

Supplementary Material

Analyzing the effect of age, time of the day and the experiment on the basal locomotor activity and light-off visual motor response assays in zebrafish larvae

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Supplementary Figures

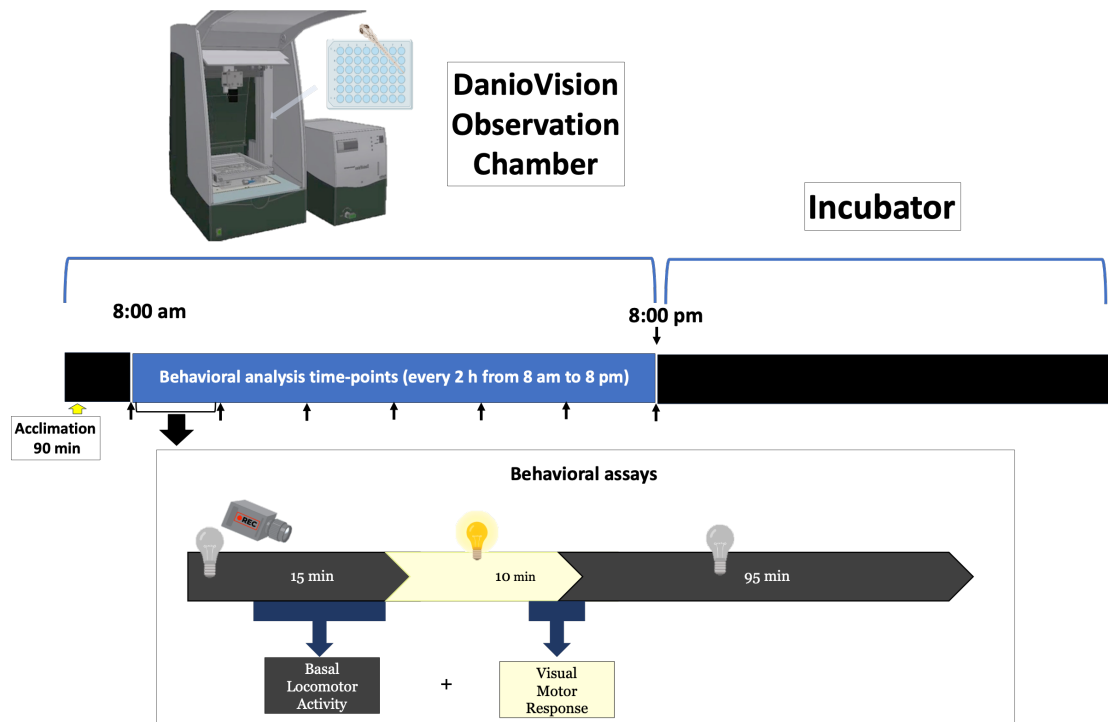


Figure S1. Experimental procedure used for the validation of the basal locomotor activity and light off-visual motor response in zebrafish larvae. The night before the validation experiment, zebrafish larvae were transferred to an incubator at 28°C in the dark (from 8 pm to 6:30 am under our conditions). On the day of the experiment, 90 min before the start of recording (8 am), the microplate containing the larvae was transferred to a DanioVision Observation Chamber for acclimation, where it remained at 28°C throughout the 12-hour procedure (from 8 am to 8 pm). A task consisting of 15 min dark/ 10 min light/ 95 min dark was programmed with Ethovision software. This task sequence was repeated 7 times. Upon completion the plate was transferred back to the incubator and the whole process was repeated on the subsequent days.

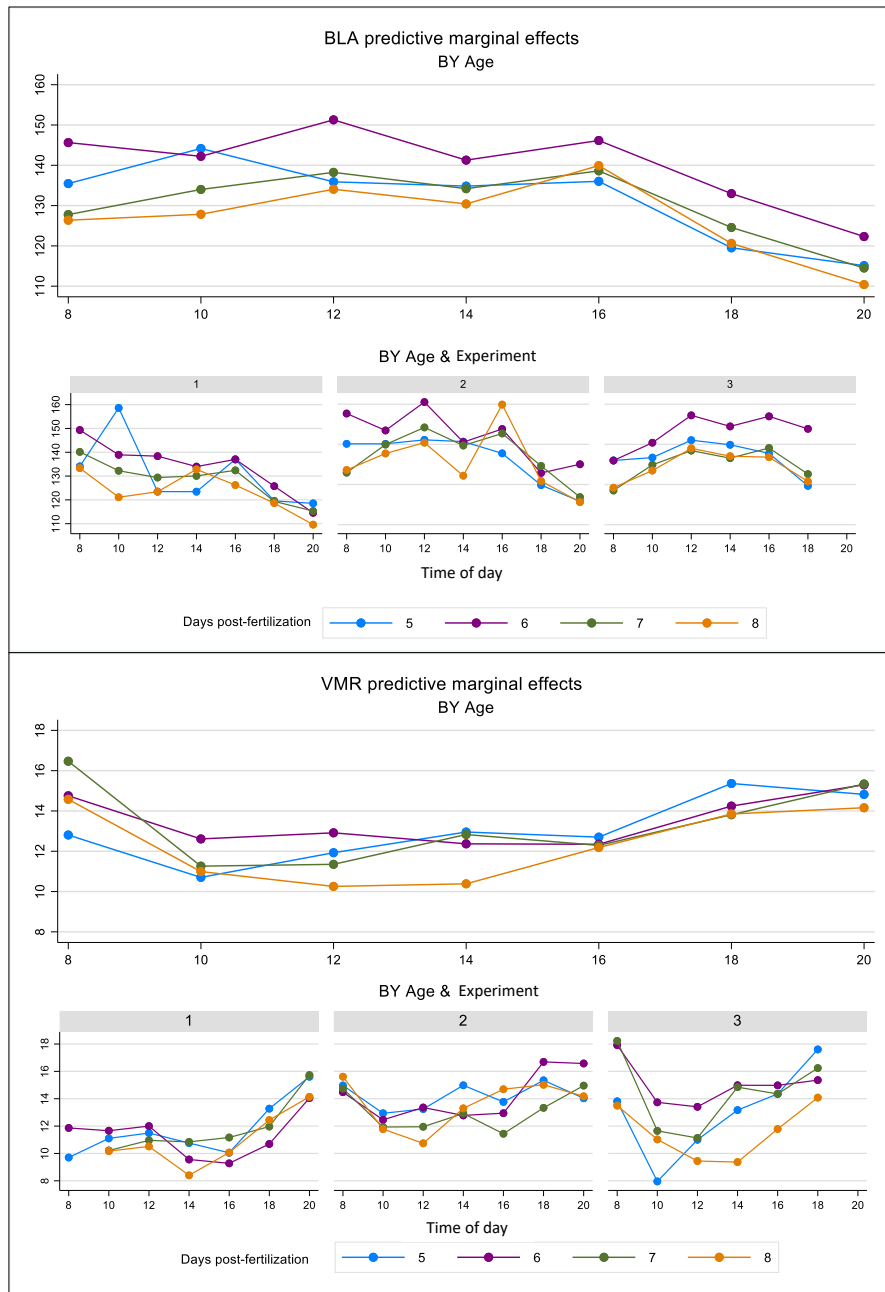


Figure S2. Understanding the 3-way interaction between age, hours and the experiment for BLA and light-off VMR in the RE linear regression model.

Supplementary Tables

Table S1. Random-Effects (RE) linear regression model estimation using generalized least squares (GLS) for BLA and light-off VMR variables. Main effects, full two-way interactions and the three-way interaction included.

Variables	Coef.	BLA (95% CI)		Coef.	VMR† (95% CI)	
Age [days]						
5	0.73	(-9.12 ;	10.57)	-5.78 *	(-11.33 ;	-0.24)
6	15.98 *	(6.14 ;	25.83)	-3.62	(-8.42 ;	1.18)
7	6.80	(-3.04 ;	16.65)	-0.06	(-4.86 ;	4.74)
8 (Ref.)						
Time [hours]						
8:00 (Ref.)						
10:00	-12.24 *	(-22.09 ;	-2.40)	-5.31 *	(-10.86 ;	0.23)
12:00	-9.93 *	(-19.78 ;	-0.09)	-4.97 *	(-10.52 ;	0.58)
14:00	-0.52	(-10.36 ;	9.33)	-7.08 *	(-12.63 ;	-1.54)
16:00	-7.14	(-16.99 ;	2.70)	-5.42 *	(-10.97 ;	0.13)
18:00	-14.71 *	(-24.56 ;	-4.87)	-3.04	(-8.60 ;	2.51)
20:00	-23.75 *	(-33.59 ;	-13.90)	-1.35	(-6.15 ;	3.46)
Experiment						
1 (Ref.)						
2	-6.14	(-16.81 ;	4.53)	0.12	(-4.82 ;	5.06)
3	-14.96 *	(-25.69 ;	-4.23)	-1.99	(-7.66 ;	3.69)
Day # Time						
5 10:00	36.78 *	(22.85 ;	50.70)	6.71 *	(0.52 ;	12.91)
5 12:00	-0.66	(-14.58 ;	13.27)	6.76 *	(0.57 ;	12.96)
5 14:00	-10.12	(-24.04 ;	3.81)	8.14 *	(1.94 ;	14.34)
5 16:00	10.07	(-3.86 ;	23.99)	5.77 *	(-0.43 ;	11.97)
5 18:00	0.21	(-13.71 ;	14.14)	6.62 *	(0.41 ;	12.82)
5 20:00	8.21	(-5.71 ;	22.13)	7.25 *	(1.71 ;	12.79)
6 10:00	1.83	(-12.09 ;	15.75)	5.11 *	(-0.43 ;	10.65)
6 12:00	-1.04	(-14.96 ;	12.89)	5.11 *	(-0.43 ;	10.65)
6 14:00	-14.84 *	(-28.77 ;	-0.92)	4.78 *	(-0.76 ;	10.32)
6 16:00	-5.19	(-19.11 ;	8.74)	2.83	(-2.71 ;	8.37)
6 18:00	-8.87	(-22.79 ;	5.06)	1.87	(-3.68 ;	7.42)
6 20:00	-11.03	(-24.95 ;	2.89)	3.53 *	(-0.39 ;	7.45)
7 10:00	4.32	(-9.61 ;	18.24)	0.11	(-5.43 ;	5.65)
7 12:00	-0.84	(-14.76 ;	13.08)	0.50	(-5.04 ;	6.05)
7 14:00	-9.56	(-23.48 ;	4.36)	2.50	(-3.04 ;	8.05)
7 16:00	-0.61	(-14.53 ;	13.31)	1.16	(-4.38 ;	6.70)
7 18:00	-6.06	(-19.98 ;	7.86)	-0.42	(-5.96 ;	5.13)
7 20:00	-1.10	(-15.02 ;	12.83)	1.65	(-2.26 ;	5.56)
Day # Experiment						
5 2	12.34 *	(-1.59 ;	26.26)	5.14 *	(-0.41 ;	10.70)
5 3	12.86	(-1.14 ;	26.86)	6.10 *	(-0.12 ;	12.32)
6 2	12.18 *	(-1.75 ;	26.10)	2.49	(-1.44 ;	6.42)
6 3	-2.45	(-16.44 ;	11.55)	8.04 *	(2.48 ;	13.61)
7 2	-8.10	(-22.03 ;	5.82)	-0.81	(-4.74 ;	3.12)
7 3	-8.18	(-22.18 ;	5.81)	4.79 *	(-0.77 ;	10.36)
Time # Experiment						
10:00 2	20.50 *	(6.58 ;	34.42)	1.48	(-4.06 ;	7.03)
10:00 3	20.78 *	(6.78 ;	34.78)	2.84	(-3.38 ;	9.07)
12:00 2	23.57 *	(9.65 ;	37.49)	0.10	(-5.45 ;	5.65)
12:00 3	29.54 *	(15.54 ;	43.54)	0.92	(-5.32 ;	7.16)
14:00 2	-2.31	(-16.23 ;	11.62)	4.77 *	(-0.78 ;	10.32)
14:00 3	16.23 *	(2.24 ;	30.23)	2.95	(-3.29 ;	9.19)
16:00 2	39.75 *	(25.83 ;	53.67)	4.51	(-1.04 ;	10.06)
16:00 3	22.36 *	(8.36 ;	36.36)	3.71	(-2.51 ;	9.93)
18:00 2	9.21	(-4.71 ;	23.14)	2.46	(-3.10 ;	8.01)
18:00 3	17.85 *	(3.85 ;	31.85)	3.63	(-2.63 ;	9.88)
20:00 2	7.74	(-6.18 ;	21.66)	-0.08	(-4.01 ;	3.84)
20:00 3	(n.e.)			(n.e.)		
Day # Time # Experiment						
5 10:00 2	-45.05 *	(-64.74 ;	-25.36)	-4.92	(-11.71 ;	1.88)
5 10:00 3	-43.88 *	(-63.67 ;	-24.09)	-10.10 *	(-17.47 ;	-2.73)
5 12:00 2	-11.01	(-30.70 ;	8.68)	-3.62	(-10.41 ;	3.17)
5 12:00 3	-8.85	(-28.64 ;	10.95)	-5.52	(-12.90 ;	1.86)
5 14:00 2	14.03	(-5.66 ;	33.72)	-5.82 *	(-12.62 ;	0.98)
5 14:00 3	2.22	(-17.57 ;	22.02)	-4.66 *	(-12.04 ;	2.72)
5 16:00 2	-47.41 *	(-67.10 ;	-27.72)	-6.06 *	(-12.85 ;	0.74)
5 16:00 3	-21.75 *	(-41.55 ;	-1.96)	-3.52	(-10.88 ;	3.84)
5 18:00 2	-15.29	(-34.98 ;	4.40)	-5.65	(-12.45 ;	1.14)
5 18:00 3	-16.01	(-35.81 ;	3.78)	-3.41	(-10.81 ;	3.99)
5 20:00 2	-20.82 *	(-40.51 ;	-1.13)	-6.75 *	(-12.30 ;	-1.21)
5 20:00 3	(n.e.)			(n.e.)		
6 10:00 2	-18.54 *	(-38.23 ;	1.15)	-3.30	(-8.84 ;	2.24)
6 10:00 3	-1.49	(-21.29 ;	18.30)	-6.82 *	(-13.64 ;	0.00)
6 12:00 2	-6.89	(-26.58 ;	12.80)	-1.36	(-6.90 ;	4.18)
6 12:00 3	3.97	(-15.83 ;	23.76)	-5.56	(-12.40 ;	1.27)
6 14:00 2	3.41	(-16.28 ;	23.10)	-4.16	(-9.71 ;	1.38)
6 14:00 3	16.13	(-3.66 ;	35.93)	-3.58	(-10.43 ;	3.27)
6 16:00 2	-35.14 *	(-54.83 ;	-15.45)	-3.45	(-8.99 ;	2.09)
6 16:00 3	12.00	(-7.79 ;	31.80)	-4.06	(-10.89 ;	2.76)
6 18:00 2	-15.27	(-34.96 ;	4.42)	0.93	(-4.62 ;	6.48)
6 18:00 3	21.53 *	(1.74 ;	41.33)	-5.02	(-11.86 ;	1.83)
6 20:00 2	1.74	(-17.95 ;	21.43)	(n.e.)		
6 20:00 3	(n.e.)			(n.e.)		
7 10:00 2	1.42	(-18.27 ;	21.11)	0.92	(-4.62 ;	6.46)
7 10:00 3	-0.08	(-19.88 ;	19.71)	-4.21	(-11.04 ;	2.61)
7 12:00 2	9.73	(-9.96 ;	29.42)	1.58	(-3.97 ;	7.12)
7 12:00 3	1.12	(-18.68 ;	20.91)	-3.55	(-10.40 ;	3.29)
7 14:00 2	25.89 *	(6.20 ;	45.58)	-2.01	(-7.55 ;	3.53)
7 14:00 3	9.93	(-9.86 ;	29.73)	-1.76	(-8.60 ;	5.09)
7 16:00 2	-12.49	(-32.20 ;	7.23)	-3.55	(-9.09 ;	1.99)
7 16:00 3	6.57	(-13.23 ;	26.36)	-3.33	(-10.15 ;	3.49)
7 18:00 2	14.91	(-4.81 ;	34.62)	-0.40	(-5.95 ;	5.14)
7 18:00 3	11.09	(-8.70 ;	30.89)	-2.16	(-9.01 ;	4.69)
7 20:00 2	4.91	(-14.78 ;	24.60)	(n.e.)		
7 20:00 3	(n.e.)			(n.e.)		
Constant	133.35 *	(125.81 ;	140.90)	15.49 *	(10.23 ;	20.74)
Sigma_u	10.28			2.79		
Sigma_e	24.60			6.89		
p	0.1487			0.1408		
R ² within	18.84%			8.17%		
R ² between	6.15%			8.95%		
R ²	16.80%			8.40%		

[†] light-off VMR restricted to the interval [-25;50]; * $P \leq 0.05$; ° $0.05 < P \leq 0.10$; a#b means interaction between a and b variables; (Ref.) means reference category; ρ is the fraction of the error variance due to the differences between individuals (Intraclass Correlation of the error); R^2_{Within} , R^2_{Between} and R^2 and are the within, between and global correlations squared expressed in percentages; n.e. means not estimable.

Table S2. Pairwise comparisons of predictive margins of BLA for age. Differences and P-values corrected using the Bonferroni method to account for multiple comparisons.

Age			A		
			6	7	8
Predictive Margins			141.16	131.06	127.91
B	5	132.39	8.77 [†] 0.000*	-1.33 1.000	-4.48 0.000*
	6	141.16		-10.10 0.000*	-13.25 0.000*
	7	131.06			-3.15 0.031*

Note. [†] Difference between the predictive margins (A-B); * $P \leq 0.05$; ° $0.05 < P \leq 0.10$; Shaded cells indicate non-statistically significant differences.

Table S3. Pairwise comparisons of predictive margins of BLA for time of the day. Differences and P-values corrected using the Bonferroni method to account for multiple comparisons.

Time of the day			A					
			10:00	12:00	14:00	16:00	18:00	20:00
Predictive Margins			137.06	139.87	135.18	140.18	124.42	115.58
B	8:00	133.81	3.25 [†] 0.536	6.06 0.001*	1.37 1.000	6.37 0.000*	-9.39 0.000*	-18.23 0.000*
	10:00	137.06		2.81 1.000	-1.88 1.000	3.12 0.672	-12.64 0.000*	-21.48 0.000*
	12:00	139.87			-4.69 0.027*	0.31 1.000	-15.45 0.000*	-24.29 0.000*
	14:00	135.18				5.00 0.012*	-10.76 0.000*	-19.60 0.000*
	16:00	140.18					-15.76 0.000*	-24.60 0.000*
	18:00	124.42						-8.84 0.000*

Note. [†] Difference between the predictive margins (A-B); * $P \leq 0.05$; ° $0.05 < P \leq 0.10$; Shaded cells indicate non-statistically significant differences.

Table S4. Pairwise comparisons of predictive margins of BLA for time of the day and age. Differences and P-values corrected using the Bonferroni method to account for multiple comparisons.

DAY 5	Time of the day			A					
				10:00	12:00	14:00	16:00	18:00	20:00
	Predictive Margins			144.17	135.89	134.83	136.02	119.53	115.10
B	8:00	135.47	8.70 [†] 0.058°	0.42 1.000	-0.64 1.000	0.55 1.000	-15.94 0.000*	-20.37 0.000*	
	10:00	144.17		-8.28 0.094°	-9.34 0.028*	-8.15 0.107	-24.64 0.000*	-29.07 0.000*	
	12:00	135.89		-1.06 1.000	0.13 1.000	-16.36 0.000*	-20.79 0.000*		
	14:00	134.83			1.19 1.000	-15.30 0.000*	-19.73 0.000*		
	16:00	136.02				-16.49 0.000*	-20.92 0.000*		
	18:00	119.53						-4.43 1.000	
DAY 6			Predictive Margins	142.23	151.28	141.29	146.15	132.97	122.32
	B	8:00	145.64	-3.41 [†] 1.000	5.64 1.000	-4.35 1.000	0.51 1.000	-12.67 0.000*	-23.32 0.000*
		10:00	142.23	9.05 0.039*		-0.94 1.000	3.92 1.000	-9.26 0.031*	-19.91 0.000*
		12:00	151.28			-9.99 0.012*	-5.13 1.000	-18.31 0.000*	-28.96 0.000*
		14:00	141.29			4.86 1.000	-8.32 0.090°	-18.97 0.000*	
		16:00	146.15				-13.18 0.000*	-23.83 0.000*	
		18:00	132.97						-10.65 0.026*
DAY 7			Predictive Margins	134.00	138.25	134.20	138.63	124.57	114.51
	B	8:00	127.76	6.24 0.673	10.49 0.007*	6.44 0.566	10.87 0.004*	-3.19 1.000	-13.25 0.001*
		10:00	134.00	4.25 1.000		0.20 1.000	4.63 1.000	-9.43 0.025*	-19.49 0.000*
		12:00	138.25			-4.05 1.000	0.38 1.000	-13.68 0.000*	-23.74 0.000*
		14:00	134.20			4.43 1.000	-9.63 0.020*	-19.69 0.000*	
		16:00	138.63				-14.06 0.000*	-24.12 0.000*	
		18:00	124.57						-10.06 0.050*
DAY 8			Predictive Margins	127.84	134.06	130.42	139.92	120.62	110.40
	B	8:00	126.37	1.47 1.000	7.69 0.174	4.05 1.000	13.55 0.000*	-5.75 1.000	-15.97 0.000*
		10:00	127.84	6.22 0.686		2.58 1.000	12.08 0.001*	-7.22 0.274	-17.44 0.000*
		12:00	134.06			-3.64 1.000	5.86 0.922	-13.44 0.000*	-23.66 0.000*
		14:00	130.42				9.50 0.023*	-9.80 0.016*	-20.02 0.000*
		16:00	139.92				-19.30 0.000*	-29.52 0.000*	
		18:00	120.62						-10.22 0.042*

Note. [†] Difference between the predictive margins (A-B); * P ≤ 0.05; ° 0.05 < P ≤ 0.10; Shaded cells indicate non-statistically significant differences.

Table S5. Pairwise comparisons of predictive margins of light-off VMR for age. Differences and P-values corrected using the Bonferroni method to account for multiple comparisons.

Age			A		
			6	7	8
		Predictive Margins	13.42	13.07	12.13
B	5	12.95	0.47 [†] 0.839	0.12 1.000	-0.82 0.066°
	6	13.42		-0.35 1.000	-1.29 0.000*
	7	13.07			-0.94 0.024*

Note. [†] Difference between the predictive margins (A-B); * $P \leq 0.05$; ° $0.05 < P \leq 0.10$; Shaded cells indicate non-statistically significant differences.

Table S6. Pairwise comparisons of predictive margins of light-off VMR for time of the day. Differences and P-values corrected using the Bonferroni method to account for multiple comparisons.

Time of the day			A					
			10:00	12:00	14:00	16:00	18:00	20:00
Predictive Margins			11.39	11.62	12.14	12.38	14.32	14.91
B	8:00	14.48	-3.09 [†] 0.000*	-2.86 0.000*	-2.34 0.000*	-2.10 0.000*	-0.16 1.000	0.43 1.000
	10:00	11.39		0.23 1.000	0.75 1.000	0.99 0.337	2.93 0.000*	3.52 0.000*
	12:00	11.62			0.52 1.000	0.76 1.000	2.70 0.000*	3.29 0.000*
	14:00	12.14				0.24 1.000	2.18 0.000*	2.77 0.000*
	16:00	12.38					1.94 0.000*	2.53 0.000*
	18:00	14.32						0.59 1.000

Note. [†] Difference between the predictive margins (A-B); * $P \leq 0.05$; ° $0.05 < P \leq 0.10$; Shaded cells indicate non-statistically significant differences.

Table S7. Pairwise comparisons of predictive margins of light-off VMR for time of the day and age. Differences and P-values corrected using the Bonferroni method to account for multiple comparisons.

DAY 5	Time of the day		A					
			10:00	12:00	14:00	16:00	18:00	20:00
	Predictive Margins		10.70	11.93	12.96	12.70	15.37	14.82
B	8:00	13.45	-2.75 [†] 0.022*	-1.52 1.000	-0.49 1.000	-0.75 1.000	1.92 0.477	1.37 1.000
	10:00	10.70		1.23 1.000	2.26 0.122	2.00 0.305	4.67 0.000*	4.12 0.000*
	12:00	11.93			1.03 1.000	0.77 1.000	3.44 0.001*	2.89 0.038*
	14:00	12.96				-0.26 1.000	2.41 0.072°	1.86 0.950
	16:00	12.70					2.67 0.024*	2.12 0.466
	18:00	15.37						-0.55 1.000
DAY 6	Predictive Margins		12.61	12.92	12.38	12.36	14.23	15.32
	8:00	15.30	-2.71 [†] 0.025*	-2.38 0.088°	-2.92 0.010*	-2.94 0.009*	-1.07 1.000	0.02 1.000
	10:00	12.61		0.31 1.000	-0.23 1.000	-0.25 1.000	1.62 0.971	2.71 0.074°
	12:00	12.92			-0.54 1.000	-0.56 1.000	1.31 1.000	2.40 0.206
	14:00	12.38				-0.02 1.000	1.85 0.508	2.94 0.034*
	16:00	12.36					1.87 0.473	2.96 0.032*
	18:00	14.23						1.09 1.000
DAY 7	Predictive Margins		11.26	11.35	12.83	12.29	13.80	15.34
	8:00	n.e.						
	10:00	11.26		0.09 1.000	1.57 1.000	1.03 1.000	2.54 0.041*	4.08 0.000*
	12:00	11.35			1.48 1.000	0.94 1.000	2.45 0.059°	3.99 0.000*
	14:00	12.83				-0.54 1.000	0.97 1.000	2.51 0.143
	16:00	12.29					1.51 1.000	3.05 0.021*
	18:00	13.80						1.54 1.000
DAY 8	Predictive Margins		10.99	10.25	10.37	12.19	13.85	14.16
	8:00	n.e.						
	10:00	10.99		-0.74 1.000	-0.62 1.000	1.20 1.000	2.86 0.011*	3.17 0.014*
	12:00	10.25			0.12 1.000	1.94 0.380	3.60 0.000*	3.91 0.001*
	14:00	10.37				1.82 0.570	3.48 0.001*	3.79 0.001*
	16:00	12.19					1.66 0.929	1.97 0.708
	18:00	13.85						0.31 1.000

Note. [†] Difference between the predictive margins (A-B); * $P \leq 0.05$; ° $0.05 < P \leq 0.10$; n.e.: not estimable. Shaded cells indicate non-statistically significant differences.