Table S1: Overview of numbers of specimens used per light treatment and per sampling timepoint. Timepoint 0 = Start of experiment; Timepoint 1 = 1 month; Timepoint 2 = 4 months (*O. cyanosoma*) / 6 months (*P. moluccensis, P. amboinensis*).

Species	Treatment												
	Baseline	Blue		Green		Red		No Filter		ND0.15		ND0.6	
	0	1	2	1	2	1	2	1	2	1	2	1	2
O. cyanosoma	18	8	8	7	5	8	5	6	n/a	10	n/a	7	n/a
P. moluccensis	18	3	3	3	5	3	9	6	n/a	6	n/a	5	n/a
P. amboinensis	8	5	5	13	5	4	5	n/a	n/a	n/a	n/a	n/a	n/a

Table S2: Primer names and sequences used for PCR and sequencing of the pool of opsins for *O. cyanosoma*; and summary of qPCR primer combinations and efficiencies for each species. Primer names and sequences for *P. amboinensis* and *P. moluccensis* were obtained from Stieb et al. (2016).

opsin	species	primer pool	sequence
SWS2Aα	*O. cyanosoma*	pSWS2AA_F1	5’-GCCATGGCTAACCTCATTGT-3’
		pSWS2AA_R4	5’-TTTGGAGACTTCAGTTACTGATGCT -3’
SWS2Aβ	*O. cyanosoma*	pSWS2AB_F3	5’-AACTTGGCCTTTTCCAACCT -3’
		pSWS2AB_R5	5’-ACTTCAGTCACCGACTGG -3’
RH2B	*O. cyanosoma*	pRH2B_F3	5’-CTCCGGCAACCTCTGAACTT -3’
		pRH2B_R3	5’-GTATGGGGTCCAAGCAACAA-3’
RH2A	*O. cyanosoma*	pSWS2AB_R5	5’-GGCAACCGCTGAACTACATC-3’
		pRH2A_R1	5’-AGCCAAAGACCATCAAGACG-3’
LWS	*O. cyanosoma*	pLWS_F1	5’-TCAGCGTATGCAACCAGTTC-3’
		pLWS_R1	5’-GGCATATCCAGGGTTAGCAG-3’
RH1	*O. cyanosoma*	pRH1_F2	5’-GCGTTGTCCGGAGTCCTTAT-3’
		pRH1_R2	5’-TCCACATGAGCACTGCATTC-3’
opsin	**species with primer efficiencies (%)**	**primer qPCR**	**sequence**
SWS1	*P. amboinensis* (90), *P. moluccensis* (94)	SWS1_forward_1	5’-CTCCAAGAGCTCCTGCGTCT-3’
		SWS1_reverse_1	5’-TGATGCAGGCGTTGAACTGTTTG-3’
SWS2B	*P. amboinensis* (91), *P. moluccensis* (91)	SWS2B_forward_1	5’-GGTGAAAGCGGTAGCAAAGG-3’
		SWS2B_reverse_1	5’-CCATCTTGGTCACCTCCCGCTC-3’
SWS2Aα	*O. cyanosoma* (103)	SWS2AA_F3	5’- ATAAACAGTTCCGTGGGTGCATGAT -3’
		SWS2AA_R3	5’- TTGGAGACTTCAGTTACTGATGCTG -3’
SWS2Aβ	*O. cyanosoma* (100)	SWS2AB_F1	5’- TAACGCTTGGTGGGATGGTGAG -3’
		SWS2AB_R1	5’- GCTAAAGCGTGGTCAGGTTTGAAC -3’
RH2B	*P. amboinensis*	RH2B_forward_1	5’-GGTGGGCTATTTCTCCTTGGG-3’
	P. moluccensis	RH2B_forward_2	5’-GATGGGCTATTTCTCCTTGGGG-3’
	P. amboinensis (93), *P. moluccensis* (96)	RH2B_reverse_1	5’-CACAGAGACACTTGACCTCCG-3’
	O. cyanosoma (95)	RH2B_F1	5’- CTGCTTTGGCTTCACCATCACC -3’
		RH2B_R5	5’-ACTTGACCTCCCAGTGTAGCCATG-3’
RH2A	*P. amboinensis* (91), *P. moluccensis* (94)	RH2A_forward_1	5’-CATTCTTGGACCCACTTTCTGCG-3’
		RH2A_reverse_1	5’-CCAGAGAGCAACTTCACCTCCA-3’
	O. cyanosoma (91)	RH2A_F2	5’-ATGCAGGAGCTGGAGTTGCTTTC-3’
		RH2A_R2	5’-GGTACCTGGACCAGCCACC-3’
LWS	*P. moluccensis*	LWS_forward_1	5’-ACACCAATCACACCAAAGATCCC-3’
	P. amboinensis	LWS_forward_3	5’-CCAATTACACCAAAGATCCC-3’
	P. amboinensis (96), *P. moluccensis* (95)	LWS_reverse_2	5’-GACAAACATCCAGGCTGTGGC-3’
	O. cyanosoma (96)	LWS_F2	5’-TTCGGATGGAGCAGGTACTGG-3’
		LWS_R2	5’-ATCATGTACGACTGGACTCCAGG-3’
RH1	*P. amboinensis* (91), *P. moluccensis* (90)	RH1_forward_1	5’-CCACTGCATGATCACCACCT-3’
		RH1_reverse_1	5’-GATGCTCCCTCCTCTTCTTCG-3’
	O. cyanosoma (81)	RH1_F3	5’-CCATCAGCAACTTCCGCTTTGG-3’
		RH1_R3	5’-GGGGTACGGAGCAAGCAGC-3’

Table S3: Summary of total relative opsin expression under different colour treatments and after different treatment durations in the three investigated reef fish species. Values are given as median fraction of total single cone opsin (%) and interquartile range for each of the SWS cone opsins present in each respective species (*O. cyanosoma*: SWS2Aα, SWS2Aβ; *P. amboinensis*/*P. moluccensis*: SWS1/SWS2B), as median fraction of total double cone opsin (RH2B, RH2A, LWS) and interquartile range, and as median fraction of cone and rod opsin of total opsin (%) and interquartile range.

Species	time spent in tank [months]	treatment	n	Opsin gene								
				SWS1	SWS2B	SWS2Aα	SWS2Aβ	RH2B	RH2A	LWS	Total Cone	Total RH1
P. amboinensis	0	baseline	8	86.6, 9.6	13.4, 9.6	-	-	45.0, 4.0	54.5, 3.9	0.4, 0.4	58.7, 6.5	41.3, 6.5
	1	blue	5	84.2, 6.1	15.8, 6.1	-	-	45.4, 2.4	53.4, 1.6	1.3, 0.2	64.4, 3.1	35.6, 3.1
		green	13	68.4, 4.1	31.6, 4.1	-	-	46.6, 5.4	46.6, 6.6	1.1, 4.9	63.9, 7.5	36.1, 7.5
		red	4	68.8, 13.2	31.2, 13.2	-	-	46.0, 2.6	52.8, 1.8	1.1, 0.4	63.2, 4.5	36.8, 4.5
	6	blue	5	75.7, 3.5	24.3, 3.5	-	-	45.6, 1.0	53.9, 0.9	0.5, 0.4	52.9, 6.1	47.1, 6.1
		green	5	58.8, 12.9	41.2, 12.9	-	-	44.5, 4.4	54.5, 5.0	0.6, 0.6	56.3, 9.1	43.7, 9.1
		red	5	45.8, 12.2	54.2, 12.2	-	-	45.3, 1.7	54.2, 1.0	0.6, 0.2	47.6, 7.9	52.4, 7.9
P. moluccensis	0	baseline	18	85.0, 12.5	15.0, 12.5	-	-	43.4, 3.1	52.2, 2.7	3.6, 2.1	63.2, 11.0	46.8, 11.0
	1	blue	3	87.8, 12.4	12.2, 12.4	-	-	44.3, 1.5	49.6, 1.4	6.6, 2.6	62.6, 1.8	37.4, 1.8
		green	3	56.1, 8.9	43.9, 8.9	-	-	40.6, 2.0	50.8, 0.8	8.7, 2.7	58.3, 2.2	41.7, 2.2
		red	3	57.3, 6.2	42.7, 6.2	-	-	41.7, 4.0	51.3, 3.9	5.1, 1.0	56.6, 5.3	33.4, 5.3
		no filter	6	69.1, 21.9	30.9, 21.9	-	-	45.5, 2.1	49.0, 2.6	6.3, 2.2	65.7, 15.5	34.3, 15.5
		0.15 ND	6	69.8, 14.4	30.2, 14.4	-	-	47.0, 2.4	48.4, 2.6	5.5, 4.0	55.1, 8.2	44.9, 8.2
		0.6 ND	5	55.5, 5.2	44.5, 5.2	-	-	44.2, 2.0	50.1, 1.2	6.1, 3.3	61.3, 0.7	38.7, 0.7
	6	blue	3	70.3, 5.9	29.7, 5.9	-	-	43.5, 1.0	49.8, 1.3	5.0, 1.3	51.0, 11.4	49.0, 11.4
		green	5	59.6, 14.3	40.4, 14.3	-	-	41.5, 0.9	52.4, 0.3	6.1, 1.0	53.1, 3.3	46.9, 3.3
		red	9	66.6, 25.9	33.4, 25.9	-	-	41.5, 3.6	49.9, 4.4	6.0, 2.6	62.3, 12.1	37.7, 12.1
O. cyanosoma	0	baseline	18	-	-	9.4, 7.5	90.6, 7.5	17.1, 8.5	82.5, 8.6	0.4, 0.9	91.2, 3.9	8.8, 3.9
	1	blue	8	-	-	9.4, 5.5	90.6, 5.5	16.5, 8.9	83.0, 8.5	0.7, 0.5	92.8, 0.8	7.2, 0.8
		green	7	-	-	6.2, 5.7	93.8, 5.7	14.5, 2.9	84.6, 4.1	0.8, 0.7	93.4, 1.3	6.6, 1.3
		red	8	-	-	2.3, 2.4	97.7, 2.4	13.1, 5.1	86.6, 5.1	0.4, 0.2	92.9, 1.6	7.1, 1.6
		no filter	6	-	-	16.6, 2.1	83.4, 2.1	33.4, 10.0	65.9, 9.4	0.5, 0.6	89.9, 3.3	10.1, 3.3
		0.15 ND	10	-	-	16.7, 17.9	83.3, 17.9	20.5, 12.5	78.6, 11.3	0.4, 1.0	91.3, 1.3	8.7, 1.3
		0.6 ND	7	-	-	13.9, 9.3	86.1, 9.3	42.0, 20.0	57.9, 19.7	0.1, 0.3	91.1, 5.6	8.9, 5.6
	4	blue	8	-	-	11.3, 5.6	88.7, 5.6	4.1, 5.6	94.3, 6.6	1.5, 1.7	94.9, 1.5	5.1, 1.5
		green	5	-	-	9.0, 6.4	91.0, 6.4	6.1, 6.2	92.2, 7.2	1.2, 0.5	95.5, 0.3	3.5, 0.3
		red	5	-	-	3.3, 1.7	96.7, 1.7	3.3, 3.9	95.4, 3.4	0.6, 0.5	94.8, 1.1	5.2, 1.1

Table S4: Summary of beta regression models showing results for baseline datasets tested against light treatments (blue, green, red, no filter, ND0.15, ND0.6) after different time points, and results for no filter datasets tested against light treatments (blue, green, red, ND0.15, ND0.6). After Bonferroni-Correction for six (baseline dataset) respectively five (no filter dataset) tested treatment hypotheses (p = α/m, with m=6 resp. 5), p-values less than or equal to 0.0083, 0.0017, and 0.00017 resp. p-values less than or equal to 0.01, 0.002, and 0.0002 were considered significant and are marked with *, **, or ***, respectively. Statistically significant P-values are shown in bold.

species	time spent in tank [months]	light & control treatment	opsin gene							
			SWS1	SWS2B	SWS2Aα	SWS2Aβ	RH2B	RH2A	LWS	RH1
			baseline	baseline	baseline	baseline	baseline	baseline	baseline	baseline
P. amboinensis	1	blue	0.385	0.385	n/a	n/a	0.508	0.865	**0.002***	0.337
		green	**2.77E-08*****	**2.77E-08*****	n/a	n/a	0.09	**0.004***	**0.007***	0.277
		red	**7.02E-05*****	**7.02E-05*****	n/a	n/a	0.404	0.245	0.009	0.194
	6	blue	**1.05E-04*****	**1.05E-04*****	n/a	n/a	0.922	0.982	0.169	0.071
		green	**1.01E-14*****	**1.01E-14*****	n/a	n/a	0.78	0.676	0.046	0.091
		red	**2.67E-13*****	**2.67E-13*****	n/a	n/a	0.697	0.823	0.999	0.015
P. moluccensis	1	blue	0.947	0.947	n/a	n/a	0.465	0.16	0.715	0.683
		green	**1.85E-05*****	**1.85E-05*****	n/a	n/a	0.108	0.452	**2.23E-04****	0.222
		red	**5.83E-06*****	**5.83E-06*****	n/a	n/a	0.902	0.22	0.009	0.126
		no filter	**7.65E-04****	**7.65E-04****	n/a	n/a	0.09	**7.68E-04****	**0.002***	0.349
		0.15 ND	**4.00E-05*****	**4.00E-05*****	n/a	n/a	0.019	**6.13E-04****	0.13	0.044
		0.6 ND	**3.5E-10*****	**3.5E-10*****	n/a	n/a	0.414	0.051	0.05	0.257
	6	blue	0.01	0.01	n/a	n/a	0.863	0.151	**0.008***	0.076
		green	**4.96E-09*****	**4.96E-09*****	n/a	n/a	0.235	0.849	**0.006***	**7.38E-05*****
		red	**3.64E-04****	**3.64E-04****	n/a	n/a	0.14	0.12	**8.25E-08*****	0.058
O. cyanosoma	1	blue	n/a	n/a	0.521	0.521	0.63	0.602	0.357	0.204
		green	n/a	n/a	0.109	0.109	0.204	0.213	0.145	0.076
		red	n/a	n/a	**1.74E-05*****	**1.74E-05*****	0.184	0.134	0.527	0.416
		no filter	n/a	n/a	**0.003***	**0.003***	**1.01E-04*****	**9.68E-05*****	0.75	0.417
		0.15 ND	n/a	n/a	**0.002***	**0.002***	0.627	0.609	0.834	0.707
		0.6 ND	n/a	n/a	0.343	0.343	**0.003***	**0.003***	**0.001***	0.156
	4	blue	n/a	n/a	0.227	0.227	**2.41E-04****	**7.62E-04****	0.029	0.015
		green	n/a	n/a	0.736	0.736	**0.002***	**0.002***	0.029	0.011
		red	n/a	n/a	**2.01E-04****	**2.01E-04****	**3.43E-05*****	**2.73E-05*****	0.836	0.013
			no filter	no filter	no filter	no filter	no filter	no filter	no filter	no filter
P. moluccensis	1	blue	0.046	0.046	n/a	n/a	0.514	0.18	0.306	
		green	0.183	0.183	n/a	n/a	**0.002***	0.03	0.349	
		red	0.121	0.121	n/a	n/a	0.365	0.304	0.995	
		0.15 ND	0.606	0.606	n/a	n/a	0.435	0.528	0.477	
		0.6 ND	0.011	0.011	n/a	n/a	0.323	0.259	0.591	
O. cyanosoma	1	blue	n/a	n/a	**5.44E-04****	**5.44E-04****	**1.25E-08*****	**9.86E-09*****	0.147	
		green	n/a	n/a	**3.34E-06*****	**3.34E-06*****	**6.49E-09*****	**8.03E-09*****	0.061	
		red	n/a	n/a	**2.97E-14*****	**2.97E-14*****	**4.52E-12*****	**7.63E-13*****	0.748	
		0.15 ND	n/a	n/a	**0.004***	**0.004***	**5.7E-04****	**3.44E-04****	0.895	
		0.6 ND	n/a	n/a	0.178	0.178	0.782	0.751	0.037	

Table S5: Overview of sites and site effects considered for λ_{max}-calculations for *Pomacentrus moluccensis*, *P. amboinensis*, and *Ostorhinchus cyanosoma*. Bottom site numbers indicate amino acid residue position of Bovine rhodopsin, top site numbers indicate amino acid (AA) residue position in the target genes. All site effects and overall sensitivities are given in nm. AA changes in sites that our λ_{max}-calculations are based on are highlighted in blue, and are either known tuning sites (SWS2B W265Y, SWS2Aβ S94A, LWS S164A, LWS Y261F: Lin et al., 1998; Takahashi and Ebrey, 2003; Yokoyama, 2008; Dungan et al., 2016) or in close vicinity (RH2B C157V: Chan et al., 1992; Wilkie et al., 2000), are estimated on MSP mesaurements (SWS2Aα L216F; RH2B I49C, RH2B S109G, LWS: Cortesi et al., 2015; Spady et al., 2006; Siebeck et al., 2010; Losey et al., 2003; Stieb et al., 2016), or are based on a change in polarity (RH2B C98A, RH2B S124A, RH2A F158/166L, RH2A F158/166A).

SWS2B

Species	Variable site							Estimated tuning effect (nm)						Estimate (nm)	Actual lmax (nm)	MSP
	49	52	124	169	174	209	271	49	52	124	169	174	271			
***P. moluccensis* SWS2B**	F	F	T	F	A	Y	Y	0	0	0	0		-15	408		(404 - 410 in other damselfish)
***P. amboinensis* SWS2B**	F	F	T	F	A	Y	Y	0	0	0	0		-15	408		
P. fuscus SWS2B	F	F	T	C	A	Y	W	0	0	0	0		0	423		
M. zebra SWS2B	F	F	T	C	A	Y	W	0	0	0	0		0	423	423	
O. niloticus SWS2B	F	F	T	C	A	Y	W	0	0	0	0		0	423	423	
O. latipes SWS2B	L	V	A	C	S	F	Y	-3	6	-10	0	4	-15	405	405	
L. goodei SWS2B	L	F	G	F	T	Y	Y	-3	0	-10	0	2	-15	397	397	
Bovine RH1	Y	L	T	M	A	F	W									
	43	46	118	163	168	203	265	43	46	118	163	168	265			

SWS2A

Species	Variable site											Estimated tuning effect (nm)										Estimate (nm)	Actual lmax (nm)
	47	49	88	100	115	169	174	222	275	298	305	47	49	100	115	169	174	222	275	298	305		
***O. cyanosoma* SWS2Aα**	A	F	A	A	A	F	A	F	A	S	A	0	0	0	0	0	0	-8	0	0	0	448	
P. fuscus SWS2Aα	A	F	A	A	A	F	S	F	A	S	A	0	0	0	0	0	0	-8	0	0	0	448	448
M. zebra SWS2A	A	F	A	A	A	F	S	L	A	S	A	0	0	0	0	0	0	0	0	0	0	456	
O. niloticus SWS2A	A	F	A	A	A	F	S	L	A	S	A	0	0	0	0	0	0	0	0	0	0	456	456
L. goodei SWS2A	A	Y	A	A	A	F	S	L	A	A	T	0	0	0	0	0	0	0	0	8	2	460	448
O. latipes SWS2A	A	F	A	A	A	C	A	F	A	S	T	0	0	0	0	0	0	-8	0	0	2	450	439
***O. cyanosoma* SWS2Aβ**	S	Y	S	T	V	I	S	L	A	S	A	0	0	14	-2	0	0	0	0	0	0	468	
P. fuscus SWS2Aβ	A	F	A	A	A	F	S	L	T	S	A	0	0	0	0	0	0	0	6	0	2	464	457
Bovine RH1	A	Y	A	L	A	A	A																
	41	43	82	94	109	163	168	216	269	292	299	41	43	94	109	163	168	216	269	292	299		

RH2B

Species	Variable site										Estimated tuning effect (nm)										Estimate (nm)	Actual lmax (nm)
	45	50	95	96	99	110	125	158	204	293	45	50	95	96	99	110	125	158	204	293		
O. cyanosoma RH2B_1	M	C	T	F	A	G	A	V	Y	A	0		0	0	-2	8	-2	-2	0	0	474	
P. amboinensis RH2B	M	C	T	I	C	G	S	C	Y	A	0		0	0	0	8	0	0	0	0	480	480
P. moluccensis RH2B	M	C	T	I	C	G	S	C	Y	A	0		0	0	0	8	0	0	0	0	480	
O. niloticus RH2B	M	I	T	I	C	S	S	C	Y	A											472	472
M. zebra RH2B	I	I	T	I	C	S	S	C	Y	A	0	0	0	0	0	0	0	0	0	0		484
O. latipes RH2A	M	L	C	I	C	G	S	C	F	S	0	0	-10	0	0	0	0	0	-1	-8	453	452
Bovine RH1	M	M	T	L	S	G	A	V	F	A												
	44	49	94	95	98	109	124	157	203	292	44	49	94	95	98	109	124	157	203	292		

RH2A

Species	Variable site			Estimated tuning effect (nm)			Estimate (nm)	Actual lmax (nm)
	117	166	298	117	166	298		
***O. cyanosoma* RH2A_1**	A	A	I	0	0	0	518	
***P_amboinensis* RH2A**	F	I	L	0	0	0	518	
***P. moluccensis* RH2A**	F	I	L	0	0	0	518	
M. zebra RH2Aa	F	F	L	0	10	0		528
O. niloticus RH2Aa	F	F	L	0	10	0		528
M. zebra RH2Ab	F	L	I	0	0			518
O. niloticus RH2Ab	F	L	I	0	0			518
Bovine RH1	G	A	I					
	109	158	290	109	158	290		

LWS

Species	Variable Site		Tuning effect (nm)		Estimate (nm)	Actual lmax (nm)
	177	274	177	274		
***O. cyanosoma* LWS**	A	F	-7	-10	544	
***P. amboinensis* LWS**	A	Y	-7	0	554	
***P. moluccensis* LWS**	A	Y	-7	0	554	
O. niloticus LWS	S	Y	0	0	561	561
L. goodei LWS	S	Y	0	0	561	573
L. goodei LWS	S	Y				
O. latipes LWS	S	Y	0	0	561	
O. latipes LWS	S	Y	0	0	561	
Bovine RH1	A	F				
	164	261	164	261		

RH1

Species	Variable site				Estimated tuning effect (nm)			Estimate (nm)	Actual lmax (nm)
	83	168	298	299	83	298	299		
***O. cyanosoma* RH1**	N	S	S	A	-6	0	-2	494	
***P. amboinensis* RH1**	N	A	S	S	-6	0	0	496	
***P moluccensis* RH1**	N	A	S	S	-6	0	0	496	
M. zebra RH1	D	A	A	A	0	8	-2	508	
O. niloticus RH1	D	A	S	S	0	0	0	502	
L. goodei RH1	D	A	A	S	0	8	0	510	
O. latipes RH1	D	A	S	S	0	0	0		502
Bovine RH1	D	A	S	A					
	83	168	298	299	83	298	299		

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