ALMA MATER STUDIORUM
UNIVERSITA' DI BOLOGNA

SCUOLA DI SCIENZE

Corso di laurea magistrale in BIOLOGIA MARINA

ALLEGATO 1a

Tesi di laurea in Adattamenti degli animali all'ambiente marino

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1. **APPENDIX 1**

1.1. EXPERIMENTAL SETUP AND SURGERIES

1.1.1. Photocells setup and tanks

Figure 1. Photocells attached to a PVC tube were then loaded inside the tanks.

Figure 2. Example of how photocells were positioned inside the tanks in vertical position.

Figure 3. Some of the tanks arranged for the experiment

1.2. OUTPUT OF QUANTASOFT SOFTWARE AND OPTIMIZATION OF PCR PROTOCOL

1.2.1. Melatonin genes and *mt1* receptor

For each gene two wells are shown as examples: the left well with template and thus with amplification while the right one is the control. Grey dots represent droplets without target (-). Blue dots represent droplets that contain target (+). The fluorescence of grey dots in a sample is higher than control owing to cDNA. Horizontal pink line represents the threshold between positive and negative droplets, based on the fluorescence detected.

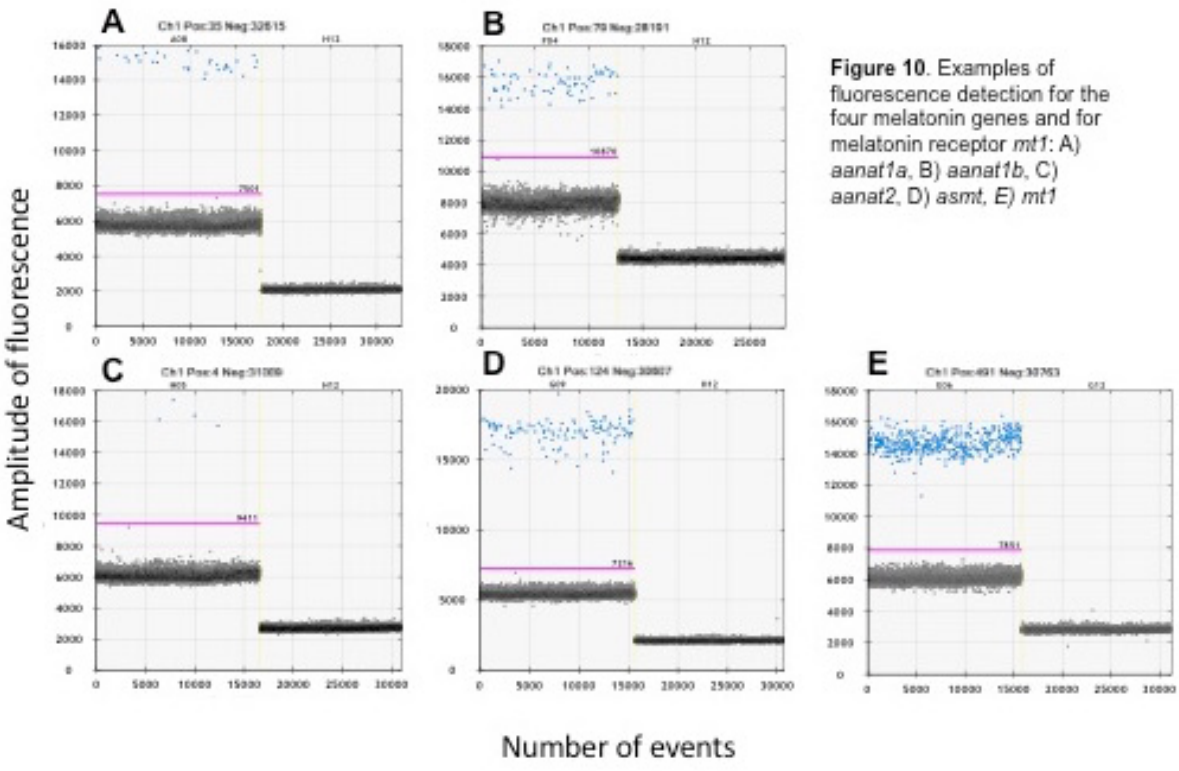

Figure 10. Examples of fluorescence detection for the four melatonin genes and for melatonin receptor *mt1*: A) *aanat1a*, B) *aanat1b*, C) *aanat2*, D) *asmt*, *E) mt1*

1.2.2. ddPCR optimization: concentration tests of the housekeeping gene *elfα*

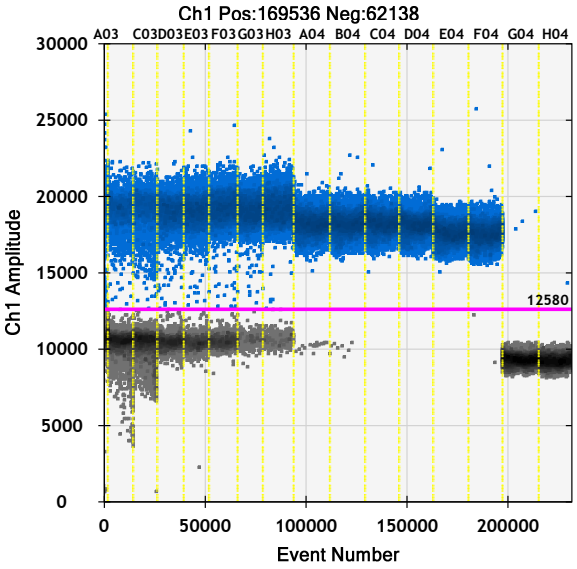

Figure 11. Range of concentrations of cDNA: 50 ng-0.75 ng. On the right of the graphs controls are shown, towards the left side the concentration of cDNA decrease as the amount of droplets without target (-) increases (grey dots).

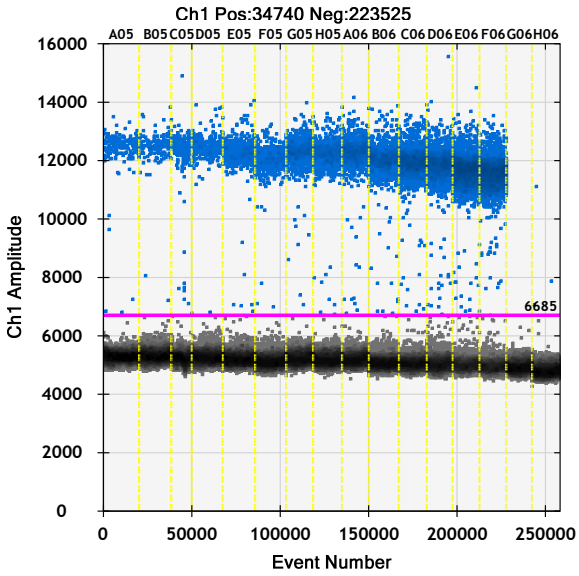

Figure 12. Range of concentrations of cDNA: 1 ng-0.015 ng. On the right of the graphs controls are shown, towards the left side the concentration of cDNA decrease as the amount of droplets with target (+) decreases (blue dots).

2. APPENDIX 2: ANOVA TABLES

Plasma melatonin

TWO-WAY ANOVA

	Df	Sum of Squares	F value	Pr (>F)
Treatment	6	7494	26.1307	$< 2.2*10^{-16}$
LD	1	694	14.5159	0.0002802
Treatment*LD	6	765	2.6665	0.0211144
Residuals	76	3633		

ONE-WAY ANOVA AT DAY

	Df	Sum of Squares	Mean of squares	F value	Pr (>F)
Treatment	6	4173.1	695.51	14.162	$6.53*10^{-08}$
Residuals	33	1620.7	49.11		

ONE-WAY ANOVA AT NIGHT

	Df	Sum of Squares	Mean of squares	F value	Pr (>F)
Treatment	6	4073.7	678.96	14.512	$5.72*10^{-09}$
Residuals	43	2011.8	46.79		

aanat1a

TWO-WAY ANOVA

	Df	Sum of Squares	F value	Pr (>F)
Treatment	3	36.45	2.0319	0.1267348
LD	1	8.32	1.3907	0.2460197
Treatment*LD	3	126.47	7.0493	0.0007566
Residuals	36	215.29		

aanat1b

ONE-WAY ANOVA AT DAY

	Df	Sum of Squares	Mean of squares	F value	Pr (>F)
Treatment	3	1.079	0.35966	0.4797	0.7006
Residuals	17	12.745	0.7497		

ONE WAY ANOVA AT NIGHT

	Df	Sum of squares	Mean of squares	F value	Pr (>F)
Treatment	3	7.6571	2.55237	5.0416	0.009755
Residuals	19	9.6189	0.50626		

aanat2

ONE-WAY ANOVA AT DAY

	Df	Sum of squares	Mean of squares	F value	Pr (>F)
Treatment	3	17.793	5.931	1.9703	0.1567
Residuals	17	51.175	3.0103		

ONE WAY ANOVA AT NIGHT

	Df	Sum of squares	Mean of squares	F value	Pr (>F)
Treatment	3	17.59	5.8649	0.3469	0.7918
Residuals	19	321.24	16.9075		

asmt

ONE-WAY ANOVA AT DAY

	Df	Sum of squares	Mean of squares	F value	Pr (>F)
Treatment	3	4.305	1.435	0.7341	0.5459
Residuals	17	33.23	1.9547		

ONE-WAY ANOVA AT NIGHT

	Df	Sum of squares	Mean of squares	F value	Pr (>F)
Treatment	3	27.709	9.2364	1.9205	0.1588
Residuals	20	96.187	4.8093		

mt1

TWO-WAY ANOVA

	Df	Sum of squares	F value	Pr (>F)
Treatment	3	1.411	2.157	0.10958
LD	1	11.09	50.8444	$1.90*10^{-08}$
Treatment*LD	3	3.963	6.0561	0.001841
Residuals	37	8.07		