

Supplementary material

Efficacy of estradiol in feminising the eastern mosquitofish, *Gambusia holbrooki*: advance towards developing a genetic control option

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Percentage of sex ratio and Chi-Square analysis of *G. holbrooki* following E2 administration during embryonic (Experiment 1) and Juvenile (Experiment 2) life stage

Table S1. Percentage of sex ratio in control and treatment groups of both experiments at 50 DAP

Experiment	Treatments	Sex ratio (%)		χ^2	d.f.	P
		Male	Female			
Experiment 1: Treatment on neonates	C1	54.5	45.5	0.802	1	>0.05
	C2	47.7	52.3	0.160	1	>0.05
	T1	-	100			
	T2	-	100			
	T3	-	100			
	T4	-	100			
	T5	-	100			
Experiment 2: Treatment on embryo by ingestion by gravid females	C1	56.5	43.5	0.360	1	>0.05
	C2	56.3	43.7	0.640	1	>0.05
	T1	39.9	60.1	4.000	1	<0.05
	T2	14.2	85.8	51.840	1	<0.05
	T3	-	100			
	T4	-	100			
	T5	-	100			

Quantity of E2 delivered to each treated fish at the end of the 30 days treatment period

Table S2. Calculated average total quantity of food and E2 (mean ± s.d.) delivered to each *G. holbrooki* at the end of the 30 days treatment period

For the Average total quantity of food given, the total food given to all fish throughout the treatment duration divided by the number of fish per treatment

Experiment	E2 Dose	Average total quantity of food given (mg)	Average total quantity of E2 delivered (mg)
Experiment 1 : treatment on neonates	C1 – without ethanol	12.94 ± 0.913	N/A
	C2 – with 70% ethanol	12.86 ± 0.796	N/A
	T1 - 50 mg kg ⁻¹ feed	12.68 ± 1.303	0.00063 ± 0.000065
	T2 - 100 mg kg ⁻¹ feed	12.80 ± 0.682	0.00128 ± 0.000068
	T3 - 200 mg kg ⁻¹ feed	12.90 ± 1.113	0.00258 ± 0.000223
	T4 - 300 mg kg ⁻¹ feed	13.26 ± 1.318	0.00398 ± 0.000395
	T5 - 400 mg kg ⁻¹ feed	13.32 ± 0.937	0.00533 ± 0.000375
Experiment 1 : treatment on embryo by ingestion by gravid females	C1 – without ethanol	300.22 ± 3.384	N/A
	C2 – with 70% ethanol	300.14 ± 1.797	N/A
	T1 - 50 mg kg ⁻¹ feed	301.12 ± 1.714	0.0151 ± 0.000086
	T2 - 100 mg kg ⁻¹ feed	298.46 ± 4.275	0.0298 ± 0.000428
	T3 - 200 mg kg ⁻¹ feed	297.34 ± 5.131	0.0595 ± 0.001026
	T4 - 300 mg kg ⁻¹ feed	299.02 ± 3.737	0.0897 ± 0.001121
	T5 - 400 mg kg ⁻¹ feed	298.98 ± 5.251	0.1196 ± 0.002100