Title: Comparative visual ecology of cephalopods from different habitats

Authors: Wen-Sung Chung & N. Justin Marshall

Supplementary materials

Microspectrophotometry (MSP) procedure

Animals were dark-adapted in aerated aquaria for at least 8 hours prior to the retinal preparation for MSP. The specimen was anesthetised in cold seawater mixed with 2% $MgCl_2$ and then decapitated. Eyes were removed under dim red illumination (690 nm LED). Retinal preparations were conducted under infrared illumination with the aid of a dissecting microscope fitted with an infrared image converter (Electrophysics, USA). The cornea and lens were removed and the eyecup was placed in 0.1 M phosphate buffer saline (PBS) (17-515DPBS, Lonza, USA) containing 6% sucrose. For large eyes (diameter 10-15 mm), each eye cup was symmetrically divided into four quadrants and three rectangular shaped retinal samples (ca. 2 x 4 mm) were selected from each quadrant. These retinal pieces were embedded in the Optimal Cutting Temperature compound (OCT) (Tissue-Tek, Sakura Finetek, USA) mixed with 10% sucrose for cryosectioning at -20°C under dim red illumination (690 nm LED). For small eyes (diameter 2-9 mm), an entire eyeball was embedded for cryosectioning. Transverse section of the retina (12 μm thickness), were collected with a coverslip (22 x 64 mm #1, Menzel-Glaser, Germany).

Three different mounting solutions were used for MSP measurement as follows: (1) The standard mounting solution (0.1M PBS mixed with 6% sucrose, pH 7.4). (2) The alkaline mounting solution is 0.1M PBS mixed with 6% sucrose and its pH value is adjusted to 10

using 0.1 M NaOH solution. pH was measured with a FiveEasy pH meter (Mettler Toledo, United Kingdom). (3) Hydroxylamine solution (50% w/v solution, Merck, Germany) was diluted into three concentrations, 25% (pH 11), 10% (pH 10.6), and 1% (pH 9.8) respectively.

A drop of the mounting solution was placed on a freshly cut retinal slice. The preparation was covered with a circular No. 0 glass coverslip (10 mm diameter #0, Chance Propper, United Kingdom). The edges of the top coverslip were then sealed with silicone vacuum grease to prevent dehydration. With the aid of dim red illumination, the preparation was then positioned on the stage of the microscope for MSP measurement. Other than brief exposure to dim red light, all procedures were performed using infrared illumination and an image converter in a constant temperature (22°C) dark room.

MSP operation and data analysis

Operation of MSP followed a standard protocol developed for vertebrate or invertebrate photoreceptors [1-3]. The measuring light beam was set to a size of 2 x 15 µm and placed parallel to the long axis of the rhabdome (main text Figure 1). The relatively large size of rhabdome (ca. 4x120 - 3x270 µm) made this easy. Baseline and sample scans were made from tissue-free and cellular regions of the preparation respectively. The scanning spectral range was between 300 and 800 nm with a measurement taken every 1 nm throughout 2 passes, a downward long-to-short wavelength scan and an upward short-to-long wavelength scan. If both scans revealed no obvious distortions and the absorbance spectra were flat for 100 nm beyond the wavelength at which the long wavelength limb first dropped to zero absorption, two curves were then averaged and saved. Subsequently, the visual pigment was bleached using a white light beam (ca. 2 x 15 µm) and the absorption spectrum of photo-

product was measured using the same MSP protocol. The bleaching process was repeated in some cases until effective visual pigment bleaching occurred.

Baseline and sample data were converted to absorbance values at each wavelength. Best fit visual pigment nomograms were used to determine the λmax of each sample following the methods developed by MacNichol [4], Govardovskii et al [5] and Hart et al [3]. Absorbance spectra from each measurement that satisfied the selection criteria established by Hart, Partridge [3] were accepted and these data in the sampling retinal region were averaged.

Spectral sensitivity and photochemistry reactions under different mountants

Comparisons of λmax values obtained from different mountants showed no significant difference within species, representing a slight variation of the averaged spectral sensitivity (< 4 nm). However, addition of irradiation made photo-chemical reactions of visual pigment behave differently in three mountants, particularly the reaction speed and changes of waveform due to different types of photo-product (main text Figure 1).

Hydroxylamine solution

The bleaching procedure using hydoroxylamine solution was remarkably fast and sensitive. The rate of reaction depended on concentration of hydroxylamine. Given a short period of 30-second irradiation of a white light beam, high concentration hydroxylamine solutions were capable of turning an extended range of retinal slice insensitive to light, forming a patch of irreversible photo-product over 4 mm diameter adjust to the light beam in 25% solution and around 2 mm diameter in 10% one. By contrast, with further diluted hydroxylamine solution (1%), 30-second white light irradiation sufficiently bleached visual pigments, resulting that the spectral peak of visual pigment dropped and consequently another peak of

the photo-product raised at short wavelengths (approx. 360 nm) (main text Figure 1b-d). Also, irradiation caused amounts of partially-bleached visual pigments near the light beam. The partially-bleached MSP curve can be visually identified by the two-peak broad spectrum, resulting disadvantages which the subsequent MSP measurements were therefore frequently rejected due to the selection criteria and exclusive in further analysis [3]. In most of measurements, repeated bleaching procedures caused the retinal slice completely lost photosensitivity within a sum of 5-minute white light irradiation.

Addition of hydroxylamine instantaneously reacts with the free-chromophore and destroys acid metarhodopsin with the result that the released chromophores from both rhodopsin and metarhodopsin are converted to retinene-oxime (photo-product λmax approx. 380 nm) [6]. This could explain that a short period of light exposure caused rapid loss of photosensitivity of an entire retinal slice. Also, the production of partially-bleached visual pigments after a short period of illumination (mainly due to the light exposure during MSP measurement) often resulted the distorted spectral curve where the long wave limb raised up in the following MSP measurement. This feature, the tilted long-wave limb of the curve and the widen half bandwidth, caused the best fit curve mismatched with the template of visual pigment suggested by Govardovskii, Fyhrquist [5], rendering suspected artefacts for further analyses.

Standard mountant (pH7.4)

In standard mountant, repeated exposure to bright white light for at least four five-minute periods was required for significant effect of bleaching. The spectrum of the mixture of photo-product (acid-metarhodopsin) and rhodopsin became gradually flattened while its maximal sensitive peak remained nearly at the same spectral peak at the λmax of the

rhodopsin (i.e. pre-bleaching λmax 492 nm versus post-bleaching λmax 494 nm in *I. notoides*; 502 versus 505 nm in *S. lessoniana*) (main text Figure 1e). The whole bleaching process usually took 15-25 minutes. In 92% of scans (n = 75), the post-bleaching spectrum did not lie near the pre-bleaching spectrum at long wavelengths, particularly beyond 600 nm. With this measuring artefact, these mismatch-paired measurements were exclusive in further analysis.

Alkaline mountant (pH10)

The alkaline mountant shortened the duration of the bleaching process and revealed distinctive changes in waveform between scan pairs (pre- and post-bleached scans) (main text Figure 1f). In 87% of scans, a 2-minute white light beam exposure was sufficient to generate a distinctive separation of spectral peaks, resulting a second peak of the photo-product (alkaline-metarhodopsin) appeared at short wavelengths (approx. 380 nm) (main text Figure 1f). Another advantage is to restrict the partially-bleached area within a small range (approx. 50 μm), allowing to measure neighbouring photoreceptors. Along with these advantages in measuring cephalopod visual pigment, investigating the selected cephalopods in this study was adopted this new protocol therefore.

Phylogenetic data:

TreeBASE access number: 19730 (http://purl.org/phylo/treebase/phylows/study/TB2:S19730)

Group	Species	Retinal Quadrant	λmax (nm)
Cuttlefish	*Sepia plangon* (n=3, ML: 10-24 mm)	AD	498 ± 1.8 (N=29)
		AV	498 ± 2.2 (N=29)
		PD	498 ± 2.7 (N=25)
		PV	500 ± 3.2 (N=29)
Octopus	*Callistoctopus dierythraeus* (n=3, ML: 65-80 mm)	AD	484 ± 1.9 (N=23)
		AV	487 ± 2.5 (N=21)
		PD	488 ± 1.8 (N=21)
		PV	487 ± 1.8 (N=18)
	Hapalochlaena maculosa (n=4, ML: 8-13 mm)	AD	486 ± 3.1 (N=18)
		AV	485 ± 2.4 (N=18)
		PD	485 ± 2.2 (N=22)
		PV	485 ± 1.7 (N=21)
	Octopus australis (n=6, ML: 10-15 mm)	AD	485 ± 1.3 (N=15)
		AV	484 ± 1.2 (N=15)
		PD	485 ± 1.1 (N=15)
		PV	485 ± 1.3 (N=15)
	Octopus tetricus (n=3, ML: 55-65 mm)	AD	487 ± 2.7 (N=20)
		AV	488 ± 2.3 (N=20)
		PD	484 ± 4.6 (N=22)
		PV	487 ± 2.3 (N=20)
Squid	*Euprymna tasmanica* (n=3, ML: 6-10 mm)	AD	498 ± 1.4 (N=25)
		AV	498 ± 1.3 (N=25)
		PD	498 ± 1.1 (N=25)
		PV	498 ± 1.4 (N=25)
	Idiosepius notoides (n=12, ML: 6-10 mm)	AD	493 ± 2.5 (N=24)
		AV	492 ± 2.1 (N=19)
		PD	493 ± 1.4 (N=27)
		PV	492 ± 1.4 (N=30)
	Sepioteuthis lessoniana (n=5, ML: 22-52 mm)	AD	505 ± 2.5 (N=21)
		AV	504 ± 1.5 (N=21)
		PD	502 ± 1.6 (N=35)
		PV	503 ± 2.0 (N=21)

table S1 Microspectrophotometrical data of four retinal quadrants in eight cephalopods. Mantle length (ML) (A: anterior; P: posterior; D: dorsal; V: ventral). Values are mean ± 1S.D.

TM1

amino acid site	1–10	11–20	21–30	31–40	41–50
Todarodes pacificus	**MGRDLRDNET**	**WWYNPSIVVH**	**PHWREFDQVP**	**DAVYYSLGIF**	**IGICGIIGCG**
Idiosepius paradoxus	.A.GTP....	.....YME..	S..KQ.....	.....C....	....
Euprymna scolopes	....IP....	....	....	.....G....	......?...
Alloteuthis subulata	-------...	.....YMDI.	S..KQ.....	A.........	.A.......A
Doryteuthis pealeii	....IP....	.....YMEIN	S..KQ.....	A.........	V
Loligo forbesii	....IP....	.....YMDI.	...KQ.....	A.........	.A.......V
Sepia latimanus	....IP....	.....TME..	...IQ.....	....	......V...
Sepia officinalis	....IP....	.....TME..	...KQ.N...	....	.........T
Entroctopus dofleini	VESTTLV.Q.	.....TVDI.	...AK..PI.	......V...	..VV....IL
Octopus bimaculoides	VESSTLV.Q.	.....TVDI.	...AK..PI.	......V...	..VV....IF
Octopus vulgaris	VESTTLV.Q.	.....TVDI.	...AK..PI.	..I...V...	..VV....IF
Nautilus pompilius	TTI.PENITS	V.D.EDVFI.	...KH.PG..	..AH.IV...	.TVV..C.VM

TM1 TM2

amino acid site	51–60	61–70	71–80	81–90	91–100
Todarodes pacificus	**GNGIVIYLFT**	**KTKSLQTPAN**	**MFIINLAFSD**	**FTFSLVNGFP**	**LMTISCFLKK**
Idiosepius paradoxus	....	....	....	....	....
Euprymna scolopes	....	....	....	....	....
Alloteuthis subulata	....	....	....	....	M........H
Doryteuthis pealeii	....	....	....	....	.........Y
Loligo forbesii	...V......	....	....	....	.......M.Y
Sepia latimanus	....	....	....	....	.......I..
Sepia officinalis	....	....	....	....	.......I..
Entroctopus dofleini	...V.....S	....	........M.	LS..AI....	.K...A.M..
Octopus bimaculoides	...V.....S	....	........M.	LS..AI....	.K...A.M..
Octopus vulgaris	...V.....S	....	........M.	LS..AI....	.K...A.M..
Nautilus pompilius	...V...I.S	R....R....	........L.	L...A.....	.LS..S.Q..

TM3 TM4

amino acid site	101–110	111–120	121–130	131–140	141–150
Todarodes pacificus	**WIFGFAACKV**	**YGFIGGIFGF**	**MSIMTMAMIS**	**IDRYNVIGRP**	**MAASKKMSHR**
Idiosepius paradoxus	....M.....	....	......S...	....	.......N..
Euprymna scolopes	.V..H.....	L	....	....	.....H....
Alloteuthis subulata	.V..Q.....	..L......L	T.....T...	........R.	.S........
Doryteuthis pealeii	.V..N.....	..L......L	......T...	....	.S........
Loligo forbesii	.V..N.....	..L......L	......T...	....	.S........
Sepia latimanus	.V..N.....	L	....	....	....
Sepia officinalis	.V..M.....	L	......S...	....	....
Entroctopus dofleini	....KV..QL	..LL......	...N......	....	....
Octopus bimaculoides	....KV..QL	..LL......	...N......	....	....
Octopus vulgaris	....KV..QL	..LL......	...N......	....	....
Nautilus pompilius	....QT..EL	..LA.....L	...N......	........A.	..V..R...K

TM4 (sites 151–174); TM5 (sites 195–200)

amino acid site	151–160	161–170	171–180	181–190	191–200
Todarodes pacificus	**RAFIMIIFVW**	**LWSVLWAIGP**	**IFGWGAYTLE**	**GVLCNCSFDY**	**ISRDSTTRSN**
Idiosepius paradoxus	...L......	....TM....	.......A..	....	....PS....
Euprymna scolopes	....	....	....	....	.T..YA....
Alloteuthis subulata	K.....V...	I..TI.....	.......Q..	....	.T..AS....
Doryteuthis pealeii	K.........	I..TT.....	.......S..	....	.....S....
Loligo forbesii	K.........	I..TI.....	....	....	.T..T.....
Sepia latimanus	..SL......	M..T..S...	.......V..	....	.T..YV....
Sepia officinalis	...L......	M..T..S...	.......V..	....	.T...A....
Entroctopus dofleini	...L......	M..IV.SV..	V.N....VP.	.I.TS.....	L.T.PS...F
Octopus bimaculoides	...L......	I..IV.SV..	V.N....VP.	.I.TS.....	L.T..S...F
Octopus vulgaris	...L......	I..IV..V..	V.N....VP.	.I.TS.....	L.T..N...F
Nautilus pompilius	K......S..	I.AAV.TLP.	L......IP.	.FQTS.T...	LT.NNYF..Y

TM5 (sites 201–244); TM6 (sites 242–250)

amino acid site	201–210	211–220	221–230	231–240	241–250
Todarodes pacificus	**ILCMFILGFF**	**GPILIIFFCY**	**FNIVMSVSNH**	**EKEMAAMAKR**	**LNAKELRKAQ**
Idiosepius paradoxus	.I..Y....M	L..I......	....	....	.........K
Euprymna scolopes	.I..YVFA.C	F..T..L...	....	....	....
Alloteuthis subulata	.V..Y.FA.M	F..VV.....	....	....	....
Doryteuthis pealeii	.V..YLFA.M	C..IV.....	....	....	....
Loligo forbesii	....Y.FA.M	C..VV.....	....	....	....
Sepia latimanus	.V..Y.FA.C	F.........	....	....	....
Sepia officinalis	.V..Y.FA.C	F.........	.....A....	....	....
Entroctopus dofleini	....YFC..M	L..I..A...	....	....	....
Octopus bimaculoides	....YFC..M	L..I..A...	....	....	....
Octopus vulgaris	....YFM..M	L.VV..A...	....	....	....
Nautilus pompilius	V..LYLF..I	T.VT..AI..	.F.FKA.AD.	......K..K	M....I.AGA

TM6 (sites 251–289); TM7 (sites 292–300)

amino acid site	251–260	261–270	271–280	281–290	291–300
Todarodes pacificus	**AGANAEMRLA**	**KISIVIVSQF**	**LLSWSPYAVV**	**ALLAQFGPLE**	**WVTPYAAQLP**
Idiosepius paradoxus	.......K..	.......T..	M.........	........P.	....
Euprymna scolopes	...S...K..	.......T..	........I.	....	....
Alloteuthis subulata	...S...K..	.......T.S	........I.	........I.	....
Doryteuthis pealeii	...S...K..	.......T..	........I.	....	....
Loligo forbesii	.......K..	.......T..	....	........I.	....
Sepia latimanus	.........P	....	....	........I.	....
Sepia officinalis	...S...K..	.......T..	....	........I.	....
Entroctopus dofleini	...S...K..	...M..IT..	M.......II	........A.	.......E..
Octopus bimaculoides	..QS...K..	...M..IT..	M.......II	........A.	.......E..
Octopus vulgaris	..QS...K..	...M..IT..	M........I	........A.	.......E..
Nautilus pompilius	SEQR...KI.	...MI.IT..	....T.....	.M.G....P.	......SEV.

TM7 (sites 301–318)

amino acid site	301–309	310	311–319	320	321–329	330	331–339	340	341–349	350
Todarodes pacificus	**VMFAKASAI**	**H**	**NPMIYSVSH**	**P**	**KFREAISQT**	**F**	**PWVLTCCQF**	**D**	**DKETEDDKD**	**A**
Idiosepius paradoxus	....	.	....	.	......AAQ	.	..I.....Y	.	E..I...R.	.
Euprymna scolopes	....	.	....	.	.......N.	.	..I....T.	.	E......R.	.
Alloteuthis subulata	....	.	....	.	......ASN	.	..I.....Y	.	E..I.....	.
Doryteuthis pealeii	....	.	....	.	......ASN	.	..I......	.	E..I..E..	.
Loligo forbesii	....	.	....	.	....R.ASN	.	..I.....Y	.	E..I.....	.
Sepia latimanus	....	.	..L......	.	...G..ADN	.	..IIA..R.	.	E..V..E..	.
Sepia officinalis	....	.	..L......	.	......AEN	.	..IIS....	.	E..V.....	.
Entroctopus dofleini	.L.......	.	..IV.....	.	......QT.	.	..L......	.	E..C..AN.	.
Octopus bimaculoides	.L.......	.	..IV.....	.	......QN.	.	..L......	N	E..C..ANE	.
Octopus vulgaris	.L.......	.	..IV.....	.	......QN.	.	..L......	N	E..C..AN.	.
Nautilus pompilius	........M	.	..IV.AL..	.	......NER	.	.FLIS..A.	.	E...AEPEE	S

amino acid site	351–359	360	361–369	370	371–379	380	381–389	390	391–399	400
Todarodes pacificus	**ETEIPAGES**	**S**	**DAAPSADAA**	**Q**	**MKEMMAMMQ**	**K**	**MQQQQAAYP**	**P**	**QGYAPPPQG**	**Y**
Idiosepius paradoxus	.A...DS.Q	T	GGGGESVD.	A	QMKE.MA.M	Q	KM.A.Q.AY	.	PQG.Y....	.
Euprymna scolopes	.A....S.Q	T	QETSPT...	.	V........	.	..A...QQQ	A	AYPPQGYPP	Q
Alloteuthis subulata	.A....A.Q	.	GGESVDA.Q	M	KEM.AM.QK	M	QA...QQPA	Y	PPQGY....	.
Doryteuthis pealeii	.A....S.Q	.	GGESADA.Q	M	KEM.AM.QK	M	QA..A.QPA	Y	PPQGY....	.
Loligo forbesii	.A......Q	.	GGETADA.Q	M	KEM.AM.QK	M	QA...QQPA	Y	PPQGY....	.
Sepia latimanus	......A.Q	.	GGGGGES.D	A	AQMKEMVAM	M	QKM..QQAA	Y	PPQGGY.PQ	G
Sepia officinalis	......A.Q	.	GGETADA.Q	M	KEM.AM.QK	M	Q...A.YP.	Q	GA.P.QGGY	P
Entroctopus dofleini	.E.VV.S.R	G	GESRD.AQM	K	EMMA.MQKM	Q	A..AAYQP.	.	PPQGY....	.
Octopus bimaculoides	.Q.VAPS.G	G	GGESADA.Q	M	KEM.AM.QK	M	QA..A.YQQ	.	PPQGY....	.
Octopus vulgaris	.A.VAPS.G	G	GGGE.V...	.	....	.	..A......	Q	PPPQGY.PQ	G
Nautilus pompilius	K.DDMRD..	.	TMSNIS.GG	.	VEMSTRGRR	G	GADTRYNDR	G	DMGVSNGEI	I

amino acid site	401–409	410	411–419	420	421–429	430	431–439	440	441–449	450
Todarodes pacificus	**PPQGYPPQG**	**Y**	**PPQGYPPQG**	**Y**	**PPPPQGAPP**	**Q**	**GAPPAAPPQ**	**G**	**VDNQAYQA-**	**-**
Idiosepius paradoxus	.......PP	A	GYPPP.AGY	P	...AGYP..	P	A--------	-	---------	-
Euprymna scolopes	GYPPP.QGY	P	.QGYP.QGA	.	..QGYPP.Q	G	AP.Q....E	.	........-	-
Alloteuthis subulata	..PPPQGYP	P	QGYPPQGYP	P	QGY.PPPQG	P	PPQGPP.QA	A	PPQGVD---	-
Doryteuthis pealeii	..PPPQGYP	P	QGYPP.QGY	P	.QGYPPPQG	P	PPQGPP.QA	A	PPQGVDNQA	-
Loligo forbesii	..PPPQGYP	P	QGYPPQGYP	P	QGY.PPPQG	P	PPQGPP.QA	A	PPQGVDNQA	Y
Sepia latimanus	GYPPQGGYP	P	QGYPP.A..	.	..AQGYP.A	.	.Y..QGY.P	P	QGAPPQG.P	P
Sepia officinalis	.QGYP..PA	Q	GGYPPQGYP	P	..QGYPPAQ	G	YP.QGY..P	Q	GAPPQGAPP	Q
Entroctopus dofleini	....AY.PP	Q	GYPPQGYPP	Q	GY....Y..	.	....QVEAP	Q	GAPPQGVDN	Q
Octopus bimaculoides	....AY.PQ	G	Y.PQGAYPP	Q	GY....Y..	.	....QGDAT	Q	GAPPQGVDN	Q
Octopus vulgaris	Y.PQGY.PQ	G	Y.PQGAYPP	Q	GY....Y..	.	....QGDAP	Q	GAPPQGVDN	Q
Nautilus pompilius	RDLLNAFVN	V	VGAQK.Q.P	S	TVSVAMPTI	P	TYL.PMY.S	H	GYYPPPP.H	Y

table S2 Alignment of 12 full-length opsin-coding amino acid sequences using the multiple sequence alignment (MUSCLE) method. Amino acid identity across species is indicated by a dot (.); (-) indicates unavailable amino acids.

Species	λmax (nm)	Habitat	Dominant light sources during forage	Max living depth (m)	Averaged distribution depth (m)
Idiosepius notoides	493	1	1	20	10
Idiosepius paradoxus	493*	1	1	20	10
Euprymna tasmanica	499	1	2	80	40
Euprymna scolopes	500*	1	2	200	100
Sepiola sp.	498	1	2	150	76
Alloteuthis subulata	505	1	2	500	275
Loligo forbesii	500	1	2	700	375
Doryteuthis pealeii	499	1	2	390	195
Sepioteuthis australis	503*	1	2	70	35
Sepioteuthis lessoniana	503	1	2	100	50
Sepia pharaonis	500*	1	2	130	70
Metasepia tullbergi	506	1	2	86	45
Sepia officinalis	499	1	2	200	125
Sepia latimanus	499*	1	2	30	15
Spirula spirula	NA	2	3	1750	1000
Bathyteuthis berryi	490	2	3	1200	1000
Pterygioteuthis microlampas	486	2	3	800	425
Histioteuthis oceanica	486	2	3	1000	750
Ommastrephes bartramii	488*	2	2	500	250
Todarodes pacificus	488	2	2	500	250
Illex coindetii	488*	2	2	600	300
Vampyroteuthis infernalis	NA	2	3	1200	900
Callistoctopus ornatus	487	1	1	80	40
Entroctopus dofleini	486	1	1	1500	750
Octopus vulgaris	481	1	1	100	50
Hapalochlaena maculosa	485	1	1	50	25
Octopus bimaculoides	487	1	1	50	25
Nautilus pompilius	473	2	3	750	400

table S3 Dataset used in phylogenetic linear regression (PGLS) analyses. * indicates that the predictions of λmax values are inferred using the averaged λmax of their relatives obtained by the MSP and the adjusted ESP results. For the habitat, 1 = coastal waters, 2 = mid water.

For dominant light sources during forage, 1 = sunlight, 2 = partial sunlight and bioluminescence, 3 = dominant bioluminescence.

Species numbers	Predictor valuables	*t-value*	*p*
28 cephalopods	Habitat	-3.6151	**0.0016**
	Dominant light source during forage	1.8685	**0.0757**
	Maximum living depth	0.14	0.8899
	Averaged distribution depth	-0.2857	0.7779
19 decapodiform coleoids	Habitat	-4.9506	**0.0002**
	Dominant light source during forage	2.2561	**0.0406**
	Maximum living depth	0.0629	0.9508
	Averaged distribution depth	-0.3003	0.7684

table S4 The results of PGLS tests for the two different opsin phylogenies. The significance levels are shown in bold.

References

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