

Supplemental material

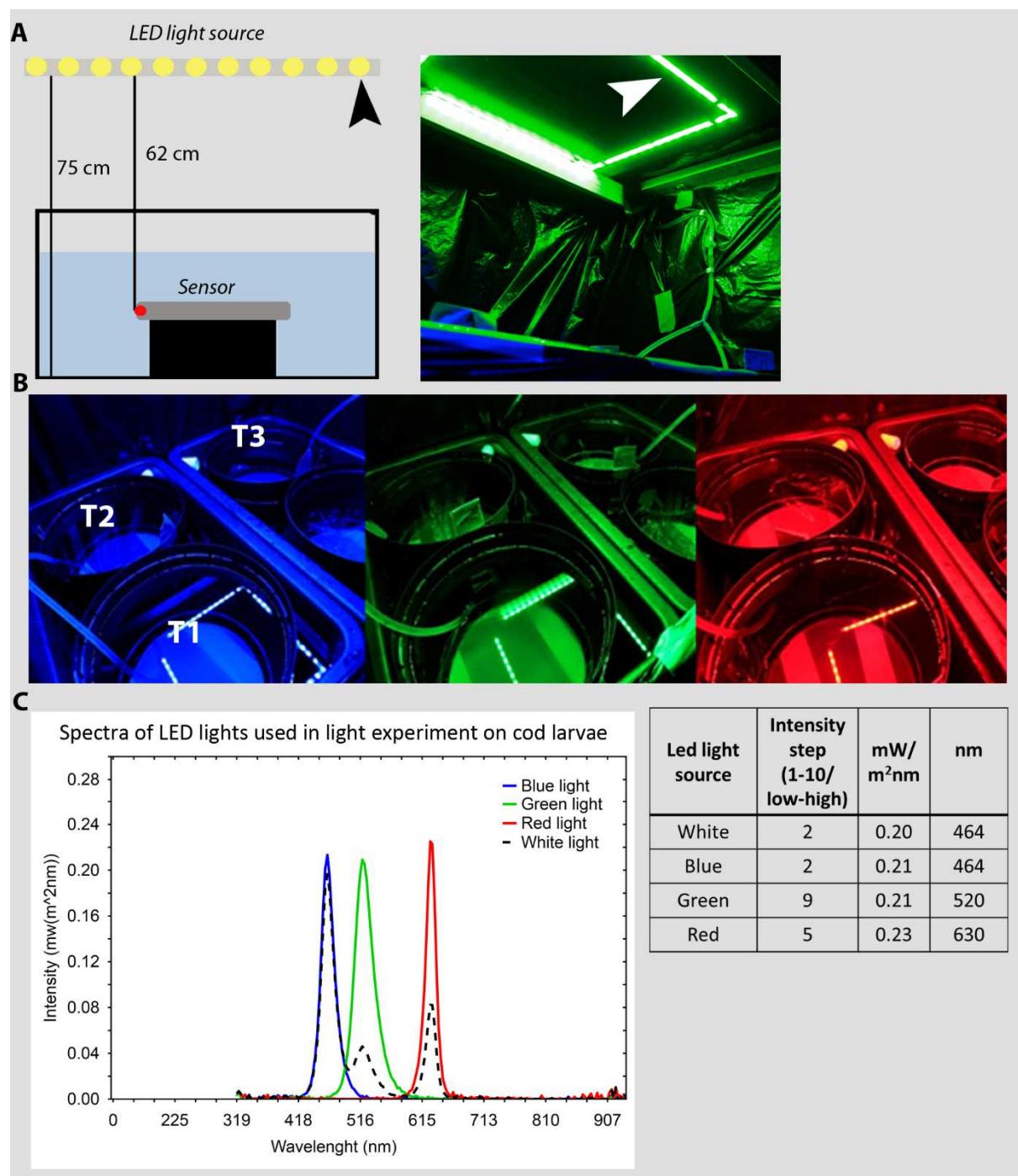


Figure S1. Experimental setup light treatments cod larvae. A) Left hand side illustrates the position at which the optical recordings were obtained within the fish tanks, and a photo of the LED light source is shown on the right hand side. B) The photos show the setup of the larval rearing tanks under different wavelenght lights of blue, green and red, respectively. C) Optical measurements were plotted for all LED lights used (left hand side), where the y-axis shows LED intensity measured in mW

$\text{m}^{-2} \text{ nm}^{-1}$ over nanometer wavelenght distribution (x-axis). The table on the right hand side shows specter data values for the used LED lights, where the chosen intensity step was normalized across the different light sources.

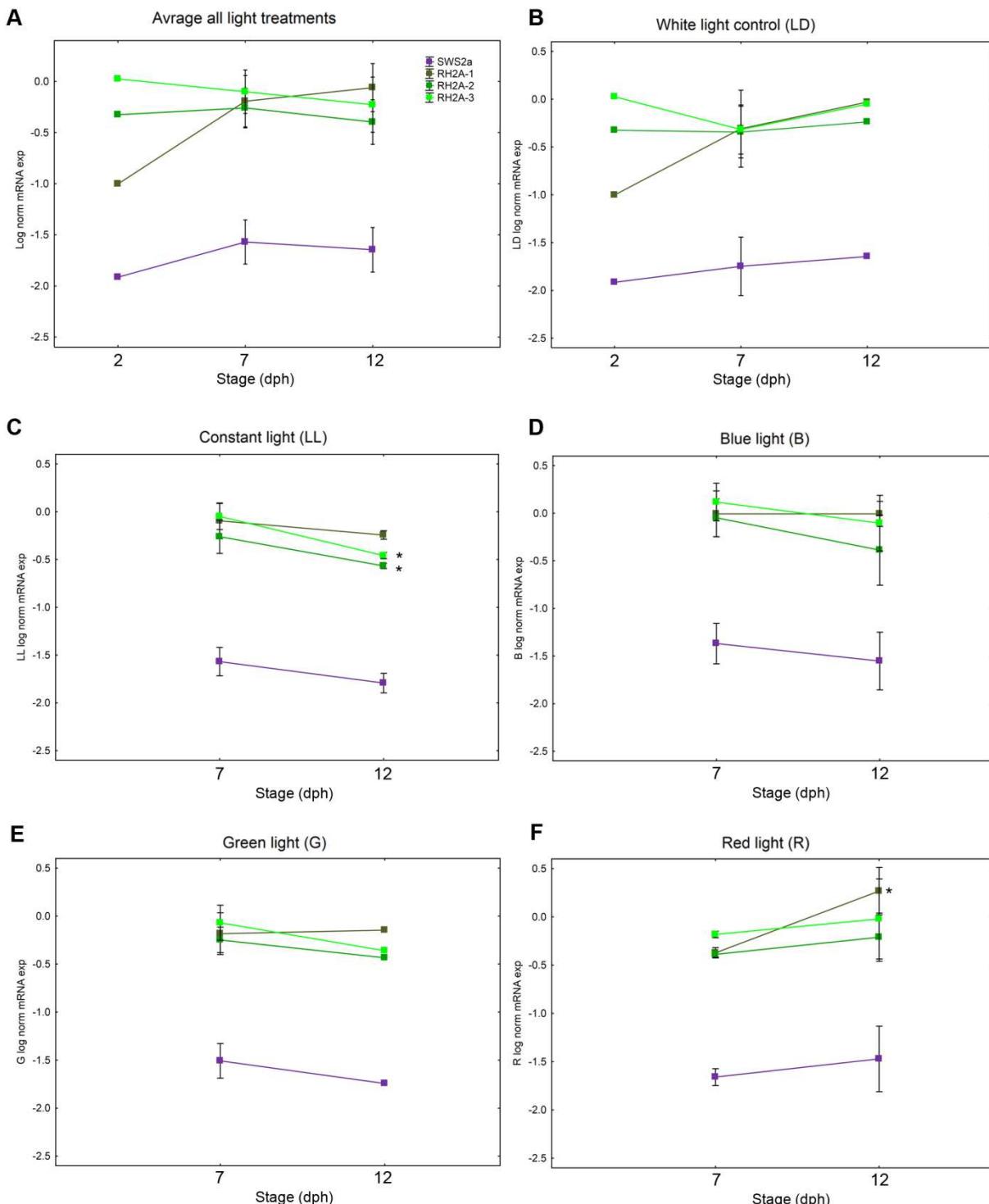


Figure S2. Temporal expression of cone opsins during light treatments (A-F). The different opsins are represented by different colors shown in A. Expression values marked with (*) in C and F represents significantly different ($p<0.05$) expression between 7- and 12 dph, using a one-way ANOVA.

Supplemental material: Statistical analyses

Light experiment NC cod larvae

Table S1. Normality test light experiment. Shapiro-Wilks test of goodness of fit on log transformed normalized qPCR expression data for all visual opsins in all stages and treatments. W=1 if data are perfectly normal in distribution. Underlined and bold W values are significantly smaller than 1 ($p<0.05$), indicating rejection of the normal distribution hypothesis. Missing cells (-) represent stages where N is too low and test could not be performed.

Stage (dph)	Treatment	SWS2A	RH2A-1	RH2A-2	RH2A-3
7	LD	W = 0.815	W = 0.943	W = 0.936	W = 0.937
12	LD	W = -	W = -	W = -	W = -
7	LL	W = 0.889	W = 0.942	W = 0.938	W = 0.827
12	LL	W = 0.886	W = 0.875	W = 0.967	W = 0.823
7	B	W = 0.992	W = 0.948	W = 0.970	W = 0.991
12	B	W = -	W = -	W = -	W = -
7	G	W = 0.899	W = 0.876	W = 0.999	W = 0.999
12	G	W = -	W = -	W = -	W = -
7	R	W = 0.958	W = 0.991	W = 0.882	W = 1.000
12	R	W = -	W = -	W = -	W = -

Table S2. Levene's Test for Homogeneity of Variances. Significant effects ($p<0.05$)

Stage (dph)	Treatment	SWS2A	RH2A-1	RH2A-2	RH2A-3
7-12	LD	P = 0.248	P = 0.354	P = 0.344	P = 0.349
7-12	LL	P = 0.421	P = 0.096	P = 0.064	P = 0.048
7-12	B	P = 0.483	P = 0.356	P = 0.195	P = 0.439
7-12	G	P = 0.230	P = 0.215	P = 0.402	P = 0.405
7-12	R	P = 0.009	P = 0.007	P < 0.001	P < 0.000

Table S3. Two-way ANOVA light treatment; stage*treatment.

Effect	Analysis of Variance. Marked effects are significant at p < .050				
	Value	F	Effect df	Error df	p
Intercept	0.005	780.006	4	15.000	0.000
Stage (dpf)	0.121	7.029	8	30.000	0.000
Treatment	0.423	0.944	16	46.463	0.528

Table S5. Tukey HSD post hoc for stage effect *sfs2a*.

Cell No.	Tukey HSD test; variable Log Relative SWS2a express . Approximate Probabilities for Post Hoc Tests Error: Between MS = .04453, df = 18.000			
	Stage (dph)	{1} -1.915	{2} -1.570	{3} -1.646
1	2		0.2780	0.464
2	7	0.280		0.677
3	12	0.464	0.677	

Table S6.1. Tukey HSD post hoc for stage effect *rh2a-1*.

Cell No.	Tukey HSD test; variable Log Relative RH2-1 express . Approximate Probabilities for Post Hoc Tests Error: Between MS = .06455, df = 18.000			
	Stage (dph)	{1} -1.002	{2} -.1932	{3} -.0430
1	2		0.017	0.006
2	7	0.017		0.361
3	12	0.007	0.3617	

Table S6.2. Bonferroni post hoc for stage effect *rh2a-1*.

	Bonferroni test; variable Log Relative RH2-1 express . Probabilities for Post Hoc Tests Error: Between MSE = .06712, df = 17.000			
Cell No.	Stage (dph)	{1} -1.002	{2} -.1932	{3} -.0601
1	2		0.023	0.010
2	7	0.023		0.771
3	12	0.010	0.771	

Table S7. Tukey HSD post hoc for stage effect *rh2a-2*.

	Tukey HSD test; variable Log Relative RH2-2 express . Approximate Probabilities for Post Hoc Tests Error: Between MS = .04333, df = 18.000			
Cell No.	Stage (dph)	{1} -.3244	{2} -.2580	{3} -.3966
1	2		0.949	0.942
2	7	0.949		0.280
3	12	0.942	0.280	

Table S8. Tukey HSD post hoc for stage effect *rh2a-3*.

	Tukey HSD test; variable Log Relative RH2-3 express . Approximate Probabilities for Post Hoc Tests Error: Between MS = .05228, df = 18.000			
Cell No.	Stage (dph)	{1} .02566	{2} -.1001	{3} -.2266
1	2		0.860	0.558
2	7	0.860		0.407
3	12	0.558	0.407	

Table S9. One-way ANOVA light treatment for stage effect.

Effect	Analysis of Variance. Marked effects are significant at p < .050				
	Value	F	Effect df	Error df	p
Intercept	0.005	921.301	4	19	0.00
Stage (dph)	0.106	9.824	8	38	0.000

Significant (p<0.05) Tukey HSD post hoc test for stage effect within treatment

Table S10.1 Tukey HSD post hoc for stage effect within treatment LL.

Stage (dph)	Treatment=LL Tukey HSD test; Variable: Log Relative RH2-2 express . Marked differences are significant at p < .050		
	{1} M=0.0000	{2} M=-.2613	{3} M=-.5667
2 {1}			
7 {2}			0.040
12 {3}		0.040	

Table S10.2 Bonferroni post hoc for stage effect within treatment LL.

Stage (dph)	Treatment=LL Bonferroni test; Variable: Log Relative RH2-2 express . Marked differences are significant at p < .050. Error: Between MS = ,01562, df = 4,0000		
	{1} M=0.0000	{2} M=-.2613	{3} M=-.5667
2 {1}			
7 {2}			0.040
12 {3}		0.040	

Table S11.1 Tukey HSD post hoc for stage effect within treatment LL.

Stage (dph)	Treatment=LL Tukey HSD test; Variable: Log Relative RH2-3 express . Marked differences are significant at p < .050		
	{1} M=0.0000	{2} M=-.0496	{3} M=-.4579
2 {1}			
7 {2}			0.008
12 {3}		0.008	

Table S11.2 Bonferroni post hoc for stage effect within treatment LL.

Stage (dph)	Treatment=LL Bonferroni test; Variable: Log Relative RH2-3 express . Marked differences are significant at p < .050. Error: Between MS = ,00992, df = 4,0000		
	{1} M=0.0000	{2} M=-.0496	{3} M=-.4579
2 {1}			
7 {2}			0.008
12 {3}		0.008	

Table S12.1 Tukey HSD post hoc for stage effect within treatment R.

Stage (dph)	Treatment=R Tukey HSD test; Variable: Log Relative RH2-1 express . Marked differences are significant at p < .050		
	{1} M=0.0000	{2} M=-.3723	{3} M=.26714
2 {1}			
7 {2}			0.018
12 {3}		0.018	

Table S12.2 Bonferroni post hoc for stage effect within treatment R.

	Treatment=R Bonferroni test; Variable: Log Relative RH2-1 express . Marked differences are significant at p < .050. Error: Between MS = ,02186, df = 3,0000		
Stage (dph)	{1} M=0.0000	{2} M=-.3723	{3} M=.26714
2 {1}			
7 {2}			0.018
12 {3}		0.018	

Maturing NC cod

Table S13. Normality maturing cod. Shapiro-Wilks test of goodness of fit on log transformed normalized qPCR expression data for all visual opsins. W=1 if data are perfectly normal in distribution. Underlined and bold W values are significantly smaller than 1 ($p<0.05$), indicating rejection of the normal distribution hypothesis.

Gene	Female	Male
RH1	<u>W = 0.841</u>	<u>W = 0.756</u>
Rh2a-1	W = 0.903	<u>W = 0.751</u>
Sws2a	W = 0.924	<u>W = 0.565</u>

Table S14. Levene's Test for Homogeneity of Variances. Significant effects ($p<0.05$)

Variable Female-Male	Levene Test of Homogeneity of Variances. Marked effects are significant at p < .05							
SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p	
Rh1 Log Norm mRNA exp	0.030	2	0.015	0.254	25	0.010	1.497	0.243
Sws2a Log Norm mRNA exp	0.052	2	0.026	1.518	25	0.061	0.431	0.654
Rh2a-1 Log Norm mRNA exp	0.033	2	0.016	0.295	25	0.012	1.387	0.268

Table S15. Main effects ANOVA Gene*Part-of-retina*Gender. Significant effect (p<0.05)

Effect	Univariate Tests of Significance for Log norm mRNA exp. Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	10.903	1	10.903	279.150	0.000
Gene	110.587	2	55.294	1415.652	0.000
Part of retina	0.009	1	0.009	0.240	0.626
Gender	0.128	1	0.128	3.273	0.076
Gene: * Part of retina	0.050	2	0.025	0.636	0.533
Gene: * Gender	0.027	2	0.013	0.344	0.710
Part of retina * Gender	0.048	1	0.047	1.215	0.275
Gene: * Part of retina * Gender	0.027	2	0.013	0.344	0.710
Error	2.109	54	0.039		

Table S16.1. Tukey HSD post hoc for gene effect.

Cell No.	Tukey HSD test; variable Log norm mRNA exp. Approximate Probabilities for Post Hoc Tests Error: Between MS = .03698, df = 61.000			
	Gene:	{1} 1.8530	{2} -1.293	{3} .67694
1	RH1		P << 0.001	P << 0.001
2	SWS2A	P << 0.001		P << 0.001
3	RH2A-1	P << 0.001	P << 0.001	

Table S16.2. Bonferroni post hoc for gene effect.

Cell No.	Bonferroni test; variable Log norm mRNA exp. Probabilities for Post Hoc Tests Error: Between MSE = ,06607, df = 81,000			
	Gene:	{1} 1.9954	{2} -1.132	{3} .9150
1	RH1		P << 0.001	P << 0.001
2	SWS2A	P << 0.001		P << 0.001
3	RH2A-1	P << 0.001	P << 0.001	

Northeast Arctic cod (NEA cod)

Table S17. Test of normality distribution Northeast Arctic cod. *Shapiro-Wilks test of goodness of fit on log transformed normalized qPCR expression data for all visual opsins. W=1 if data are perfectly normal in distribution. Underlined and bold W values are significantly smaller than 1 (p<0.05), indicating rejection of the normal distribution hypothesis.*

Season	Part of retina	RH1	SWS2A	RH2A-1	RH2A-2	RH2A-3
Summer	Dorsal	W = 0.902	W = 0.883	W = 0.926	W = 0.955	W = 0.923
Summer	Ventral	W = 0.945	W = 0.856	W = 0.934	W = 0.954	W = 0.929
Winter	Dorsal	W = 0.946	W = 0.930	W = 0.808	W = 0.959	W = 0.951
Wintre	Ventral	W = 0.953	W = 0.731	W = 0.893	W = 0.943	W = 0.929

Table S18. Levene's Test for Homogeneity of Variances. Significant effects (p<0.05)

Gene	Levene's Test for Homogeneity of Variances: Effect: Season Degrees of freedom for all F's: 1, 138			
	MS Effect	MS Error	F	p
RH1	0.000	0.025	0.012	0.915
Sws2a	0.007	0.049	0.145	0.704
Rh2a-1	0.230	0.021	18.7	0.000
Rh2a-2	0.001	0.10	0.014	0.907
Rh2a-3	0.037	0.030	1.221	0.276

Table S19. Levene's Test for Homogeneity of Variances. Significant effects (p<0.05)

Season	All Groups Levene's Test for Homogeneity of Variances: Effect: "Part of retina" by gene Degrees of freedom for all F's: 1, 131			
	MS Effect	MS Error	F	p
Summer Log Norm. mRNA exp (ubiq)	0.247	0.368	0.672	0.414

Table S20. Levene's Test for Homogeneity of Variances. Significant effects (p<0.05)

Season	All Groups Levene's Test for Homogeneity of Variances: Effect: "Part of retina" by gene Degrees of freedom for all F's: 1, 138			
	MS Effect	MS Error	F	p
Winter log norm mRNA exp	0.675	0.387	1.742	0.189

Table S21. Main effects ANOVA Gene*Part-of-retina*Season. Significant effect ($p < 0.05$)

Effect	Univariate Tests of Significance for Log Norm. mRNA exp. Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	123.368	1	123.368	1268.362	0.000
Gene:	693.768	5	138.754	1426.542	0.000
Part of retina	2.228	1	2.228	22.910	0.000
Season	0.343	1	0.343	3.529	0.062
Gene:*Part of retina	3.990	5	0.798	8.203	0.000
Gene:*Season	0.168	5	0.034	0.344	0.885
Part of retina*Season	0.187	1	0.187	1.925	0.167
Gene:*Part of retina*Season	0.121	5	0.024	0.248	0.940
Error	20.426	210	0.097		

Table S22.1. Tukey HSD post hoc for Gene*Part of retina Winter.

Cell No.	Tukey HSD test; variable log norm mRNA Winter. Approximate Probabilities for Post Hoc Tests Error: Between MS = .07208, df = 108.00														
	gen e3	Part of retina3	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	
			1.642	1.638	-	-	-	-	1.095	1.213	-	-	-	-	
1	RH1	Dorsal		1.000	P << 0.001										
2	RH1	Ventral	1.000		P << 0.001										
3	SW S2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.978	P << 0.001	P << 0.001	P << 0.001
4	SW S2A	Ventral	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.716	P << 0.001	P << 0.001	P << 0.001	P << 0.001
5	SW S2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.993	P << 0.001						
6	SW S2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.993		P << 0.001						
7	RH2 A-1	Dorsal	P << 0.001		0.998	P << 0.001									
8	RH2 A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.998		P << 0.001				
9	RH2 A-2	Dorsal	P << 0.001	P << 0.001	0.978	0.716	P << 0.001	P << 0.001	P << 0.001	P << 0.001		P << 0.001	P << 0.001	P << 0.001	P << 0.001
10	RH2 A-2	Ventral	P << 0.001		P << 0.001	P << 0.001									
11	RH2 A-3	Dorsal	P << 0.001			0.489									
12	RH2 A-3	Ventral	P << 0.001	0.489											

Table S22.2. Bonferroni post hoc test for Gene*Part of retina Winter.

Cell No.	Bonferroni test; variable log norm mRNA Winter. Probabilities for Post Hoc Tests Error: Between MS = ,07208, df = 108,00															
	gen e3	Part of retina3	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}		
			1.642 4	1.638 4	- .9213	.8416	3.287	3.152	0	8	1.077	.1692	2.339	2.064		
1	RH1	Dorsal		1.000	P << 0.001											
2	RH1	Ventral	1.000		P << 0.001											
3	SW S2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	
4	SW S2A	Ventral	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	
5	SW S2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001		P << 0.001	P << 0.001	P << 0.001	
6	SW S2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001		P << 0.001	P << 0.001	P << 0.001	
7	RH2 A-1	Dorsal	P << 0.001		1.000	P << 0.001										
8	RH2 A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001					
9	RH2 A-2	Dorsal	P << 0.001	P << 0.001	1.000	1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001		P << 0.001	P << 0.001	P << 0.001	P << 0.001	
10	RH2 A-2	Ventral	P << 0.001		P << 0.001	P << 0.001										
11	RH2 A-3	Dorsal	P << 0.001		1.000											
12	RH2 A-3	Ventral	P << 0.001		1.000											

Table S23.1. Tukey HSD post hoc for Gene*Part of retina Early fall.

Cell No.	Tukey HSD test; variable log norm mRNA exp Early fall. Approximate Probabilities for Post Hoc Tests Error: Between MS = .12394, df = 102.00													
	Gene	Part of retina	{1} 1.679 5	{2} 1.644 5	{3} -1.018 -1.051	{4} -1.051 -3.326	{5} -3.326 -3.242	{6}	{7} 1.071 4	{8} 1.114 8	{9} -1.033 -1.033	{10} -.4316 -.4316	{11} -2.381 -2.381	{12} -2.210 -2.210
1	RH1	Dorsal		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
2	RH1	Ventral	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
3	SW S2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
4	SW S2A	Ventral	P << 0.001	0.000 118	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
5	SW S2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.999 996	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
6	SW S2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.999 996		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
7	RH2 A-1	Dorsal	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001
8	RH2 A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001
9	RH2 A-2	Dorsal	P << 0.001	P << 0.001	1.000	1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001		P < 0.05	P << 0.001	P << 0.001
10	RH2 A-2	Ventral	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P < 0.05		P << 0.001	P << 0.001
11	RH2 A-3	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		0.996
12	RH2 A-3	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	0.996	

Table S23.2. Bonferroni post hoc test for Gene*Part of retina Early fall.

Cell No.	Bonferroni test; variable log norm mRNA exp Early fall. Probabilities for Post Hoc Tests Error: Between MS = .12394, df = 102.00													
	Gene	Part of retina	{1} 1.679 5	{2} 1.644 5	{3} -1.018 -	{4} -1.051 -	{5} -3.326 -	{6} -3.242 -	{7} 1.071 4	{8} 1.114 8	{9} -1.033 -	{10} -.4316 -	{11} -2.381 -	{12} -2.210 -
1	RH1	Dorsal		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001
2	RH1	Ventral	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001
3	SW S2A	Dorsal	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
4	SW S2A	Ventral	P << 0.001	0.000 118	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	P << 0.001	P << 0.001	P << 0.001
5	SW S2B	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
6	SW S2B	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001
7	RH2 A-1	Dorsal	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001
8	RH2 A-1	Ventral	P < 0.05	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000		P << 0.001	P << 0.001	P << 0.001	P << 0.001
9	RH2 A-2	Dorsal	P << 0.001	P << 0.001	1.000	1.000	P << 0.001	P << 0.001	P << 0.001	P << 0.001		P < 0.05	P << 0.001	P << 0.001
10	RH2 A-2	Ventral	P << 0.001	P << 0.001	P < 0.05	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P < 0.05		P << 0.001	P << 0.001
11	RH2 A-3	Dorsal	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001		1.000
12	RH2 A-3	Ventral	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	P << 0.001	1.000	