Supplementary Figures:

Seasonal variation in UVA light drives hormonal and behavioral changes in a marine annelid via a ciliary opsin

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SupplFig. 1. Temporal schematic of illumination for long day and short day experiments. (a,b) longday (16:8 LD) experiment entrained under "white" light including UVA (a) and "white" light with filter-reduced UVA (B). (c-f) short day (c,d- LD8:16) and equal daylength (e,f- LD12:12) experiments. As worms are normally grown at LD 16:8, shorter daylength are achieved by gradually decreasing daylength from longday. The gradual decrease is performed to avoid confounding the worms' circalunar entrainment. PD – Preparation day, ED – Experimental day. For light spectra: Extended Data Fig.4.

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c longday + UVA experiment : *c-opsin1*^{Δ8/Δ8}

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Supplementary Figure 2 *cont.* Page 2



Supplementary Figure 2 Page 3

SupplFig. 2. Individual actograms of *c-opsin1*^{$\Delta 8$} homozygous, heterozygous and wildtype siblings under long day, including strong UVA. Individual double-plotted actograms. 5 days of LD followed by 5 days of DD (shaded). Genotypes as indicated. Y-axis: days.



a longday + UVA : *c-opsin1*^{+/+}

Supplementary Figure 3 *cont.* Page 1

b longday + UVA : *c-opsin1*^{Δ8/Δ7}



Supplementary Figure 3 *cont.* Page 2

SupplFig.3. Individual actograms of *c-opsin1*^{$\Delta 8/\Delta 7$} transheterozygous and wildtype under longday, including strong UVA. 3 days of LD. Individual double-plotted actograms plot of *c- opsin1*^{+/+} (A) and *c-opsin1*^{$\Delta 8/\Delta 7$} (B). Genotypes as indicated. Y-axis: days.



Supplementary Figure 4 *cont.* Page. 1



SupplFig.4. Individual double-plotted actograms of *c-opsin1*^{$\Delta 8$} and corresponding wt under long day, with filter-reduced UVA. 5 days of LD. Genotypes as indicated. Y-axis: days.



SupplFig.5. Individual double-plotted actograms of *c-opsin1*^{$\Delta 8$} and corresponding wt under short day, including strong UVA. 5 days of LD. Genotypes as indicated. Y-axis: days.





Supplementary Figure 6 cont. Page. 1



Supplementary Figure 6 Page. 2

SupplFig.6. Individual double-plotted actograms of *c-opsin1*^{$\Delta 8$} and corresponding wt under shortday, with filter-reduced UVA. 5 days of LD. Genotypes as indicated. Y-axis: days.

Longday experiment - 16:8LD				
Comparison	One-way ANOVA (Sidak's multiple correction test)	Significant?	Summary	Adjusted P-value
	c-opsin1 ^{+/+} LD+UVA(0-8hrs)vs. c-opsin1 ^{+/+} LD-UVA(0-8hrs)	No	ns	0.9837
c-opsin1*/* LD+UVA vs LD-UVA	c-opsin1 ^{+/+} LD+UVA(8-16hrs)vs. c-opsin1 ^{+/+} LD-UVA(8-16hrs)	No	ns	0.9998
	<i>c-opsin1</i> ^{+/+} LD+UVA(16-24hrs)vs. <i>c-opsin1</i> ^{+/+} LD-UVA(16-24hrs)	Yes	****	<0.0001
	c-opsin1- ^{,,,} LD+UVA(0-8hrs)vs. c-opsin1- ^{,,,} LD-UVA(0-8hrs)	No	ns	>0.9999
c-opsin1"" LD+UVA vs LD-UVA	c-opsin1- ^{,,,} LD+UVA(8-16hrs)vs. c-opsin1- ^{,,,} LD-UVA(8-16hrs)	No	ns	0.9957
	c-opsin1 ^{-/-} LD+UVA(16-24hrs)vs. c-opsin1 ^{-/-} LD-UVA(16-24hrs)	No	ns	0.9761
	c-opsin1 ^{+/+} LD+UVA(0-8hrs)vs. c-opsin1- ^{/-} LD+UVA(0-8hrs)	No	ns	0.9898
c-opsin1 ^{+/+} vs c-opsin1 ^{-/-}	<i>c-opsin1</i> ^{+/+} LD+UVA(8-16hrs)vs. <i>c-opsin1</i> ^{-/-} LD+UVA(8-16hrs)	No	ns	0.9616
	<i>c-opsin1</i> ^{+/+} LD+UVA(16-24hrs)vs. <i>c-opsin1</i> ^{-/-} LD+UVA(16-24hrs)	Yes	****	<0.0001
	<i>c-opsin1</i> ^{+/+} LD-UVA(0-8hrs) vs. <i>c-opsin1</i> ^{-/-} LD-UVA(0-8hrs)	No	ns	0.9111
c-opsin1 ^{+/+} vs c-opsin1 ^{-/-}	c-opsin1 ^{+/+} LD-UVA(8-16hrs) vs. c-opsin1 ^{-/-} LD-UVA(8-16hrs)	No	ns	0.9859
	c-opsin1+/+ LD-UVA(16-24hrs)vs. c-opsin1-/- LD-UVA(16-24hrs)	No	ns	0.2644

Shortday experiment – 8:16LD				
Comparison	One-way ANOVA (Sidak's multiple correction test)	Significant?	Summary	Adjusted P-value
	c-opsin1 ^{+/+} SD+UVA(0-8hrs) vs. c-opsin1 ^{+/+} SD-UVA(0-8hrs)	No	ns	0.7789
c-opsin1*/* SD+UVA vs SD-UVA	c-opsin1 ^{+/+} SD+UVA(8-16hrs) vs. c-opsin1 ^{+/+} SD-UVA(8-16hrs)	No	ns	0.9328
	c-opsin1 ^{+/+} SD+UVA(16-24hrs) vs. c-opsin1 ^{+/+} SD-UVA(16-24hrs)	No	ns	0.9863
c-opsin1 ^{-/-} SD+UVA vs SD-UVA	<i>c-opsin1-</i> SD+UVA(0-8hrs)vs. <i>c-opsin1-</i> SD-UVA(0-8hrs)	No	ns	0.9989
	c-opsin1- ^{,,,} SD+UVA(8-16hrs) vs. c-opsin1- ^{,,,} SD-UVA(8-16hrs)	No	ns	0.1658
	c-opsin1 ^{-/-} SD+UVA(16-24hrs)vs. c-opsin1 ^{-/-} SD-UVA(16-24hrs)	No	ns	0.2459
SD+UVA	c-opsin1⁺/+ SD+UVA(0-8hrs) vs. c-opsin1-/- SD+UVA(0-8hrs)	No	ns	0.8137
c-opsin1 ^{+/+} vs c-opsin1 ^{-/-}	c-opsin1 ^{+/+} SD+UVA(8-16hrs)vs. c-opsin1 ^{-/-} SD+UVA(8-16hrs)	No	ns	0.3913
	<i>c-opsin1</i> ^{+/+} SD+UVA(16-24hrs)vs. <i>c-opsin1</i> ^{-/-} SD+UVA(16-24hrs)	No	ns	0.0525
SD-UVA	<i>c-opsin1</i> ^{+/+} SD-UVA(0-8hrs) vs. <i>c-opsin1</i> ^{-/-} SD-UVA(0-8hrs)	No	ns	>0.9999
c-opsin1 ^{+/+} vs c-opsin1 ^{-/-}	c-opsin1+/+ SD-UVA(8-16hrs) vs. c-opsin1-/- SD-UVA(8-16hrs)	No	ns	>0.9999
	c-opsin1 ^{+/+} SD-UVA(16-24hrs) vs. c-opsin1 ^{-/-} SD-UVA(16-24hrs)	No	ns	0.0603

Equinox experiment – 16:8LD				
Comparison	One-way ANOVA (Sidak's multiple correction test)	Significant?	Summary	Adjusted P-value
	<i>c-opsin1</i> ^{+/+} LD+UVA(0-8hrs)vs. <i>c-opsin1</i> ^{+/+} LD-UVA(0-8hrs)	No	ns	0.9981
c-opsin1+/+ LD+UVA vs LD-UVA	<i>c-opsin1</i> ^{+/+} LD+UVA(8-16hrs) vs. <i>c-opsin1</i> ^{+/+} LD-UVA(8-16hrs)	No	ns	>0.9999
	<i>c-opsin1</i> ^{+/+} LD+UVA(16-24hrs) vs. <i>c-opsin1</i> ^{+/+} LD-UVA(16-24hrs)	Yes	**	0.0056
c-opsin1 ^{-/-}	c-opsin1- ^{,,,} LD+UVA(0-8hrs) vs. <i>c-opsin1</i> - ^{,,,} LD-UVA(0-8hrs)	No	ns	0.8926
	c-opsin1- ^{,,,} LD+UVA(8-16hrs) vs. <i>c-opsin1</i> - ^{,,,} LD-UVA(8-16hrs)	No	ns	0.9927
	c-opsin1 ^{-,,,} LD+UVA(16-24hrs)vs. c-opsin1 ^{-,,,} LD-UVA(16-24hrs)	No	ns	0.3264
LD+UVA c-opsin1*/* vs c-opsin1*/-	<i>c-opsin1</i> ^{+/+} LD+UVA(0-8hrs) vs. <i>c-opsin1</i> ^{-/-} LD+UVA(0-8hrs)	No	ns	0.9969
	<i>c-opsin1</i> ^{+/+} LD+UVA(8-16hrs)vs. <i>c-opsin1</i> ^{-/-} LD+UVA(8-16hrs)	No	ns	>0.9999
	<i>c-opsin1</i> ^{+/+} LD+UVA(16-24hrs)vs. <i>c-opsin1</i> ^{-/-} LD+UVA(16-24hrs)	Yes	**	0.0045
	<i>c-opsin1</i> ^{+/+} LD-UVA(0-8hrs) vs. <i>c-opsin1</i> - ^{/-} LD-UVA(0-8hrs)	No	ns	0.9647
c-opsin1 ^{+/+} vs c-opsin1 ^{-/-}	c-opsin1+/+ LD-UVA(8-16hrs) vs. c-opsin1-/- LD-UVA(8-16hrs)	No	ns	>0.9999
	c-opsin1+/+ LD-UVA(16-24hrs) vs. c-opsin1-/- LD-UVA(16-24hrs)	No	ns	0.3128

Equinox experiment - 12:12LD				
Comparison	One-way ANOVA (Sidak's multiple correction test)	Significant?	Summary	Adjusted P-value
<i>c-opsin1</i> ^{+/+} 12:12LD+UVA vs 12:12LD-UVA	c-opsin1 ^{+/+} LD+UVA(0-12hrs)vs.c-opsin1 ^{+/+} LD-UVA(0-12hrs)	No	ns	0.9234
	<i>c-opsin1</i> ^{+/+} LD+UVA(12-24hrs) vs. <i>c-opsin1</i> ^{+/+} LD-UVA(12-24hrs)	No	ns	0.0828
c-opsin1 ^{-/-}	c-opsin1 ^{-,,} LD+UVA(0-12hrs)vs.c-opsin1 ^{-,,} LD-UVA(0-12hrs)	No	ns	0.9970
12:12LD+UVA vs 12:12LD-UVA	c-opsin1 ^{-/-} LD+UVA(12-24hrs)vs. c-opsin1 ^{-/-} LD-UVA(12-24hrs)	No	ns	0.5743
12:12LD+UVA c-opsin1+/+ vs c-opsin1-/-	c-opsin1 ^{+/+} LD+UVA(0-12hrs) vs. c-opsin1 ^{-/-} LD+UVA(0-12hrs)	No	ns	0.9975
	c-opsin1+/+ LD+UVA(12-24hrs) vs. c-opsin1+/ LD+UVA(12-24hrs)	No	ns	0.4974
12:12LD-UVA	c-opsin1 ^{+/+} LD-UVA(0-12hrs)vs. c-opsin1 ^{-/-} LD-UVA(0-12hrs)	No	ns	0.8849
c-opsin1+/+ vs c-opsin1-/-	c-opsin1+/+ LD-UVA(12-24hrs) vs. c-opsin1+/- LD-UVA(12-24hrs)	No	ns	0.8163

Supplementary Figure 7

SupplFig.7. Statistical analyses and comparisons for different photoperiods, UVA intensities and *c-opsin1* wildtype/mutant.





Supplementary Figure 8 cont. Page 1















Supplementary Figure 8 cont. Page 2



d Equinox experiment - 16:8 LD-UVA *c-opsin1*-/

Supplementary Figure 8 cont. Page 3

SupplFig.8. Individual double-plotted actograms of *c-opsin1*^{$\Delta 8$} and corresponding wt under long day, strong versus filter-reduced UVA. The same worms were subsequently analyzed under LD12:12 (see Suppl.Fig.1e,f and 9), and hence part of the "equinox experiment".





C Equinox experiment - 12:12 LD-UVA c-opsin1*/*







Supplementary Figure 9 *cont.* Page 2 d Equinox experiment - 12:12 LD-UVA *c-opsin1 c-opsin1* - #9



Supplementary Figure 9 cont. Page 3

RAMSES-ACC-VIS

Hyperspectral UV-VIS Irradiance Sensor

320..950 nm

Features:

RAMSES-ACC is a stand-alone highly integrated hyperspectral radiometer for the UV and/or VIS spectral range. Small size and very low power consumption make it suitable for hand-held and autonomous applications. The sensor is part of the **RAMSES radiometer family**, which is especially designed for combining precision hyperspectral light measurements with a maximum of flexibility.

Applications:

- monitoring
- water quality
- field measurements
- satellite data validation
- biology
- photosynthesis
- climatology







Sensors are delivered with our free graphical easy-touse software for measurement, data display and storage.

TriOS GmbH Werftweg 15 D-26135 Oldenburg Germany, info@trios.de fon +49 (0) 441 - 4 85 98-0 fax +49 (0) 441 - 4 85 98-20

www.trios.de

RAMSES-ACC

Features:

- stand-alone hyperspectral radiometer
- low power consumption
- small size
- autorange function
- free Windows-based acquisition and control software

Accessories:

- power supply
- interface for simultaneous operation of up to 4 radiometers
- data logger units
- sensor frames

-	Technical specifications
	VIS
optical	
wavelength range*	320 – 950 nm
detector type*	channel silicon photodiode array
spectral sampling*	3.3 nm/pixel
spectral accuracy*	0.3 nm
usable channels	190
typical saturation	10 W m ⁻² nm ⁻¹ (at 400nm)
(4ms integration time)	8 W m ⁻² nm ⁻¹ (at 500nm)
	14 W m ⁻² nm ⁻¹ (at 700nm)
typical NEI	0.4 μW m ⁻² nm ⁻¹ (at 400nm)
(8s integration time)	0.4 μW m ⁻² nm ⁻¹ (at 500nm)
	0.6 μW m ⁻² nm ⁻¹ (at 700nm)
detection	
collector type	cosine response
accuracy	better then 6 – 10% (depending on spectral
-	range)
electrical	
integration time	4 ms – 8 sec.
telemetry data	RS-232
interface	
data rate (RS-232)	1,200 – 19,200 baud
power requirements	1.5 – 11 VDC
	0.85 W (data acquisition active)
	100 mW (interface active)
	0.5 mW (stand-by modus)
connector	SUBCONN-Micro 5 pins, male connector
physical	
size	Ø 4.7 cm x 26 cm (without connector)
weight in air	< 1.0 kg (stainless steel/POM housing)
depth rating	300 m
operating temperature	-10 to +50°C

* specifications from Carl ZEISS, Germany

Specifications may change due to technical improvements without notification.



typ. cosine response at different wavelengths

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SupplFig.10. Information data sheet of the Ramses hyperspectral radiometers used in the study (TriOS GmbH), new address: TriOS Mess- und Datentechnik GmbH, Buergermeister-Broetje-Str.25, D-26180, Rastede, Germany