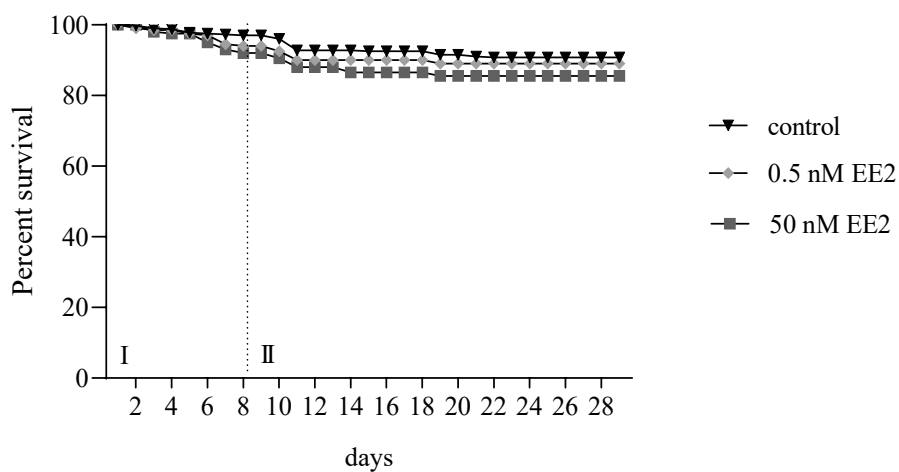


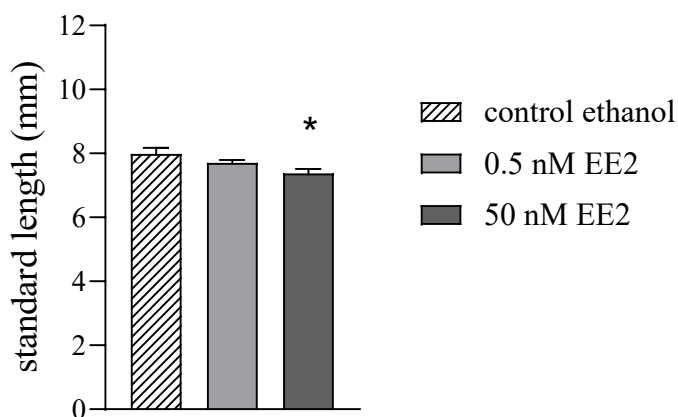
1 Supplementary Material

2 Supplementary I

3
4 **I-a:** Results from the experimental replicate 1 (October 2019 - not shown in the article). Authors
5 chose to show results from only one experimental replicate to facilitate the article's lecture. Relative
6 expression trends and significances were compared between experimental replicates. Eventual
7 discrepancies are commented in the "Discussion" section of the article.
8



9
10 *Survival (%) of sea bass larvae during phase I (from Day 0 until Day 8, I) and phase II (from*
11 *day 9 to day 28, II) of the experiment. Survival rate is presented as daily cumulative survival*
12 *percentage for each treatment.*
13

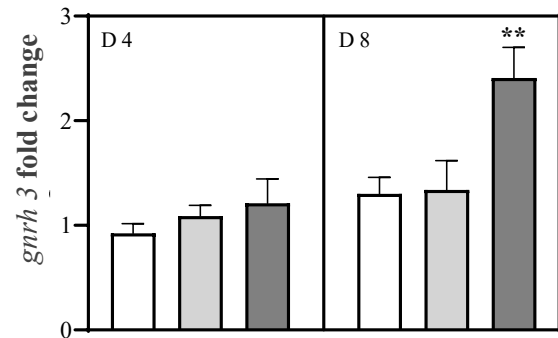
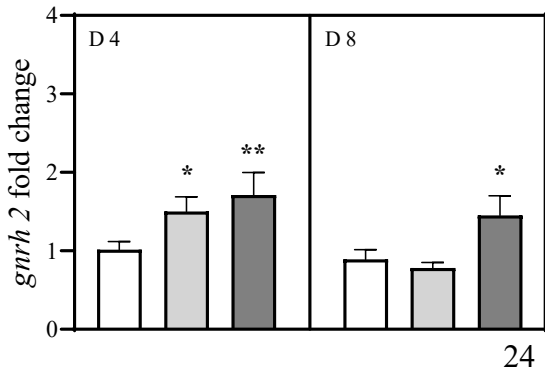
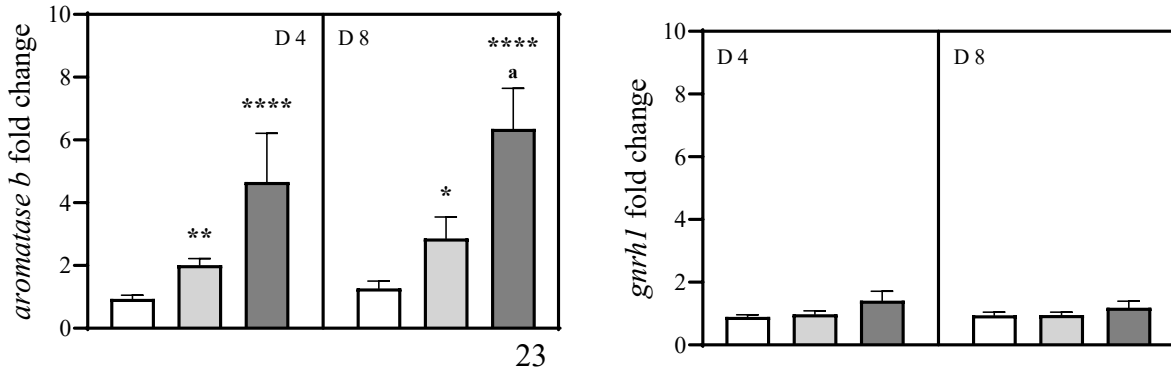


14
15
16 *Standard length measured (cm) in fish after 8 days of exposure to EE2 (~ 38 dph; end of phase*
17 *I). Values are presented as mean \pm SEM. *: $p < 0.05$ if compared to control conditions.*

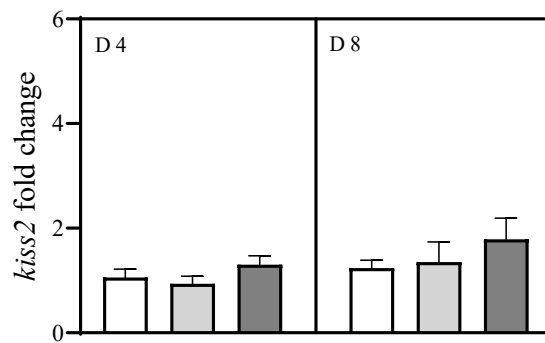
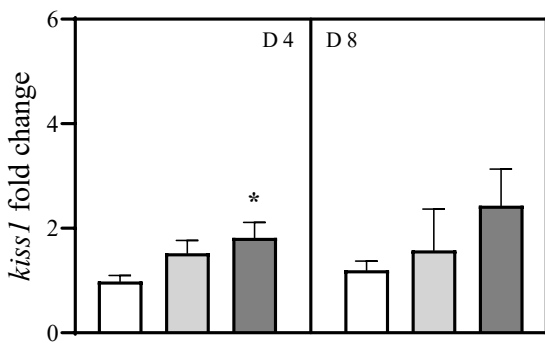
18

19 **I-b:** Gene expression results from the experimental replicate 1 (October 2019) not shown in the
20 article. Eventual discrepancies (e.g. *gnrh3* expression) are discussed in the “Discussion” section of
21 the article.

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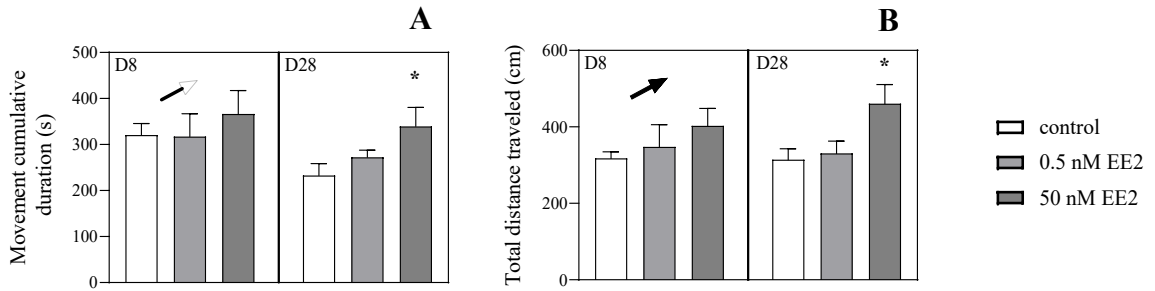
31 Data are presented as average fold change \pm SD relative to control conditions. *: $p < 0.05$; **: p
32 < 0.01 ; ****: $p < 0.0001$ if compared to control conditions.

33

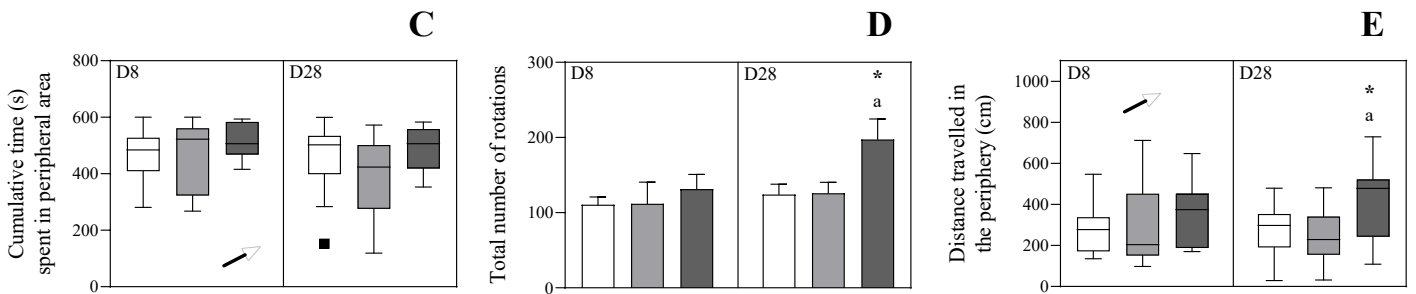
34

35 **I-c:** Behavior test's results from the experimental replicate 1 (October 2019) not shown in the article.

36



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40 Data are presented as mean \pm SEM (A, B and D) or Tukey boxplots (C and E). Graph A (movement
41 cumulative duration) and B (total distance traveled) show results for proxies of activity/locomotion
42 while C (cumulative time spent in the periphery) D (total rotations) and E (total distance traveled
43 in the periphery) are indexes of anxiety-like behaviors. *: $p < 0.05$; **: $p < 0.01$; if compared to
44 control conditions. a: $p < 0.05$ if compared to 0.5 nM EE2. Arrows describe non-significant trends.

45

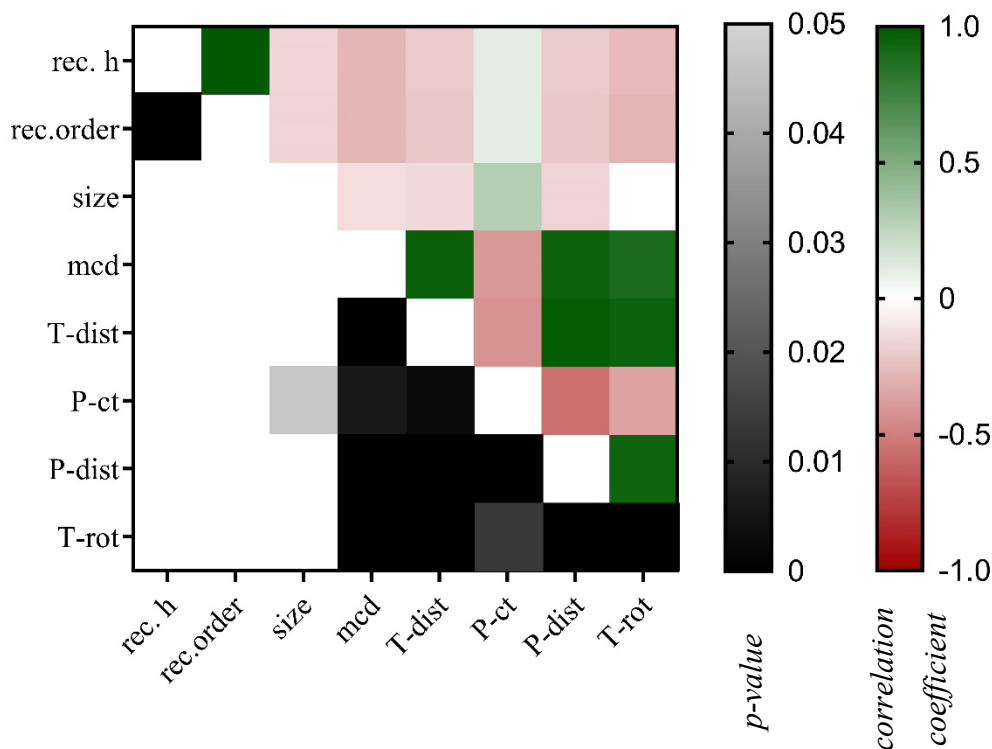
46

47 **Supplementary II**

48

49 Results of Pearson correlation analyses between behavioral endpoints and experimental variable
 50 to validate the stability of our behavioral protocol. *p*-values are represented in gray-scale colors
 51 while correlation coefficients are shown in red (negative correlation) or green (positive
 52 correlation). No significant correlations were found between experimental variables and
 53 behavioral endpoints, with the only exception of size (standard length) vs. cumulative time spent
 54 in the peripheric area ($p = 0.047$; $r = 0.29$).

55



56

57

58

59 *Behavior endpoints*: T-rot (total rotations); P-dist(distance travelled in the periphery); P-ct
 60 (cumulative time spent in the peripheric area); T-dist (total distance travelled); mcd (movement
 61 cumulative duration). *Experimental variables*: size; rec.order (recording order); rec.h (recording
 62 hour).

63

64

65 **Supplementary III**

66 Akaike information criterion for mortality analyses. All statistics were performed with R.

67 Codes

68 **#dataset preparation**

69 phaseI\$day<- as.factor(phaseI\$day)

70 phaseI\$group <- as.factor(phaseI\$group)

71

72 **#binary glm**

73 DT <-glm(event~day,data= phaseI)

74 DE <-glm(event~group,data= phaseI)

75 DI <-glm(event~day+group,data= phaseI)

76 DR <-glm(event~day*group,data= phaseI)

77 DR.aov<-aov(DR)

78 DI.aov<-aov(DI)

79 DT.aov<-aov(DT)

80 DE.aov<-aov(DE)

81

82 **#select the anova**

83 library(AICcmodavg)

84 model.set <- list(DT,DE,DI, DR)

85 model.names <- c("DT","DE", "DI", "DR")

86 aictab(model.set, modnames = model.names)

87

88 Results for *phaseI*

89

90 **#Model selection based on AICc:**

glm	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
DT	9	-7202.39	0.79	0.79	0.79	3610.23
DI	11	-7198.64	3.75	0.12	0.91	3610.37
DE	4	-7197.93	4.46	0.09	1.00	3602.97
DR	25	-7186.11	16.27	0.00	1.00	3618.33

91

92 *#read DI results*

Variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	7	0.043	0.006131	2.113	0.0392 *
Group	2	0.001	0.000417	0.144	0.8663
Residuals	2390	6.936	0.002902		

93 Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.0

94

95 *#read DT results*

Variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	7	0.043	0.006131	2.114	0.0391 *
Residuals	2390	6.937	0.002900		

96 Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.0

97

98 Results for *phaseII*

99

100 **#Model selection based on AICc:**

101

glm	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
DI	31	-23249.23	0.00	0.52	0.52	11655.73
DT	29	-23248.50	0.73	0.36	0.87	11653.35
DE	4	-23246.41	2.82	0.13	1.00	11627.21
DR	85	-23197.24	51.98	0.00	1.00	11684.50

102

103 *#read DI results*

104

Variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	27	0.209	0.007738	2.113	0.000657 ***
Group	2	0.017	0.008690	2.373 0	0.093310
Residuals	8370	30.659	0.003663		

105 Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.0

106

107 **Supplementary IV**

108
 109 Results of behavioural analysis of sea bass larvae recorded at day 8 (end of experimental phase
 110 I) and day 28 (end of experimental phase II).

	Control	0.5 nM EE2	50 nM EE2
<i>Phase I</i>			
<i>Proxies of locomotor activity</i>			
Time spent swimming (s) – (geometric mean + 95% CI)	33.2 [22.1 – 49.8]	66.0 [27.0 – 161.5]	86.5 [47.5 – 157.7]
Total distance travelled (cm) – (geometric mean + 95% CI)	85.5 [54.4 – 134.3]	175.9 [70.7 – 437.7]	214.9 [121.3 – 380.9]
<i>Proxies of anxiety-like behaviours</i>			
Time spent in the periphery of the arena (s) – (median -min-max)	551.8 [301.0 – 600.0]	572.6 [382.8 – 600.0]	599.4 [573.8 – 600.0]
Distance travelled in the periphery of the arena (cm) – (geometric mean + 95% CI)	72.4 [43.9 – 122.0]	166.4 [6.17 – 412.5]	205.2 [115.8 – 363.5]
Total number of rotations (n) – (median - min-max)	10 [4 – 23]	10 [0 – 65]	33 [1 – 129]
<i>Phase II</i>			
<i>Proxies of locomotor activity</i>			
Time spent swimming (s) – (geometric mean + 95% CI)	37.9 [22.3 – 64.4]	64.4 [41.0 – 101.8]	95.1 [60.0 – 150.7]
Total distance travelled (cm) – (geometric mean + 95% CI)	99.4 [56.0 – 176.4]	167.9 [116.8 – 241.4]	236.5 [160.8 – 347.7]
<i>Proxies of anxiety-like behaviours</i>			

Time spent in the periphery of the arena (s) – (median -min-max)	568.0 [450.1 – 600.0]	597.5 [473.0 – 600.0]	570.0 [499.2 – 600.0]
Distance travelled in the periphery of the arena (cm) – (geometric mean + 95% CI)	90.6 [51.6 – 159.0]	156.2 [107.2 – 227.5]	223.6 [152.7 – 327.3]
Total number of rotations (n) – (median -min-max)	4.5 [0 – 23]	11 [2 – 61]	17 [8 – 38]

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