Blue light regulation of host pigment expression in reef-building corals

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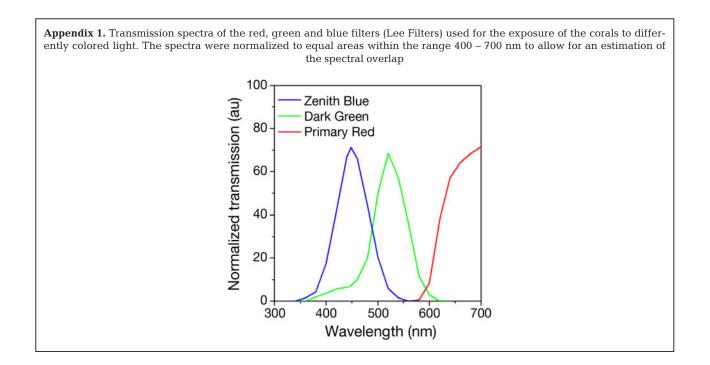
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Appendix 2. Light response of primary polyps, methodology

Planula larvae were obtained from crossing 2 colonies of *Acropora millepora*. Developing larvae were shielded from light. Settlement and metamorphosis were induced in culture dishes by the addition of calcareous red algae (1). Primary polyps were incubated under red and blue light at a photon flux of 20 µmol m⁻² s⁻¹ with a 12 h light:dark cycle for 5 d. Polyps were photographed with a Leica MZ FL III microscope (Leica Microsystems) equipped with a mercury lamp, a set of filters for epifluorescence and a Spot Insight QE digital camera (Diagnostic Instruments). The intensity of the green fluorescence was quantified using Adobe Photoshop 5.0 Software (Adobe Systems) for grey-scale analysis.

(1) Heyward AJ, Negri dAP (1999) Natural inducers for coral larval metamorphosis. Coral Reefs 18:273–279

Appendix 3. Oligonucleotide primers sequences

amilFP484/497/512	5'tttggatccatgtcttattcaaagcaaggcatcg- tacaag3' 5'tttaagcttatttaaccttcaaagggttaacatga- gcc3'
amilFP597	$5'tttggatccatggctctgtcaaagcacggtttaac3'\\5'tttaagcttatccgggcaatgcggatcgg3'$
apCP584	5'tttggatccatgagtgtgatcgctaaacaaatg- acc3' 5'tttaagcttaggcgaccacaggtttgcgtg3'
GAPDH	5'accattgtcagcaatgcatcctgc3' 5'cacacggaaagccataccagtc3'
AmES	5'gagatggacgaaaagcctatacgaatg3' 5'catttgcaggatccagcttacgc3'

Appendix 4. Probability values (p) calculated by independent *t*-test of the difference between values obtained from the different light treatments and the number of data points (n); RL: red light, GL: green light, BL: blue light; nd: not determined

Protein]	—— Light intensity (μmol photons m ⁻² s ⁻¹)——						Light quality				
	$80 \rightarrow 2$		$100 \rightarrow 4$		$400 \rightarrow 0$		$RL \rightarrow 0$	GL	$GL \rightarrow E$	BL Î	$RL \rightarrow E$	3L
hgraCFP493	0.705	30	< 0.001	30	0.641	30	< 0.001	16	< 0.001	16	< 0.001	16
mdigCFP486	nd	nd	0.002	12	0.7834	12	< 0.001	12	< 0.001	12	< 0.001	12
mdigOFP574	nd	nd	< 0.001	12	0.1848	12	< 0.001	12	< 0.001	12	< 0.001	12
shysCP564	nd	nd	3ª	3^{a}	nd	nd	3ª	3^a	3ª	3^{a}	3 ^a	3^{a}
apulCFP483	0.001	33	< 0.001	33	0.859	33	< 0.001	18	< 0.001	18	< 0.001	18
apulCP584	nd	nd	3ª	3^{a}	nd	nd	3ª	3^a	3ª	3^{a}	3 ^a	3^{a}
amilCFP484	< 0.001	21	< 0.001	21	< 0.001	21	< 0.001	14	< 0.001	14	< 0.001	14
amilGFP497	< 0.001	21	< 0.001	21	$21^{\rm b}$	$21^{\rm b}$	< 0.001	14	0.474	14	< 0.001	14
amilGFP512	< 0.001	21	< 0.001	21	< 0.001	21	< 0.001	14	< 0.001	14	< 0.001	14
amilRFP597	$21^{\rm b}$	$21^{\rm b}$	< 0.001	21	< 0.001	21	< 0.001	14	< 0.001	14	< 0.001	14

^ap-value was not calculated because the number of independent samples was too low for statistical analysis

Appendix 5. Summary of the change in GFP-like protein content upon various exposures to light, as determined from the ratios of emission (for FPs) or absorption (for CPs) maxima recorded before and after changing light conditions. RL: red light, GL: green light, BL: blue light; nd: not determined; n.c.: not calculated because the denominator was approximately zero

Protein										
	$80 \rightarrow 100$	$100 \rightarrow 400$	$400 \rightarrow 700$	$RL \rightarrow GL$	$GL \rightarrow BL$	$RL \to BL$				
hgraCFP492	0	60	0	60	70	160				
mdigCFP486	nd	50	-10	60	30	110				
mdigOFP574	nd	560	0	370	40	580				
shysCP562	nd	n.c.	nd	n.c.	280	n.c.				
apulCFP483	100	40	0	460	30	630				
apulCP584	nd	n.c.	nd	n.c.	140	n.c.				
amilCFP484	80	210	-40	230	110	600				
amilGFP497	n.c.	-70	n.c.	910	0	950				
amilGFP512	680	230	40	1040	220	n.c.				
amilRFP597	n.c.	540	150	270	640	n.c.				

^bProtein was not detectable