Supplementary data

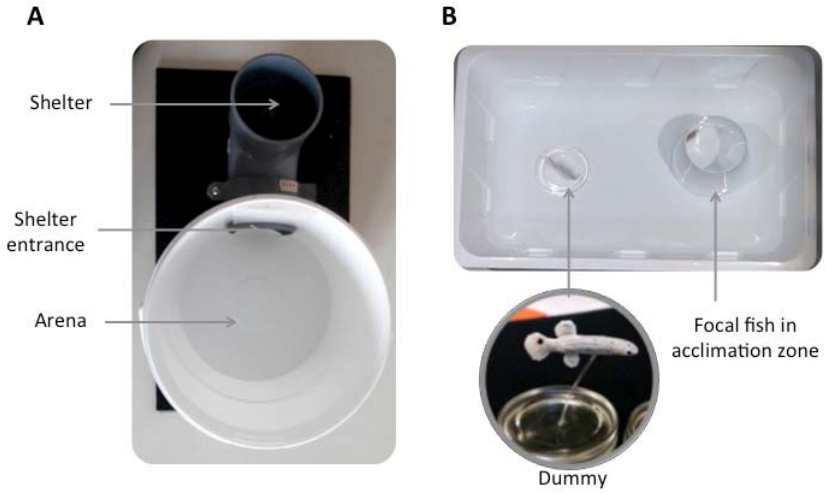

Figure S1 – Shelter test (A) and model test (B) used to respectively assess boldness and aggressiveness levels in the killifish mangrove rivulus.

Table S1
List of genes studied in qPCR, the according primer sequence used and characteristics

Genes	Primer sequence	Primer length	%GC	Tm (°C)	Amplicon lenght	Efficiency (%)
Glula	F : ACATACGTGCCTACGATCCG	20	59	55	160	104.81
	R: AAGTAGCCCCGTTTCTCCTG	20	59	55		
Scl17a7	F : CCTGTCTGGTCTGGGATTCT	20	55	59	210	103.61
	R : ATAAAGCCACCAGGGATCTG	20	50	59		
RTN 4	F : GTGCTGATGTGGATCCTGAC	20	55	59	281	104.44
	R : GAACCTTTCCCACAACGTCT	20	50	59		
Ependymin	F : CTATGACGGCCTGAACCACA	20	55	56	130	100.16
	R : TTTGGTCTTCTGGTCGATCT	20	45	52		
DRD4	F : TGATGAGTGTCGTGACATGG	20	50	59	173	94.84
	R : GCTGCTGTTCATTCAGCATT	20	45	59		
CaM	F : GGCGAGGAAGATGAAGGACA	20	55	56	135	100.37
	R : GTCAGCTTCTCTCCCAGGTT	20	55	55		
MAOA	F : AGGGCCACCTTCCAAAGTTC	20	55	56	112	94.69
	R : TGTTCCACTCAGCCTGTCAA	20	50	55		
Reference genes						
18S	F :GAACTCACCGACACCAGCA	19	58	56	105	106.4
	R : ATCATCGACGCTCCTGGA	18	56	54		
β-actin	F:CTTGCGGAATCCACGAGACC	20	60	57	150	104.42
	R :CCAGGGCTGTGATCTCCTTCTG	22	59	58		

Table S2
ELISA results

BMAA sample	Day of exposure	BMAA diluted concentration (mg/L)	Dilution factor	BMAA concentration (mg/L)
Solution 15 mg/L	4/01/18	0.125995	150	18.89925
Solution 15 mg/L	4/04/18	0.15781	150	23.6715
Solution 15 mg/L	4/06/18	0.145489	150	21.82335
Solution 15 mg/L	4/08/18	0.127726	150	19.1589
Solution 15 mg/L	4/10/18	0.123573	150	18.53595
Solution 15 mg/L	4/12/18	0.110573	150	16.58595
Solution 15 mg/L	4/14/18	0.136851	150	20.52765
Solution 15 mg/L	4/18/18	0.138842	150	20.8263
Solution 15 mg/L	4/20/18	0.151836	150	22.7754
Solution 15 mg/L	4/24/18	0.158835	150	23.82525
Solution 15 mg/L	4/26/18	0.134644	150	20.1966
Solution 15 mg/L	4/30/18	0.108577	150	16.28655
Solution 15 mg/L	5/01/18	0.117282	150	17.5923
Solution stock (382,08 mg/L)	4/01/18	0.12976	3820.82	495.7896032
Solution stock (382,08 mg/L)	4/04/18	0.152208	3820.82	581.5593706
Solution stock (382,08 mg/L)	4/06/18	0.144362	3820.82	551.5812168

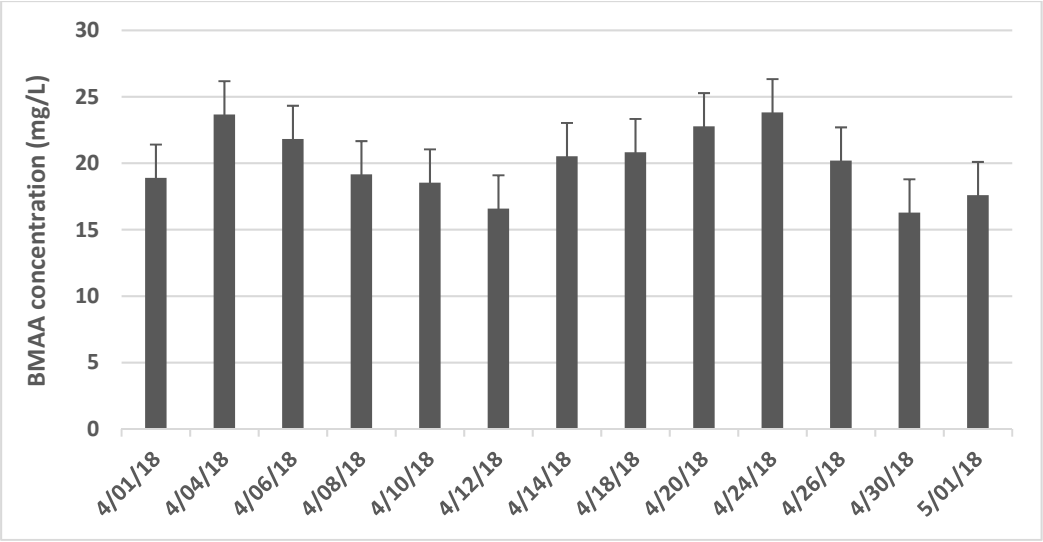

Figure S2 - Evolution of the actual BMAA concentration across the experiment for the group of fish exposed to 15 mg/L BMAA. Results were acquired by ELISA. Mean ± SD.

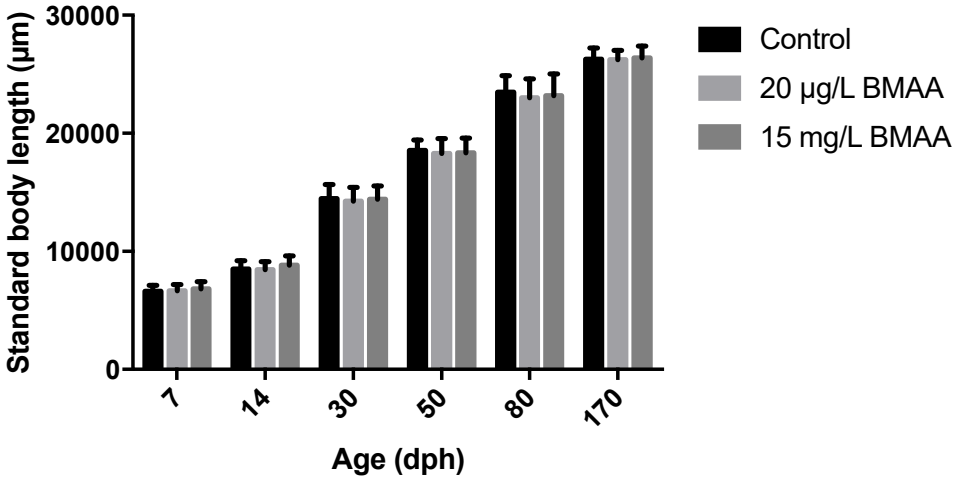

Figure S3 - Effects of BMAA during fish development (14 dph) on standard length of mangrove rivulus (Kryptolebias marmoratus) at 7, 14, 30, 50, 80, 170 dph. Results are represented as mean ± SEM.

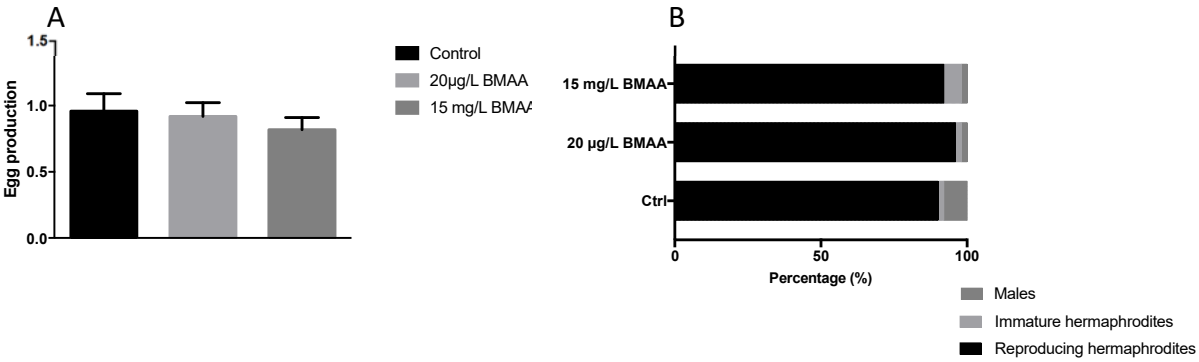

Figure S4 - BMAA effects on the reproduction of the mangrove rivulus (Kryptolebias marmoratus) A) Number of eggs layed by fish in each treatment from 70 to 120 dph (mean ± SEM). B) Percentage of reproducing hermaphrodites, males and immature hermaphrodites per treatments at 120 dph. No significant difference was shown (Friedman test for A and Chi-square test for B).

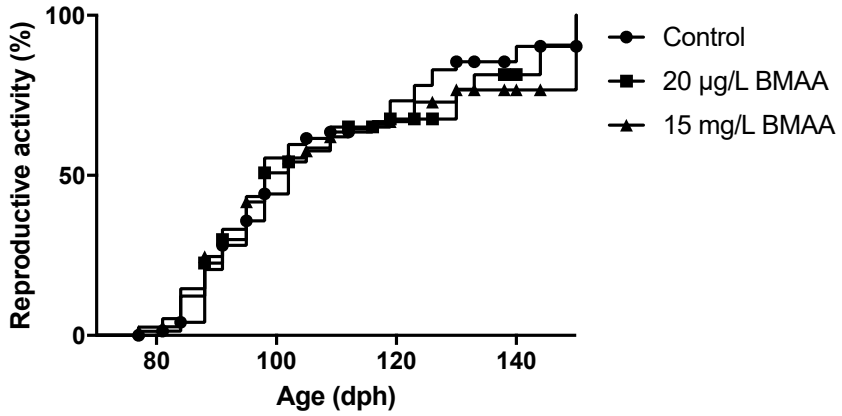

Figure S5 - Percentage of reproductively active individuals over time (days post hatching) in groups exposed to 0 (control), 20 µg/L BMAA and 15 mg/L BMAA. Reproduction was checked from 70 dph to 120 dph. Survival curves were created using the cumulative percentage of reproducing hermaphrodites against age with the Kaplan- Meier method and curves were compared with the logrank tests. No reproducing individuals at 120 dph were censored from the survival analysis. Medians age at maturity are : Control = 96.57 dph – 20 µg/L = 92.68 dph ; 15 mg/L = 92.00 dph.

Table S3
Correlation matrix of behavioral variables measured during the first replicate of shelter and model tests. Data represents Spearman coefficient. Significance levels: * p < 0.05; ** p < 0.01 and *** p < 0.001

	Distance (cm)	Latency out shelter (s)	Shelter time (s)	Number of bites	Number of attack postures	Bite latency (s)	Attack posture latency (s)
Distance (cm)		-0.7466***	-0.7985***	0.0189	0.0985	0.0242	-0.1138
Latency out shelter (s)			0.6179***	0.0440	-0.1253	-0.1059	0.0869
Shelter time (s)				-0.0452	-0.1315	-0.0031	0.1108
Number of bites					0.3338***	-0.9094***	-0.2078**
Number of attack postures						-0.3415***	-0.3503***
Bite latency (s)							0.2086**
Attack posture latency (s)							

Table S4
Shelter test results. Data are expressed as mean ± SEM for each treatment (BMAA vs control) across tests applied on fish at 120, 134 and 148 days post hatching (dph).

Treatment	Fish age (dph)	Total distance moved (cm)	Exit shelter latency (s)	Total time in shelter (s)
Ctl	120	671.2 ± 55.06	553.8 ± 81.93	1217 ± 44.67
	134	654.9 ± 51.94	399.1 ± 61.83	1329 ± 38.47
	148	777.2 ± 70.11	189.4 ± 37.88	1272 ± 36.50
20 µg/L BMAA	120	635.5 ± 51.08	544.1 ± 79.47	1196 ± 52.02
	134	692.3 ± 61.59	417.7 ± 78.59	1251 ± 50.07
	148	878.6 ± 56.59	170.5 ± 27.20	1128 ± 38.44
15 mg/L BMAA	120	645.2 ± 53.34	603.4 ± 72.19	1173 ± 52.44
	134	670.9 ± 55.23	386.2 ± 57.79	1325 ± 39.37
	148	840.9 ± 57.49	164.8 ± 38.90	1151 ± 46.43

Table S5
Model test results. Data are expressed as mean ± SEM for each treatment (BMAA vs control) across across tests applied on fish at 120, 134 and 148 days post hatching (dph).

Treatment	Fish age (dph)	Bites	Attack postures	Bite latency (s)	Attack posture latency (s)
Ctl	120	1.267 ± 0.245	4.578 ± 0.350	387.9 ± 32.37	86.90 ± 16.37
	134	0.422 ± 0.108	1.067 ± 0.154	476.0 ± 29.93	376.5 ± 33.23
	148	0.222 ± 0.100	0.733 ± 0.125	535.2 ± 23.29	402.6 ± 30.37
20 µg/L BMAA	120	1.000 ± 0.208	4.271 ± 0.338	407.0 ± 30.28	98.58 ± 17.19
	134	0.417 ± 0.148	1.083 ± 0.173	527.1 ± 21.71	333.9 ± 34.93
	148	0.417 ± 0.133	0.625 ± 0.151	525.6 ± 23.14	445.3 ± 31.67
15 mg/L BMAA	120	0.917 ± 0.188	4.000 ± 0.301	436.6 ± 28.92	101.8 ± 15.87
	134	0.479 ± 0.126	0.979 ± 0.159	496.3 ± 26.65	349.6 ± 36.69
	148	0.479 ± 0.146	1.080 ± 0.181	528.8 ± 21.72	368.1 ± 34.51