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Supplemental Information

A Unique Advantage for Giant Eyes in Giant Squid

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Supplemental Theory

Discrimination criteria Detection of a target against a background requires discrimination of signals from visual channels sampling light from the target and the background respectively. We assume that the channels being compared have identical properties. A target channel detects a mean of N_T photons per integration time, and the corresponding mean count for a background channel is N_B. The photon counts are sums of real photons and intrinsic noise. We follow Land [24] and assume Gaussian distribution of photon samples. Discrimination between the signals in the two channels is possible when the difference is greater than or equal to a reliability constant R times the standard deviation of the difference (which is the square root of the sum of the two means; see Land [24]: $|N_T - N_B| \geq R\sqrt{N_T + N_B}$. The discrimination threshold is then given by:

$$|N_T - N_B| = R\sqrt{N_T + N_B} \qquad \text{Eq. 1}$$

Variables and constants are defined in the Table on page 5. For confidence levels and values of R see the Table on page 6.

Case 1: Detection of a point-source on a black target We assume a pair of visual channels optimally suited to discriminate a point source against a dimmer background. A target channel is aimed at the bioluminescent point source, and its signal is compared to that of a channel aimed at the background next to the point source (Fig. 2A). The target channel is assumed to receive all light that enters the eye from the bioluminescent point source. For both channels, the target blocks background space-light from behind the target, but new space-light is scattered into the line of sight between the target and the observer. The target channel will receive an average of N_{bio} photons per integration time from the point source and N_{black} photons scattered in along the line of sight, whereas the background channel only receives N_{black} photons from the line of sight. Each channel also generates an average of X_{ch} false photons per integration time. The total average signal in the target channel will thus be $N_T = N_{bio} + N_{black} + X_{ch}$ and in the background channel, $N_B = N_{black} + X_{ch}$. Inserting this into Eq. 1 gives:

$$N_{bio} = R\sqrt{N_{bio} + 2N_{black} + 2X_{ch}}\,. \qquad \text{Eq. 2}$$

Before we derive expressions for N_{bio} and N_{black}, we need to consider the angular size of the two channels. We know that aquatic eyes typically have focal lengths of 2.55 lens radii [1] (Matthiessen's ratio, $M=2.55$). Expressed in pupil diameters (A), the focal length, f, is $0.5MA$, and the angular diameter of the Airy disc becomes $2.44\lambda M / 2nf$ radians. Multiplied by the focal length to get the actual size on the retina we get $2.44\lambda M / 2n$. This means that if M is constant, the diffraction blur spot has a constant size on the retina irrespective of eye size. For a wavelength (λ) of 480 nm, a refractive index (n) of 1.33, and M = 2.55 the Airy disc is 1.1 μm, and for M as high as 3, the Airy disc is still only 1.3 μm. But photoreceptor diameters in giant squid are larger (5 μm), which implies that realistic angular dimensions of the spatial channels should be given by the actual receptor diameter. We assume a Gaussian profile of the angular sensitivity of the receptor [S1], where the half-width is d/f (radians) and its solid angle is $1.13(d/f)^2$ (steradians).

We are interested in the relation between the pupil diameter A and the range r, and search for expressions relating these to N_{bio} and N_{black}. Following Warrant [S2], light divergence from an isotropic point source of bioluminescence gives a photon flux density of $E/4\pi r^2$, attenuation by water is given by $e^{-\alpha \cdot r}$ and the pupil area accepting the light is $\pi A^2/4$. The product of these factors multiplied by the efficiency of the retina and the integration time provides the desired expression of N_{bio} (see the Table on page 5 for explanation of variables):

$$N_{bio} = \frac{EA^2}{16r^2} e^{-\alpha \cdot r} q \Delta t \qquad \text{Eq. 3}$$

The space-light is an extended source, and the sensitivity [1] of a retinal channel is simply the product of the pupil area, $\pi\,(A/2)^2$, the solid angle in visual space of the channel, $1.13(d/f)^2$, and the efficiency q by which the eye detects photons. For an observer at constant depth in the sea, space-light enters the line of sight at the rate $1-e^{(\kappa-\alpha)r}$, where κ depends on the viewing angle [17, S3]. The radiance seen in the direction of a black target then becomes $I_{space}\left(1-e^{(\kappa-\alpha)r}\right)$, where I_{space} is the background radiance at the depth of the observer. We arrive at N_{black} by multiplying the sensitivity by the radiance and the integration time Δt:

$$N_{black} = 1.13\left(\frac{\pi}{4}\right)A^2\left(\frac{d}{f}\right)^2 q\Delta t \cdot I_{space}\left(1-e^{(\kappa-\alpha)r}\right). \qquad \text{Eq. 4}$$

We know from above that $f = \left(M \cdot A\right)/2$ and get:

$$N_{black} = 3.55\left(\frac{d}{M}\right)^2 q\Delta t \cdot I_{space}\left(1-e^{(\kappa-\alpha)r}\right). \qquad \text{Eq. 5}$$

The dark noise per integration time is simply:

$$X_{ch} = X\Delta t\,. \qquad \text{Eq. 6}$$

We now combine Eqs. 3, 5 and 6 with Eq. 2, and solve for A to obtain the desired relation for detection of point sources:

$$A = \sqrt{R^2\left(1+\sqrt{1+\frac{8\left(3.55\left(\frac{d}{M}\right)^2 q\Delta t\cdot I_{space}\left(1-e^{(\kappa-\alpha)r}\right)+X\Delta t\right)}{R^2}}\right)\frac{8r^2}{Eq\Delta t}e^{\alpha\cdot r}} \qquad \text{Eq. 7}$$

Cases 2 and 3: Detection of an extended black target, and an extended luminous target
We again assume an equal pair of visual channels, but now optimally sized to detect an extended object against the background space-light. To maximise the signal, the target channel fills the width of the object (Fig. 2A), and both channels have square rather than Gaussian sensitivity profiles. We thus assume that the angular size of the visual channels is dynamic, and suited to the object at all times. The angle in visual space of such a channel is the target width divided by its distance, T/r (radians), and with a square profile its solid angle is $(\pi/4)(T/r)^2$ (steradians). The channel is formed as a circular pool of photoreceptors, where each photoreceptor occupies a solid angle of $(\pi/4)(d/f)^2$ in visual space (see the Table on page 5 for definition of variables). The number of photoreceptors forming a channel is then $(Tf/rd)^2$, and its diameter on the retina is Tf/r.

The target itself is assumed to be black (zero reflectance), but as it moves through the water it may trigger bioluminescent flashes within its profile, but not in the visual field of the background channel. The signal of the target channel comes from target bioluminescence attenuated on its way to the eye, space-light having entered the line of sight between the target and the eye, and dark noise from the contributing photoreceptors: $N_T = N_{bio} + N_{black} + X_{ch}$, and the background channel sums background space-light and channel noise: $N_B = N_{space} + X_{ch}$. The discrimination threshold from Eq. 1 now becomes

$$\left|N_{bio} + N_{black} - N_{space}\right| = R\sqrt{N_{bio} + N_{black} + N_{space} + 2X_{ch}} \qquad \text{Eq. 8}$$

We are now ready to work out expressions for N_{bio}, N_{black}, N_{space} and X_{ch}, which happens to be easier in the reverse order. Note that the signal parameters used in this case will have to be derived anew, because they are not identical to those derived for the point source case. The channel noise X_{ch} is derived as for the point source case, but here multiplied by the number of photoreceptors in the pool, and combined with the relation $f = MA/2$:

$$X_{ch} = \left(\frac{TMA}{2rd}\right)^2 X\Delta t. \qquad \text{Eq. 9}$$

The background detector is now directly monitoring the unblocked spacelight I_{space}. To arrive at the photon count of the detector, we put together factors corresponding to those used for Eq. 4,

but with T/r replacing d/f, and assuming a square rather than Gaussian profile of the angular sensitivity (replacing 1.13 with $\pi/4$):

$$N_{space} = \left(\frac{\pi}{4}\right)^2 A^2 \left(\frac{T}{r}\right)^2 q\Delta t \cdot I_{space} = 0.617 A^2 \left(\frac{T}{r}\right)^2 q\Delta t \cdot I_{space}. \qquad \text{Eq. 10}$$

The amount of detected light entering the line of sight between the target and the eye, N_{black}, can be worked out by replacing I_{space} of Eq. 10 with $I_{space}\left(1 - e^{(\kappa-\alpha)r}\right)$ as in Eq. 4:

$$N_{black} = 0.617 A^2 \left(\frac{T}{r}\right)^2 q\Delta t \cdot I_{space}\left(1 - e^{(\kappa-\alpha)r}\right) \qquad \text{Eq. 11}$$

Bioluminescence triggered by the target is likely to be composed of randomly distributed point sources. With the mean distance x between nearest neighbours, the number of point sources per unit area of the target is $1/(4x^2)$ [S4]. The product of this density and the area viewed by the target channel, $(\pi/4)T^2$, yields the total number of point sources seen by the target channel: $\pi \cdot T^2/16x^2$. We can now multiply the expression of Eq. 3 with the number of viewed point sources to obtain N_{bio} for the extended source case:

$$N_{bio} = \frac{\pi \cdot T^2 E A^2}{256 x^2 r^2} e^{-\alpha \cdot r} q\Delta t \qquad \text{Eq. 12}$$

This expression also holds for small targets seen at long distances, because the modulation transfer function of deep oceanic water is practically flat from zero spatial frequency up to 10 cycles per degree [21]. We can thus safely ignore effects caused by spatial degradation of the image.

We now substitute Eqs. 9-12 for X_{ch}, N_{space}, N_{black} and N_{bio} in Eq. 8 and solve for A to obtain:

$$A = \frac{R\sqrt{q\Delta t\left\{\frac{\pi \cdot T^2 E}{256 x^2 r^2} e^{-\alpha \cdot r} + 0.617\left(\frac{T}{r}\right)^2\left[I_{space}\left(2 - e^{(\kappa-\alpha)r}\right)\right]\right\} + 2\left(\frac{TM}{2rd}\right)^2 X\Delta t}}{\left|q\Delta t\left\{\frac{\pi \cdot T^2 E}{256 x^2 r^2} e^{-\alpha \cdot r} - 0.617\left(\frac{T}{r}\right)^2\left[I_{space}\left(e^{(\kappa-\alpha)r}\right)\right]\right\}\right|}, \qquad \text{Eq. 13}$$

which is the desired relation between A and r for detection of extended sources. The visibility of non-luminous extended black targets (dark silhouettes) can also be analysed by Eq. 13, simply by allowing $E = 0$.

Definition of variables (units in brackets)

N_T	Mean number of real and false photons detected per integration time in a visual channel aimed at the target (photons)
N_B	Mean number of real and false photons detected per integration time in a visual channel viewing the background space-light (photons)
N_{bio}	Mean photon count (per integration time) originating from bioluminescent sources (photons)
N_{space}	Mean photon count (per integration time) from background space-light (photons)
N_{black}	Mean photon count (per integration time) originating from light scattered into the line of sight between target and observer (photons)
X_{ch}	Number of false photons (dark noise) per integration time in a visual channel (photons)
X	Dark noise rate per photoreceptor (photons s^{-1})
A	Pupil diameter (m)
r	Range: maximum visibility distance to target (m)
E	Number of photons emitted by bioluminescent point source in all directions per second (photons s^{-1})
I_{space}	Radiance of space-light background in the direction of view at the position (depth) of the eye (photons m^{-2} s^{-1} sr^{-1})
T	Width of extended target (m)
x	Average distance between point sources across an extended object (m)
α	Beam attenuation coefficient of sea water (m^{-1})
κ	Attenuation coefficient of background radiance (backscattering coefficient) (m^{-1})
λ	Wavelength of light, taken as 480 nm for bioluminescence and transmitted daylight
n	Refractive index in object and image space, taken as the value for water, 1.33
d	Photoreceptor diameter (m)
Δt	Integration time (s)
q	Detection efficiency: ratio of detected to incident photons, which depends on losses in the ocular media, the fraction absorbed by photopigment and the transduction efficiency
f	Focal length (m)
M	Matthiessen's ratio, $2f/A$
R	Reliability coefficient

Values assumed for modelling We used values that in our opinion are the most realistic (see Table on this page). Values for radiance of down-welling daylight, and absorption in the sea are based on measurements in oceanic water [21]. The original data come from Dr Andrew Barnard, Dr Scott Pegau and Dr Ronald Zaneveld (College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, OR, USA), who collected them using a dual path, multiband absorption/ attenuation meter (ac-9, Wetlabs Inc.) and fluorometer in the Equatorial Pacific (10.05 local time, 30 April 1996; 0°0_ N, 177°21_ W). Absorption and beam attenuation coefficients (at 412, 440, 488, 510, 532, 555, 650 and 676 nm) and chlorophyll concentration were measured at 1 m intervals to a depth of 199 m (after which depth inherent optical properties and chlorophyll concentration values were assumed to remain constant). These values were then input into a radiative transfer software package to compute the relevant radiances and irradiances as a function of depth. The energy values per nm were converted to quanta, and the number of photons available to photoreceptors was spectrally integrated over 390-510 nm using a spectral sensitivity curve calculated for 300 μm long *Architeuthis* photoreceptors from a rhodopsin template [S5] peaking at 470 nm. At a depth of 200m the number of quanta (per m^2, s and sr) was $6.28 \cdot 10^{15}$ for down-welling radiance, $5.11 \cdot 10^{13}$ for horizontal radiance and $2.90 \cdot 10^{13}$ for up-welling radiance. Below 200 m the log radiances decrease linearly with depth, and the intensity reduction per 100 m was 1.638 log units for down-welling radiance, 1.677 log units for horizontal radiance and 1.668 log units for up-welling radiance. The attenuation and backscatter coefficients for 488 nm were assumed constant below 200 m, with $\alpha = 0.0468$, and $\kappa = 0.0385$ for looking up, $\kappa = 0$ for horizontal viewing, $\kappa = -0.0385$ for looking downwards.

Rhabdom diameters ranging between 5 and 6 μm in an *Arciteuthis* sp. (mantle length 1.43 m, caught on December 4, 2006) were measured from semi-thin sections of a central piece of retina embedded in histological Araldite. The piece of retina was prepared from an eye preserved in 4% formalin, and kindly put at our disposal by Dr Tsunemi Kubodera.

Video recordings of live *Architeuthis* [11] together with typical foraging depths of sperm whales [S6, S7], suggest that giant squid normally inhabit depths of 600-1000 m during the day. A recent investigation [S8] indicates occasional presence at moderate depths (200-400 m).

List of values used for modelling

R	1.96 for 95% confidence [24]
E	$1 \cdot 10^{11}$ quanta s^{-1} for gelatinous zooplankton [S2, 14, S9]
x	0.3 m assumed for gelatinous zooplankton [18]; can be much smaller in dinoflagellate and copepod layers of shallow water)
T	0.1 m for prey; 0.5 m for conspecific; 2 m for predator (sperm whale)
d	5 μm (measured histologically in *Architeuthis* sp., see above)
Δt	0.16 s (mysid) [S10, S11]
q	0.36 [S12]
X	$1 \cdot 10^{-4}$ s^{-1} [14, 16]
M	2.55 [S13]

Sensitivity analysis Apart from the values given in the Table on page 6, we also used alternative values to test if the conclusions were critically sensitive to variations in variables within reasonable bounds. The Table below list alternative values and the effect these have on three cases, taken from the traces presented in Fig 2B. We also tested the alternative values from the Table below on all diagrams presented in the paper (Figs. 2B, C and 3A-D), and found that the conclusions of the investigation are surprisingly robust, and remain valid for each individual substitution of alternative values. We also analysed the effect of random variation of input values, within the tabulated ranges, and confirmed that large extended targets provide the best growth return under all permutations of possible input values (Figure 3 C, D).

Alternative values and their consequence in % of the calculated visual range

		Detection principles and conditions		
		Point source A=50 mm 350 m depth horizontal viewing	Extended dark A=50 mm 250 m depth horizontal viewing 0.5 m target	Extended luminous A=50 mm 550 m depth horizontal viewing 0.5 m target
E	$1 \cdot 10^9$ dinoflagellate and copepod layers*	-66%	not applicable	-81%
x	60 cm	not applicable	not applicable	-28%
d	3 μm	+1.8%	0%	0%
	7 μm	-0.8%	0%	0%
Δt	0.016 s	-36%	-22%	-38%
	1.6 s	+40%	+24%	+40%
q	0.05	-31%	-19%	-35%
X	$1 \cdot 10^{-3}$	0%	0%	-7%
M	3.00	+0.2%	0%	0%

*The lower intensity of dinoflagellate and copepod bioluminescent flashes is often compensated by much higher densities than those typical of bioluminescent gelatinous zooplankton [14, S9].

Supplemental References

S1. Snyder, A.W. (1975). Photoreceptor optics - Theoretical principles. In Photoreceptor optics, A.W. Snyder and R. Menzel eds. (Berlin, Heidelberg, New York: Springer), pp. 38-55.

S2. Warrant, E.J. (2000). The eyes of deep-sea fishes and the changing nature of visual scenes with depth. Phil. Trans. R. Soc. Lond. B. *355*, 1155-1159.

S3. Johnsen, S. (2002). Cryptic and conspicuous coloration in the pelagic environment. Proc. R. Soc. Lond. B. *269*, 243-256.

S4. Clark, P.J. and Evans, F.C. (1954). Distance to Nearest Neighbor as a Measure of Spatial Relationships in Populations. Ecology *35*, 445-453.

S5. Govardovskii, V.I., Fyhrquist, N., Reuter, T., Kuzmin, D.G. and Donner, K. (2000). In search of the visual pigment template. Visual Neurosci. *17*, 509–528.

S6. Watkins, W.A., Daher, M.A., Dimarzio, N.A., Samuel, A., Wartzok, D., Fristrup, K.M., Howey, P.W. and Maiefs, R.R. (2002). Sperm whale dives tracked by radio telemetry. Mar. Mammal Sci. *18*, 55-68.

S7. Watwood, S.L., Miller, P.J.O., Johnson, M., Madsen, P.T. and Tyack, P.L. (2006). Deep-diving foraging behaviour of sperm whales (*Physeter macrocephalus*). J. Animal Ecol. *75*, 814–825.

S8. Landman, N.H., Cochran, J.K., Cerrato, R., Mak, J., Roper, C.F.E. and Lu, C.C. (2004). Habitat and age of the giant squid (*Architeuthis sanctipauli*) inferred from isotopic analyses. Mar. Biol. *144*, 685-691.

S9. Nicol, J.A.C. (1971). Physiological investigations of oceanic animals. In Deep oceans, P.J. Herring and M.R. Clarke eds. (London: Arthur Barker), pp. 225-246.

S10. Moeller, J.F. and Case, J.F. (1994). Properties of visual interneurons in a deep-sea mysid, *Gnathophausia ingens*. Mar. Biol. *119*, 211-219.

S11. Moeller, J.F. and Case, J.F. (1995). Temporal adaptations in visual systems of deep-sea crustaceans. Mar. Biol. *123*, 47-54.

S12. Warrant, E.J. (1999). Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. Vision Res. *39*, 1611-1630.

S13. Matthiessen, L. (1882). Über die Beziehung, welche zwischen dem Brechungsindex des Kemcentrums der Krystallinse und dem Dimension des Auges bestehen. Pflügers Arch. *27*, 510-523.