SUPPLEMENTARY FIGURES

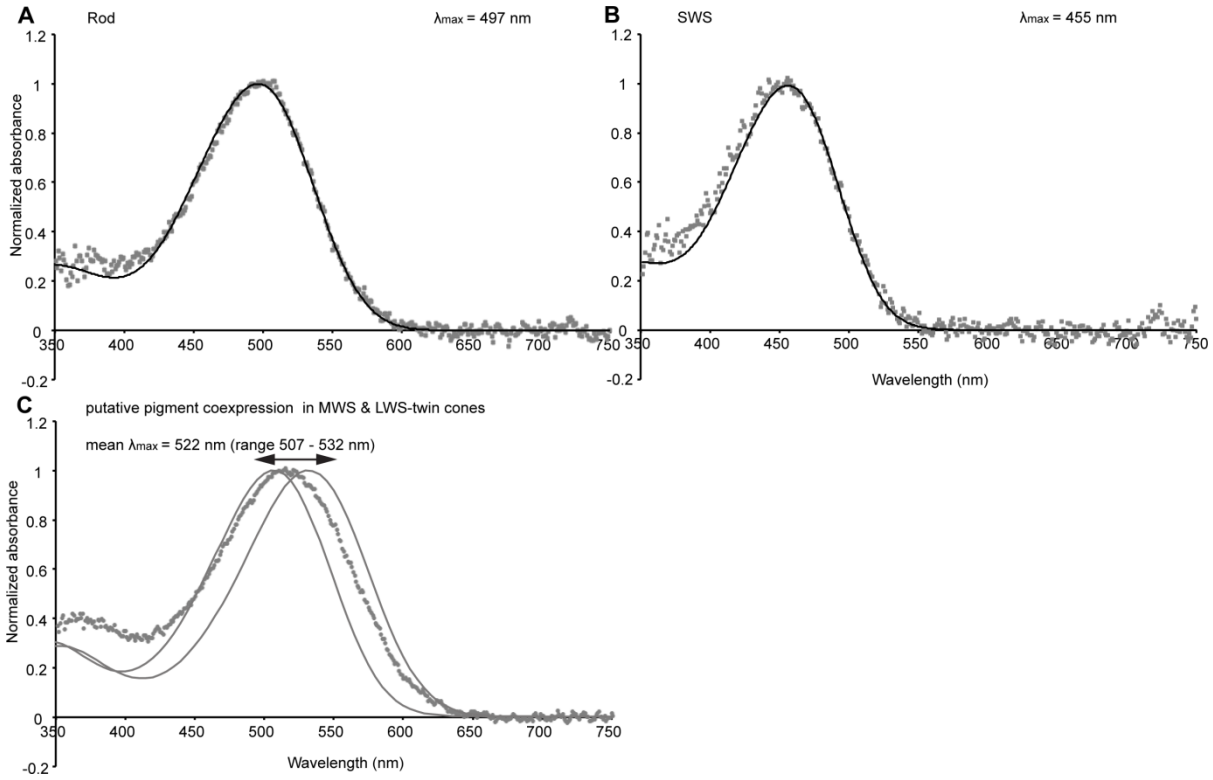

Fig. S1. Normalized pre-bleach absorbance spectra of the coral trout visual pigments (measured with MSP). (A) The visual pigment found in the rod photoreceptor used for scotopic vision (n = 22), (B) the 'blue' SWS single cone (n = 10), (C) the mean of the broad absorbance spectra found in the twin cones (MWS and LWS) and thought to be the result of a coexpression of two visual pigments with a range of 507 – 532 nm λ_{max} (n = 48). Spectra are fitted with Vitamin A1 rhodopsin templates of the appropriate λ_{max} calculated using the equations of Stavenga et al., 1993. Note that in (C) no visual templates were fitted, instead the A1 based visual templates for 507 nm and 532 nm λ_{max} are shown in grey.

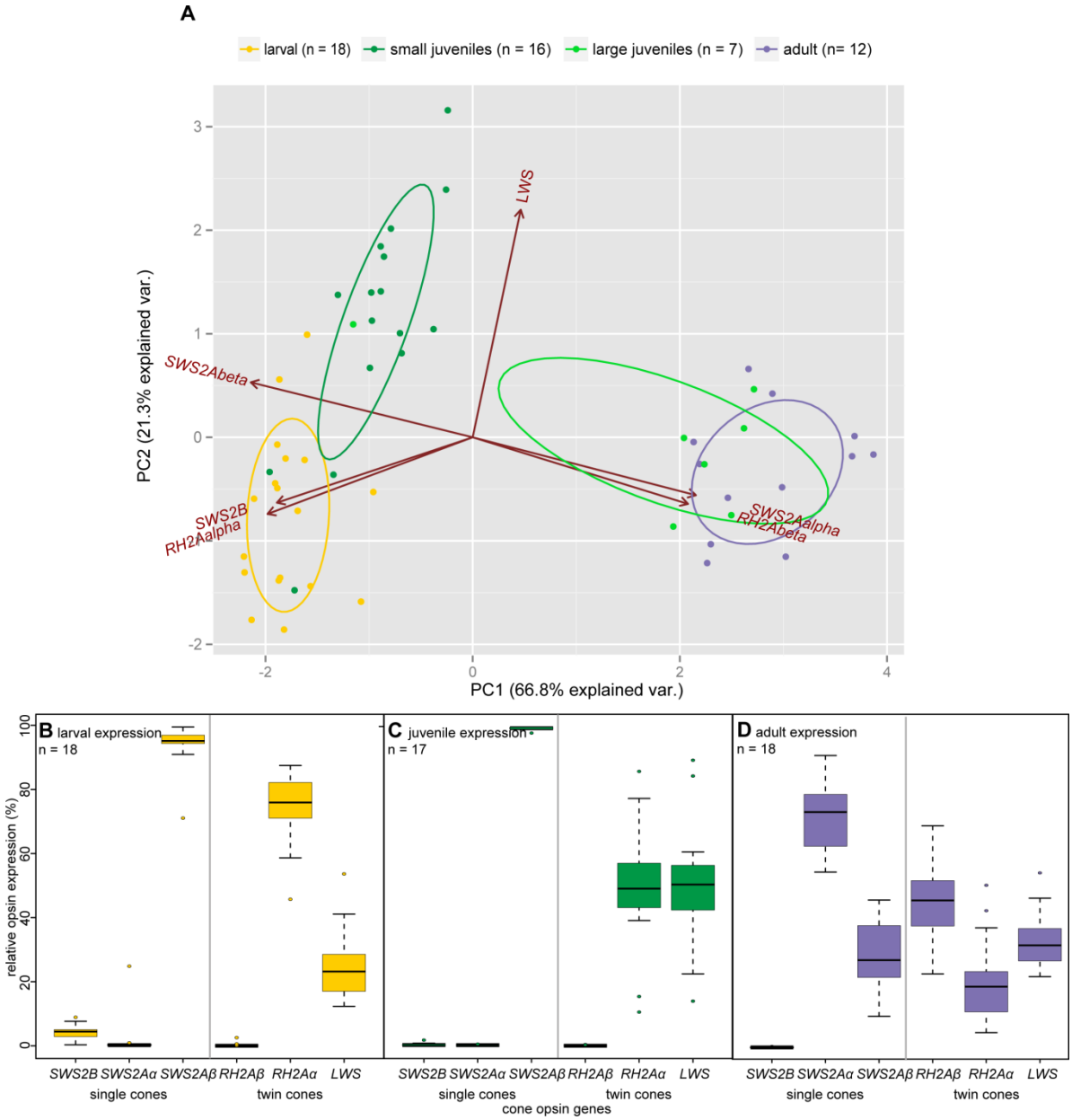

Fig. S2. Difference in opsin gene expression throughout dottyback ontogeny.

(A) A principle component analysis (PCA) shows dottyback cone opsin expression of larvae prior to settlement and one day post settlement (dps) in yellow (n = 18), small juveniles (7 – 9 dps and 34 dps) in dark green (n = 16), large juveniles in bright green (n = 7), and adults in violet (n = 12). The lines indicate differences in gene expression between individuals, separating ontogenetic stages into three distinct expression profiles: (B) larval-expression (n = 18), (C) juvenile-expression (n = 17), and (D) adult expression (n = 18). Note that the smallest of the large juveniles at 19 mm standard length (SL) clusters together with individuals of the juvenile-expression profile, while the remaining large juveniles (> 26 mm SL) already show an adult-expression profile. Gene expression was calculated for single and twin cone genes separately.

Table S1. Spectral characteristics of visual pigment found in the scotopic rod, and the photopic single cone and twin cone photoreceptors of the coral trout, *Plectropomus leopardus*. Both twin cone members showed broad absorbance spectra that are likely to be caused by pigment coexpression within outer segments with a range of 507 – 532 nm λ_{max} (also see discussion in the main article; Fig. S1).

	single cone	**twin cone**	**rod**
Morphological distinction	SWS	broad spectra (coexpression?) MWS & LWS	
λ_{max} mean ± s.e.			
pre-bleach absorbance spectra (nm)	455.4 ± 0.7	522.1 ± 0.9	496.5 ± 0.6
difference spectra (nm)	457.3 ± 1.6	522.8 ± 1.1	501.9 ± 1.2
no. cells pre-bleach/difference spectra	10 / 11	48 / 39	22 / 23

Table S2. qRT-PCR and pool primers used in this study

method	**gene (efficiency)**	**primer name**	**orientation**	**primer sequence**
qRT_PCR	*SWS1* (90%) qPCR primers	*Pfus_SWS1_2F*	forward	TTTTGGAGCCTTCAAGTTCACCAG
		Pfus_SWS1_23R	reverse	GATGTACCTGCTCCAGCCAAAG
qRT_PCR	*SWS2B* (94%) qPCR primers	*Pfus_SWS2B_1F1*	forward	CCGTGGGCTCCTTCACCTG
		Pfus_SWS2B_12R1	reverse	GGCTCACCATGCCTCCAATC
qRT_PCR	*SWS2Aα* (96%) qPCR primers	*Pfus_SWS2Aalfa_12F1*	forward	CATGGCAACACTCGGGGGTATG
		Pfus_SWS2Aalfa_2R1	reverse	CGCAAACACCCAGGTGAACC
qRT_PCR	*SWS2Aβ* (96%) qPCR primers	*Pfus_SWS2Abeta_1F2*	forward	GGTGAACTTGGCTGCCGCG
		Pfus_SWS2Abeta_12R1	reverse	CCATACCTCCAAGTGTTGCTAC
qRT_PCR	*RH2B* (91%) qPCR primers	*Pfus_RH2B_23R_new*	forward	TGTACCTCGACCAGCCCACC
		Pfus_RH2B_2F_new	reverse	TGTGGTCTGTAAACCTATGGGC
qRT_PCR	*RH2Aα* (tba) qPCR primers	*qPCR_RH2Aa_ex4_F1*	forward	GCTGCCTTCACCGCCCTC
		qPCR_RH2Aa_ex45_R1	reverse	GTCAGCATGCAGTTACGGAAC
qRT_PCR	*RH2Aβ* (tba) qPCR primers	*qRH2Abeta_ex2_F1*	forward	GGAGCTTCAAGTTCGGTGGAT
		qRH2Abeta_ex23_R1	reverse	ATGTACCTGGACCAGCCAGC
qRT_PCR	*LWS* (91%) qPCR pool	*PFus_LWS_34_F1*	forward	TGTCTCAACCTGTGGTATTACTGC
		PFus_LWS_4_R1	reverse	GGATCCCACCTGTGGCCCAT
Sanger sequencing	*SWS1* qPCR pool	*POOL_Pfus_SWS1_F*	forward	CTGTGTGCCATGGAGTCTGCC
		SWS1_R2d_dam	reverse	TCGTTGTGGGTGTACCAGTC
Sanger sequencing	*SWS2B* qPCR pool	*POOL_Pfus_SWS2B_F*	forward	GTGACTGGTACTGCCATCAATATC
		POOL_Pfus_SWS2B_R	reverse	AACGATGGTGAAGAAGGGGATGGAA
Sanger sequencing	*SWS2Aα* qPCR pool	*POOL_Pfus_SWS2Aalfa_F*	forward	CTCACTATTGCATGCACCGCC
		POOL_Pfus_SWS2Aalfa_R	reverse	GCCCATGCCCAGCATCGCT
Sanger sequencing	*SWS2Aβ* qPCR pool	*POOL_Pfus_SWS2Abeta_F*	forward	CTTACCGTTGCATGCACCGTG
		POOL_Pfus_SWS2Abeta_R	reverse	TCCACTCATCCCCAGCATCTTC
Sanger sequencing	*RH2B* qPCR pool	*RH2B_F2_Fuscus*	forward	TTA TCCTGGTTAACTTGGC
		Rh2B_R2c_dam	reverse	ATCACATAGGATTCGTTGTTG
Sanger sequencing	*RH2Aα* qPCR pool	*poolRH2Aalpha_ex1_F1*	forward	TCCAACAGGACTGGGATAAC
		poolRH2Aalpha_ex5_R1	reverse	CCATCCCAATAGTCGTCAG
Sanger sequencing	*RH2Aβ* qPCR pool	*poolRH2Abeta_ex1_F1*	forward	CCAACAGGACGGGGATTGT
		poolRH2Abeta_ex5_R1	reverse	GCCACCCATTCCAATAGTG
Sanger sequencing	*LWS* qPCR pool	*LWS_R4dFin_dam*	forward	CCCAAAACGAAGAACATGGA
		LWS_F6d_dam	reverse	AAGTTCAAGAAACTCCGTCA