Supplementary Materials

Figure S1. Amino acid sequence alignment showing part of the AmhN domain and the canonical cleavage site of Amh. GenBank/ UniProtKB/ Ensembl accession numbers: *Homo sapiens*, AAC25614.1; *Mus musculus*, CAA44912.1; *Gallus gallus*, NP_990361.1; *Alligator sinensis*, XP_006037226.1; *Xenopus laevis*, BAO04196.1; *Protopterus annectens*, AWT24621.1; *Latimeria menadoensis* (partial),

CCP19124.1; *Callorhinchus milii*, XP_007896900.1; *Acipenser baerii*, AKE47491.1; *Gasterosteus aculeatus*, G3PGH3; *Dicentrarchus labrax*, CAJ78431.1; *Oreochromis niloticus*, XP_013130583.1; *Acanthopagrus schlegelii*, ADB22521.1; *Epinephelus coioides*, AJW76790.1; *Monopterus albus*, AHF27393.1; *Oncorhynchus mykiss*, CDQ57255.1; *Oryzias latipes*, ABG24272.1; *Takifugu rubripes*, H2V975**;** *Danio rerio*, NP_001007780.1. The numbers on the right refer to amino acid position. Light blue font color indicates a proprotein convertase subtilisin/kexin type 2 cleavage site predicted by iProt-Sub server that could not be proven. Mandarine font color exposes a conserved N-glycosylation site. The primary proteolytic cleveage sites in human AMH (Arg^{445}-Ala-Gln-Arg/ Ser; yellow) and predicted subtilisin/kexin type 2 cleavage site in sea bass Amh (Arg^{426}-Ala-Thr-Arg/ Ala; red) are indicated. The light red box highlights conservation of the sea bass predicted cleavage site Arg^{426}-Ala-Thr-Arg among nine distantly related teleost species and gar. Amino acid similarities (80%) are boxed in grey.

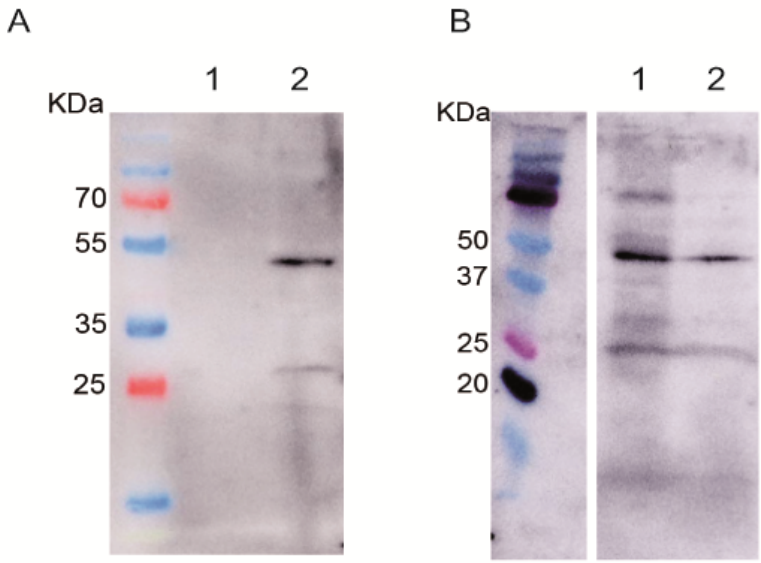

Figure S2. Specificity of the rabbit anti- sea bass Amhr2 antibody. (**A**) Total protein extracts from cells expressing the pcDNA3 empty vector as control (**A**, lane 1) and sea bass Amhr2 (**A**, lane 2). (**B**) Total protein extracts from sea bass previtellogenic ovary (**B**, lane 1) and from follicular cells (**B**, lane2). Approximately 40 μg of total protein from all extracts were analyzed. Protein standards (in kDa) were used to estimate protein molecular weight.

Table S1. Primers used in this study[a].

Primer	Position	Sequence (5′ → 3′) [b,c]		Annealing temp. [d]
*Bam*HI-amh1	29 [e]	ATAGGATCCCAGAGCAGGATGATGGTTGTG	sense	58 ºC → 68 ºC 70 ºC → 65 ºC
amh2	1460 [e]	ACTGTAAGGTGGTCAGCTGGA	antisense	64 ºC → 59 ºC
amh3	863 [e]	CTGAAGCGGTTCCTGGGCG	sense	52 ºC → 62 ºC
amh4-*Eco*RI	1704 [e]	ATCGAATTCACCAACTAAAGACTATGTGAAAC	antisense	58 ºC → 68 ºC 70 ºC → 65 ºC
amh10	1 [f,g]	AATGGGAATTCCTGCAGGTCTCACAAGGACT	sense	58 ºC → 68 ºC 65 ºC
amh12	1210 [g]	GAGAAGCGAGCTGACCCCAACAACCCA	sense	58 ºC → 68 ºC
amh13	1574 [f]	TACGACCTAGGTTTAGCGGCATCCACACTCC	antisense	58 ºC → 68 ºC 65 ºC
amh14	1247 [f]	gtggtggtgatggtggtgTCGCTTCTCCAGTCCTCTCTGCACCTCAT	antisense	58 ºC → 68 ºC
amh15	1210 [f]	GAGAAGCGAcaccaccatcaccaccacGCTGACCCCAACAACCCAG	sense	58 ºC → 68 ºC
amh16	1567 [g]	TACGACCTAGGTTAgtggtggtgatggtggtgACGACCTTCGATGCGGCATCCACACTCCCGTG	antisense	58 ºC → 68 ºC 65 ºC
amh17	1249 [g]	ACTGGGTTGTTGGGGTCAGCTCGCTTCTCCAGTCCTCTCTGCACCTCAT	antisense	58 ºC → 68 ºC
5′*AOX1*	855 [h]	GACTGGTTCCAATTGACAAGC	sense	54 ºC
3′ *AOX1*	1347 [h]	GGCAAATGGCATTCTGACATCCT	antisense	54 ºC

[a] Primers were obtained from Invitrogen or Isogen (Thermo Fisher Scientific; Isogen Life Science).
[b] Underlined sequences indicate restriction enzyme sites for cloning purposes. [c] Lower case letters code for Histidines.
[d] Touch-down or -up PCR maximum and minimum annealing temperatures.
[e] Position in *Dicentrarchus labrax amh* cDNA sequence with GenBank accession no. AM232701.1. [f] Position in *Dicentrarchus labrax amh* cDNA sequence in pPICK9-His6Amh.
[g] Position in *Dicentrarchus labrax amh* cDNA sequence pPICK9-AmhHis6. [h] Position in pPICK9 vector.

Table S2. Primers and hydrolysis probe used for quantitative real-time PCR.

Gene	Sequence (5′ → 3′) [a]	nM [d]	Amplicon size	Efficiency	First published
amhr2 (JQ801443.1) [b]	fw: CCATCCTGCGTTCTTGTTCA	300	67 bp	0.97	[A] ([30])
	rv: TGAGCAAGACCCATGTTTGC	300			
	pr: [6~FAM]AATCGCCACTGGTCGAGCCACAC[TAMRA]	125			
amh (AM232701.1) [b]	fw: TCCAAACACTGCTAACATCAACAA	50	74 bp	0.94	[B] ([21])
	rv: TGGCGTGGTTCTTGGGATT	300			
	pr: [6~FAM]CCATGGCTCATGTGCTTTCCCCCT[TAMRA]	125			
cyp19a1 (AJ311177.1) [b]	fw: TCCTCGCCGCTACTTCCA	300	65 bp	0.98	[C] ([61])
	rv: TGGCGATGTGCTTACCAACA	300			
	pr: [6 ~ FAM]CATTCGGTTCAGGCCCTCGCG[TAMRA]	100			
rpl13a (DT044539) [b]	fw: TCTGGAGGACTGTCAGGGGCATGC	100	148 bp	0.91	[D] ([72])
	rv: AGACGCACAATCTTGAGAGCAG	100			
luc1 (MH759210.1) [c]	fw: TACAACACCCCAACATCTTCGA	900	67 bp	0.96	This study
	rv: GGAAGTTCACCGGCGTCAT	900			
	pr: [6 ~ FAM]CGGGCGTGGCAGGTCTTCCC[TAMRA]	200			

[a] Forward (fw) and reverse (rv) primers were obtained from Invitrogen™ (Life Technologies). Hydrolysis probe (pr) was purchased from Eurofins Genomics, Germany.
[b] GenBank accession no. for sea bass gene.
[c] GenBank accession no. for *Photinus pyralis* voucher KSH 11044
[c] Amount of primer or probe in the PCR.

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