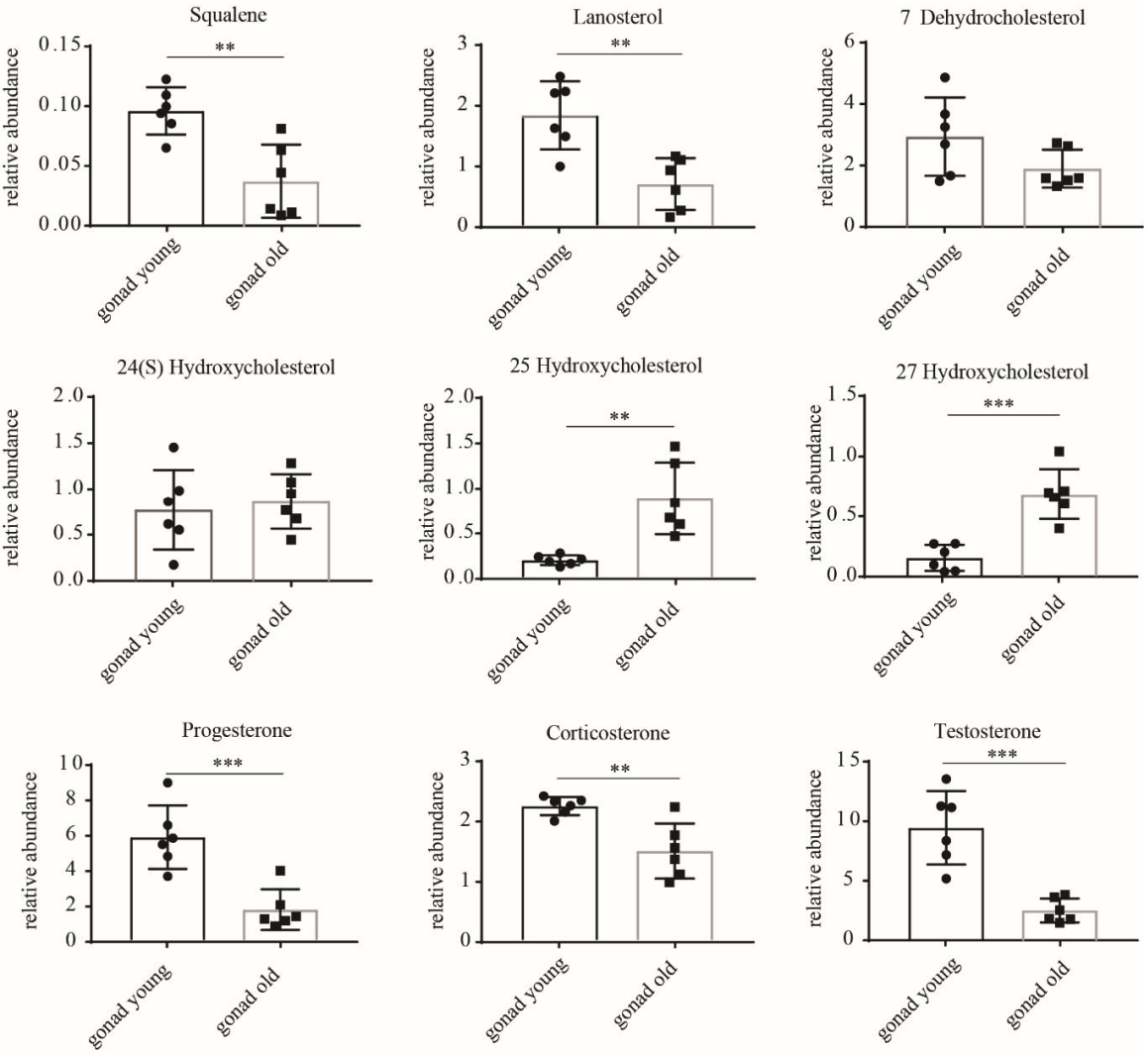

Fig. S4 Quantification of steroid in gonad tissue of young and old killifish. Statistical test were performed using t-test. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Table S10 Comparison with existing LC-MS based method

Compound	LOQ	Ionisation/Setup	References
Cortisol	2.5nmol/L	HPLC-ESI-MS/MS	(1)
17OH pregnelone	43 pg/mL	HPLC-ESI-MS/MS	(2)
Testosterone	22 pg/mL	HPLC-ESI-MS/MS	(2)
Pregnelone	250 pg/mL	HPLC-ESI-MS/MS	(3)
Testosterone	72 pg/mL	HPLC-ESI-MS/MS	(4)
Testosterone	20 pg/mL	HPLC-ESI-MS/MS	(5)
Testosterone	20 pg/mL	HPLC-ESI-MS/MS	(6)
Testosterone	20 pg/mL	HPLC-ESI-MS/MS	(7)
Testosterone	50 pg/mL	HPLC-ESI-MS/MS	(8)
Lanosterol	50 ng/mL	APCI	(9)
Squalene	50 µg/mL	GC-MS	(10)
Lanosterol	20 µg/mL	HPLC-ESI-MS/MS	(10)
7 Dehydrocholesterol	200 µg/mL	HPLC-ESI-MS/MS	(10)
7-α/β-OHC,	0.5 ng/ml	HPLC-ESI-MS/MS	(10)
25-hydroxycholesterol	4.0 ng/mL	HPLC-ESI-MS/MS	(11)
27-hydroxycholesterol	5 ng/mL	HPLC-ESI-MS/MS	(11)
27-hydroxycholesterol	4 ng/mL	HPLC-ESI-MS/MS	(11)
7-dehydrocholesterol	26.0 ng/mL	HPLC-ESI-MS/MS	(11)
Several sterols	0.01 to 20 ng/mL	HPLC-ESI-MS/MS	(12)

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