Biosynthesis of LC-PUFA and VLC-PUFA in *Pampus argenteus*: Characterization of Elovl4 elongases and regulation under acute salinity

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Contents:

Table S1. Primers used in this study

Aim	Primer name	Primer sequence (5'-3')
Partial fragment cDNA cloning	Elovl4a-F	TTGCAGACAAGCGGGTGGA
	Elovl4a-R	CCGAAGAGGATGATGAAGGTGA
	Elovl4b-F	TTGTGATGGGCTTGCCTCCCTTAT
	Elovl4b-R	ACCCTCATAGTCTACAACTTCAGC
3' RACE PCR	Elovl4a-3R-F1	CATCTGCTACGCCATCACCTT
	Elovl4a-3R-F2	GCAGGAGGGCAGTCATTCTTT
	Elovl4a-3R-F3	GGACTATTCGGACGACCCC
	Elovl4b-3R-F1	ATGATGTCAATGAAGTCAGGATAGCA
	Elovl4b-3R-F2	CACGTCCTCATGTACGGTTACTATGG
	Elovl4b-3R-F3	ACCACGCTTACCGACGTAAACCTTCC
5' RACE PCR	Elovl4a-5R-R1	AGGAACAGCAGGTAGGAGGTG
	Elovl4a-5R-R2	AGTCCACTGGTTGGCAAATGT
	Elovl4a-5R-R3	TTGATGCCAATCCACCAGAGC
	Elovl4b-5R-R1	AGGTGACAGCATAGCCAATCAGAGC
	Elovl4b-5R-R2	GCACCAAAAAAAGATTGTCCACCTG
	Elovl4b-5R-R3	GCAGATGCTATCCTGACTTCATTGA
ORF cloning for functional characterization	Elovl4a-V-F	CCCAAGCTTATGGAGATTGTAACACATTTAAT
	Elovl4a-V-R	CCCCTCGAGCTAATCTCTTTTGGCTCTTCCTTT
	Elovl4b-V-F	CCCAAGCTTATGGAGGCTGTAACACATCTGG
	Elovl4b-V-R	CCCGAATTCTTACTCCCTTTTCGCTCTTCC
	Fads2-V-F	CGGGGTACCATGGGTGGTGGAGGCCAACT
	Fads2-V-R	CCGCTCGAGTCATTTATGAAGATATGCAT
qPCR	*Elovl4a*-Q-F	ACTTCTACTACCAGACCTACCG
	Elovl4a-Q-R	TTTTGGCTCTTCCTTTCC
	Elovl4b-Q-F	TTGGCTATGCTGTCACCT
	Elovl4b-Q-R	TTACTCCCTTTTCGCTCT
	18S-F	AGTTGGTGGAGCGATTTGTCTGGTTA
	18S-R	CTAAGAAGTTGGACGCCGACCGCACG
	Elovl5-F	GGGGATTTCTGCTGCTGGAC
	Elovl5-R	AGATTGTAGAGCACCAGGAGGC
	Fads2-F	ATGCTGACGCTGCTGTGCTC
	Fads2-R	ATGCTGGAAATGTCGATGGTT
	Srebp-F	CAGGACATGGAGTTTGGAGG

Srebp-R	AGGATGGAGGAGGACTTGGAT
Lxrα-F	TCTGCAGGGAGAACCGAATG
Lxrα-R	CGCTGCACACTTCATTACCC
Pparα-F	CCACTACCAACCCCCTTCAC
Pparα-R	GGAGCTAAGGACGCTGTTGT
Hnf4α-F	GTGGACAAAGACAAGCGAAAT
Hnf4α-R	GTTCTTATGTCGCCGTTCAG

Table S2. Fatty acid compositions (mg/g dry matter) of experimental diet

Parameter	Diet
14:0	2.79
16:0	18.82
18:0	5.29
20:0	0.24
ΣSFA[a]	27.15
16:1n	4.41
18:1n-9	12.25
20:1n-9	0.59
22:1n-11	0.06
ΣMUFA[b]	17.32
18:2n-6	3.33
18:3n-6	0.39
20:2n-6	0.11
20:4n-6	0.34
22:4n-6	0.09
Σn-6 PUFA[c]	4.27
18:3n-3	0.53
18:4n-3	0.29
20:4n-3	0.28
20:5n-3	3.82
22:5n-3	0.80
22:6n-3	8.43
Σn-3 PUFA[d]	14.16
n-3/n-6 PUFA[e]	3.31
Σn-3 LC-PUFA[f]	13.34
Σn-6 LC-PUFA[g]	0.55
ΣLC-PUFA[h]	13.88

Some fatty acids, of which the contents are minor, trace amount or not detected, such as 22:0, 24:0, 14:1n-7, 22:1n-9, 20:3n-6, 20:5n-6, 20:3n-3 were not listed in Table S2.

[a] SFA, saturated fatty acids.

[b] MUFA, mono-unsaturated fatty acids.

[c] n-6 PUFA, n-6 polyunsaturated fatty acids.

[d] n-3 PUFA, n-3 polyunsaturated fatty acids.

[e] n-3/n-6 PUFA, n-3 polyunsaturated fatty acids: n-6 polyunsaturated fatty acids.

Table S3. Fatty acid compositions (mg/g dry matter) of *P. argenteus* in liver cultured in different salinity .

Parameter	Salinity		
	12 ppt	22 ppt	32 ppt
14:0	3.31±0.30	4.05±3.09	3.09±0.17
16:0	27.53±0.68^{ab}	28.32±0.77^{a}	24.85±0.12^{b}
18:0	7.37±0.01^{a}	6.55±0.50^{b}	6.38±0.08^{b}
20:0	0.21±0.04	0.20±0.01	0.16±0.01
ΣSFA[a]	38.41±0.33^{a}	39.13±0.67^{a}	34.49±0.13^{b}
16:1n	6.90±0.79	8.89±0.05	6.70±0.60
18:1n-9	48.31±3.43	44.16±5.90	34.63±4.72
20:1n-9	1.72±0.16	1.46±0.24	1.02±0.08
22:1n-11	0.65±0.05^{a}	0.44±0.01^{b}	0.49±0.06^{ab}
ΣMUFA[b]	57.58±4.09	54.95±6.09	42.85±5.45
18:2n-6	1.59±0.18	1.85±0.04	1.80±0.09
18:3n-6	0.13±0.01	0.13±0.01	0.11±0.01
20:2n-6	0.19±0.02	0.20±0.02	0.18±0.00
20:4n-6	2.48±0.08	2.46±0.10	2.33±0.05
22:4n-6	0.4±0.03^{a}	0.28±0.02^{b}	0.19±0.01^{c}
Σn-6 PUFA[c]	4.79±0.15	4.92±0.11	4.62±0.07
18:3n-3	0.35±0.04	0.44±0.05	0.34±0.04
18:4n-3	0.35±0.02	0.41±0.08	0.35±0.08
20:4n-3	0.93±0.02	0.93±0.09	0.75±0.05
20:5n-3	6.12±0.09	6.77±0.62	6.13±0.07
22:5n-3	6.22±0.01^{a}	4.94±0.63^{ab}	4.32±0.34^{b}
22:6n-3	30.22±0.98^{a}	29.03±1.03^{a}	26.72±0.29^{b}
Σn-3 PUFA[d]	44.20±1.01^{a}	42.52±1.70^{ab}	38.60±0.54^{b}
n-3/n-6 PUFA[e]	9.25±0.49	8.66±0.51	8.37±0.17
Σn-3 LC-PUFA[f]	43.50±1.06^{a}	41.67±1.60^{ab}	37.91±0.64^{b}
Σn-6 LC-PUFA[g]	3.07±0.05^{a}	2.94±0.07^{a}	2.70±0.04^{b}
ΣLC-PUFA[h]	46.57±1.10^{a}	44.62±1.54^{ab}	40.61±0.67^{b}

Data represent mean ± S.E.M. of three replicates (n=3). Values in the same column with different superscripts are significantly different ($P < 0.05$). Some fatty acids, of which the contents are minor, trace amount or not detected, such as 22:0, 24:0, 14:1n-7, 22:1n-9, 20:3n-6, 20:5n-6, 20:3n-3 were not listed in Table S2.

[a] SFA, saturated fatty acids.

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[d] n-3 PUFA, n-3 polyunsaturated fatty acids.

[e] n-3/n-6 PUFA, n-3 polyunsaturated fatty acids: n-6 polyunsaturated fatty acids.

[f] n-3 LC-PUFA, n-3 long-chain polyunsaturated fatty acids.
[g] n-6 LC-PUFA, n-6 long-chain polyunsaturated fatty acids.
[h] LC-PUFA, long-chain (C_{20-24}) polyunsaturated fatty acids.

Table S4. Fatty acid compositions (mg/g dry matter) of *P. argenteus* in gill cultured in different salinity.

Parameter	Salinity		
	12 ppt	22 ppt	32 ppt
14:0	6.42±0.62	7.65±0.55	7.24±0.45
16:0	25.31±2.30	26.61±1.99	27.32±0.61
18:0	6.46±0.49	5.93±0.40	6.42±0.36
20:0	0.55±0.02	0.59±0.06	0.49±0.02
ΣSFA[a]	38.73±3.38	40.78±2.92	41.47±1.24
16:1n	8.68±0.95	10.3±1.09	9.74±0.58
18:1n-9	42.52±4.08	46.71±5.61	46.81±1.9
20:1n-9	1.96±0.25	2.27±0.28	1.94±0.13
22:1n-11	0.88±0.14	0.83±0.02	0.91±0.05
ΣMUFA[b]	54.04±5.28	60.11±6.94	59.41±2.55
18:2n-6	2.58±0.26	3.23±0.53	3.47±0.28
18:3n-6	0.13±0.01[a]	0.22±0.02[b]	0.17±0.02[ab]
20:2n-6	0.17±0.02	0.24±0.04	0.2±0.01
20:4n-6	1.82±0.04	1.80±0.1	1.95±0.07
22:4n-6	0.46±0.03	0.61±0.11	0.60±0.01
Σn-6 PUFA[c]	5.16±0.31	6.09±0.79	6.39±0.30
18:3n-3	0.68±0.08	0.85±0.12	0.80±0.07
18:4n-3	1.20±0.17	1.43±0.14	1.25±0.09
20:4n-3	1.31±0.14	1.37±0.08	1.43±0.05
20:5n-3	7.54±0.58	7.53±0.66	7.46±0.41
22:5n-3	4.60±0.36	4.97±0.59	4.66±0.34
22:6n-3	16.34±0.92	16.52±1.30	15.23±0.60
Σn-3 PUFA[d]	31.67±2.23	32.68±2.74	30.93±1.47
n-3/n-6 PUFA[e]	6.13±0.15[a]	5.44±0.29[ab]	4.85±0.15[b]
Σn-3 LC-PUFA[f]	29.80±1.99	30.39±2.48	28.88±1.38
Σn-6 LC-PUFA[g]	2.45±0.06	2.64±0.25	2.76±0.05
ΣLC-PUFA[h]	35.64±2.34	36.73±3.02	34.98±1.54

Data represent mean ± S.E.M. of three replicates (n=3). Values in the same column with different superscripts are significantly different ($P < 0.05$). Some fatty acids, of which the contents are minor, trace amount or not detected, such as 22:0, 24:0, 14:1n-7, 22:1n-9, 20:3n-6, 20:5n-6, 20:3n-3 were not listed in Table S3.

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[e] n-3/n-6 PUFA, n-3 polyunsaturated fatty acids: n-6 polyunsaturated fatty acids.

[f] n-3 LC-PUFA, n-3 long-chain polyunsaturated fatty acids.

[g] n-6 LC-PUFA, n-6 long-chain polyunsaturated fatty acids.

[h] LC-PUFA, long-chain (C_{20-24}) polyunsaturated fatty.