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RESEARCH ARTICLE

Chromogenic behaviors of the Humboldt squid (*Dosidicus gigas*) studied *in situ* with an animal-borne video package

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ABSTRACT

Dosidicus gigas (Humboldt or jumbo flying squid) is an economically and ecologically influential species, yet little is known about its natural behaviors because of difficulties in studying this active predator in its oceanic environment. By using an animal-borne video package, National Geographic's Crittercam, we were able to observe natural behaviors in free-swimming *D. gigas* in the Gulf of California with a focus on color-generating (chromogenic) behaviors. We documented two dynamic displays without artificial lighting at depths of up to 70 m. One dynamic pattern, termed 'flashing' is characterized by a global oscillation (2–4 Hz) of body color between white and red. Flashing was almost always observed when other squid were visible in the video frame, and this behavior presumably represents intraspecific signaling. Amplitude and frequency of flashing can be modulated, and the phase relationship with another squid can also be rapidly altered. Another dynamic display termed 'flickering' was observed whenever flashing was not occurring. This behavior is characterized by irregular wave-like activity in neighboring patches of chromatophores, and the resulting patterns mimic reflections of down-welled light in the water column, suggesting that this behavior may provide a dynamic type of camouflage. Rapid and global pauses in flickering, often before a flashing episode, indicate that flickering is under inhibitory neural control. Although flashing and flickering have not been described in other squid, functional similarities are evident with other species.

KEY WORDS: Cephalopod, Chromatophore, Flashing, Signaling, Flickering, Crypsis

INTRODUCTION

Dosidicus gigas d'Orbigny 1835, commonly known as the Humboldt or jumbo flying squid, is a large pelagic squid of the family Ommastrephidae with a range extending from British Columbia to southern Chile. It is an ecologically important species as both predator and prey and is associated with highly productive oceanic systems – the California Current, Peru (Humboldt) Current, Costa Rica Dome and Equatorial Upwelling Zone. *D. gigas* feeds primarily on mesopelagic micronekton throughout life, particularly myctophid lanternfishes, krill, pteropods and mesopelagic squids (Nigmatullin et al., 2001; Markaida and Sosa-Nishizaki, 2003), but larger individuals also prey on much larger demersal fish, particularly at higher latitudes in the northern hemisphere (Field et al., 2013). Its most common predators are sperm whales, sharks and large teleost fishes (Nigmatullin et al., 2001). *D. gigas* is frequently associated with the oxygen minimum zone (OMZ), a hypoxic mesopelagic region that is hostile to most large marine animals that are not air-breathers. The presence of the Humboldt squid in or near the OMZ off California appears to be directly related to an abundance of myctophids associated with this environmental feature (Stewart et al., 2014). *D. gigas* also has a major economic presence, currently standing as the world's twelfth largest single-species fishery (FAO, 2012).

Despite the ecologic and economic importance of *D. gigas*, relatively little is known about its natural history or social behaviors, and the oceanic distribution and largely mesopelagic habitat of this species (and other ommastrephids) complicate such studies. This squid typically spends the majority of night-time hours at depths of 10 to 50 m, but during the day it maintains an average depth of several hundred meters (Stewart et al., 2012). Rapid excursions throughout the water column are common at all hours (Gilly et al., 2006; Davis et al., 2007).

Pop-up archival transmitting (PAT) tags have revealed a great deal about natural vertical movements and swimming behaviors of *D. gigas* (Gilly et al., 2012; Bazzino et al., 2010; Stewart et al., 2012), but these devices do not reveal the behavioral context for these movements, e.g. foraging, predator evasion or social interactions. Acoustic sampling suggests that *D. gigas* hunts cooperatively at night (Benoit-Bird and Gilly, 2012), thus providing non-invasive information on social structure and schooling, but there are many nuances of behavior that acoustic methods cannot resolve. Observations made with remotely operated vehicles (ROVs) or manned submersibles provide unique *in situ* observations at mesopelagic depths (Hunt and Seibel, 2000; Kubodera et al., 2007; Vechionne et al., 2002), but bright illumination and vehicle-generated noise can skew behaviors of squid in unknown ways.

A unique feature of all coleoid cephalopods (squid, cuttlefish and octopus) is the ability to change skin color through the use of neuromuscular chromatophores. These small organs are composed of an elastic pigment sac that can be expanded, and thereby made visible to an observer, through the action of a ring of radial muscle fibers (see Florey, 1969). These muscle fibers are directly innervated by motor neurons with cell bodies in the chromatophore lobes of the brain (Bullock and Horridge, 1965). Our knowledge of natural color-changing (chromogenic) behaviors in squid is based almost entirely on several species in the family Loliginidae that inhabit coastal or shelf environments and on a few mesopelagic species (Bush et al., 2009). In loliginid squid changes in spatial patterning appear to be primarily related to camouflage, intraspecific signaling and deimatic displays (Hanlon and Messenger, 1996). Unlike loliginid squids, which generally have chromatophores of several colors, oceanic squid such as *D. gigas* have only reddish-brown chromatophores (Packard, 2011). We are unaware of any work on chromogenic behavior of *D. gigas* or other ommastrephids published in the scientific literature.

A previous study of vertical movements of *Dosidicus gigas* (Gilly et al., 2012) utilized an animal-borne video and instrument package,

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the National Geographic Crittercam (Marshall et al., 2007). The present paper describes data on chromogenic behaviors collected during these Crittercam deployments. Three general types of behavior were observed: static patterning (similar to that in loliginid squids), rapid and rhythmic 'flashing' of the entire body during intra-specific encounters (possibly unique to ommastrephids) and a generally present, more subtle 'flickering'. Both flashing and flickering can be rapidly and globally interrupted or 'paused'. *D. gigas* thus differs from loligind squids in that its chromogenic displays appear to primarily involve the time domain and temporal coding, whereas other squid may rely more on spatial patterning.

RESULTS

General behaviors of squid carrying a Crittercam

All three Crittercam deployments showed an immediate descent of the squid upon release at velocities of 0.5–1.0 m s^{-1} (Fig. 1A). These descents were characterized by continuous downward movement rather than the 'climb-and-glide' pattern of locomotion that is characteristic of *D. gigas* (Gilly et al., 2012). Climb-and-glide movements are evident after the squid reaches its maximum depth, except in the case of deployment 1, because the Crittercam was torn off by conspecifics during the descent. Squid 2 slowly ascended from its maximum depth to the thermocline region (50–60 m), whereas squid 3 remained close to its maximum depth for ~45 min before it quickly ascended to the thermocline. Here, it encountered a large group of conspecifics and maintained its depth over an extremely narrow range for the remainder of the deployment. During this period many interactions with other squid occurred.

A number of these interactions involved arms-to-arms contact of squid with the appearance of spermatophores between the arms of the camera-bearing ('primary') squid (Fig. 2A). We take these interactions to be mating attempts, but we cannot ascertain whether they were successful. A total of five such encounters occurred, and in no case did the actual arms-to-arms contact last for more than a few seconds. We do not know the sex of the primary squids, but we assume that they were female based on their extremely large size. Although we observed several pairs of 'secondary' squid that were interacting, there were no arms-to-arms contacts of the type described above. Events in which a secondary squid extended one arm (or possibly tentacle in some cases) to touch the buccal area of another individual were observed several times (Fig. 2B,C), with one observation of this behavior involving the primary squid (Fig. 2D). Exactly which arm (or tentacle) was used could not be determined, but in some events it appeared to be one of the more ventral arms, consistent with the possibility that it was the hectocotylized arm. Although this behavior might also represent mating, in no case could the transfer of spermatophores be confirmed.

Splaying of arms was also observed in several instances during interactions that did not appear to be directly related to mating. In one case, a pair of secondary squid were interacting and as the primary squid approached, they both turned to face the oncoming squid and opened their arms as far as possible for several seconds and then jetted backward, away from the camera (supplementary material Movie 1).

Eight static chromogenic displays were observed in either the primary squid or secondary squid. These patterns were very similar to those described by Trueblood (Trueblood, 2010) under artificial lighting in ROV observations and involved displays along the edges of the fins, the prominent keel on arms III and the dorsal surface of the head (not illustrated). Although we observed considerably fewer patterns than previously reported, it is likely that this difference simply reflects our much smaller sample size. Different conditions for daytime Crittercam observations at <50 m depth under natural lighting compared with ROV observations made at much greater depths with artificial daylight illumination complicate direct comparison of these data sets.

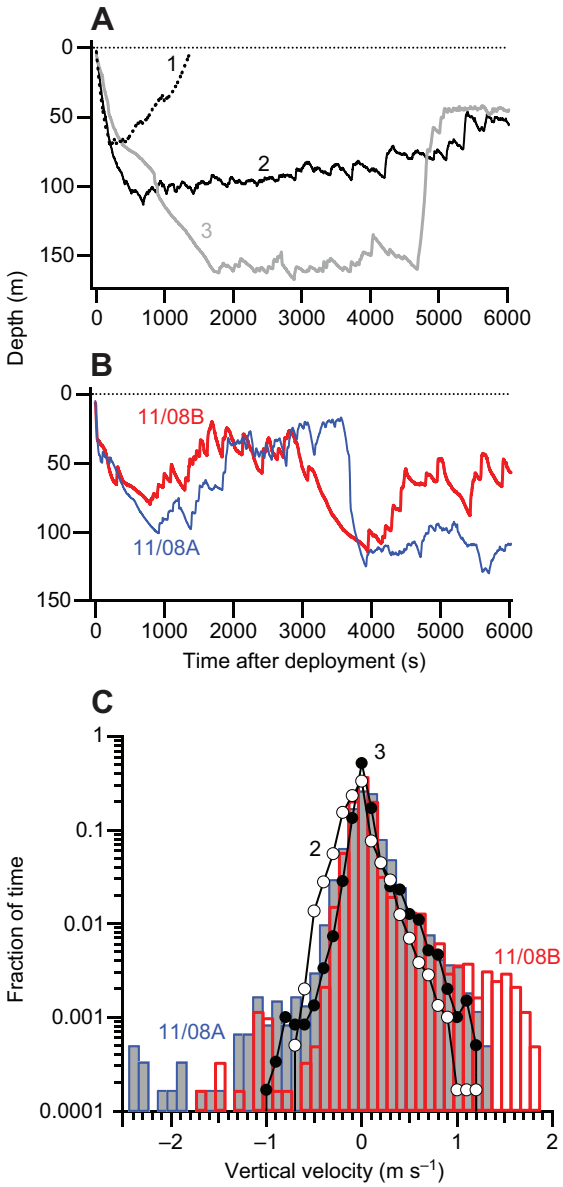

Fig. 1. Comparison of vertical movements recorded by PAT tags and Crittercam attached to the Humboldt squid *Dosidicus gigas*. (A) In each Crittercam deployment (1–3), the squid rapidly descended to a depth of about 70 m after release. Squids 2 and 3 then continued to descend more slowly to depths of 100 m and 160 m, respectively, and then commenced climb-and-glide swimming. Both squid moved to the thermocline depth at about 50 m in the late afternoon. The descent of squid 1 was prematurely terminated by attack from conspecifics. (B) Squid carrying PAT tags also dive immediately after release (at night) and then engage in climb-and-glide swimming and more rapid vertical movements. (C) Vertical velocity distributions (means in 0.1 m s^{-1} bins) for squid carrying PAT tags (bars) and Crittercam (circles) are similar over the range of ±1 m s^{-1}. Crittercam-bearing squid did not show high-velocity jetting, but these events are extremely rare.

Vertical movements recorded by Crittercam versus PAT tags

Because the Crittercam records depth and temperature, the nature of vertical movements of the primary squid can be compared with

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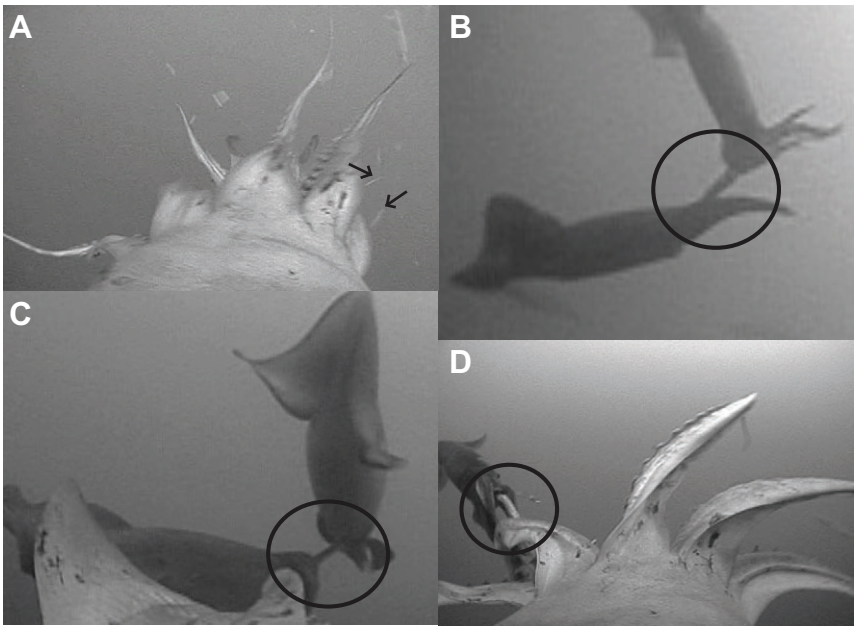

Fig. 2. Potential mating attempts observed during Crittercam deployment 3. (A) Multiple spermatophores (arrows) are visible between the arms of the primary squid. The presence of spermatophores was accompanied by an arms-to-arms interaction between the primary squid and a secondary squid; the combination of these factors is strongly indicative of a mating attempt. (B,C) One secondary squid makes contact with the buccal area of another secondary squid by extending one of its arms (circled). (D) The primary squid makes contact with the buccal area of a secondary squid by extending either an arm or a tentacle (circled).

those of squid carrying much smaller PAT tags. Squid carrying PAT tags also show a sustained descent immediately after release (at night) followed by a variety of climb-and-glide movements as well as more rapid descents and ascents (Fig. 1B). Comparison of vertical-velocity distributions for squid carrying either type of logging device (for similar amounts of time) shows that over 95% of the time is spent at velocities of between −1 m s^{-1} and 1 m s^{-1}, with no apparent difference between squid carrying a Crittercam versus a PAT tag. This strongly suggests that the Crittercam does not seriously impair the squid or grossly alter its behavior.

Virtually no fast-swimming events (>1 m s^{-1} in either vertical direction) for the two Crittercam-bearing squid released during daytime (see Materials and methods) are indicated by data in Fig. 1C. Most descents greater than −1.2 m s^{-1} were displayed only by PAT 11/08A, and they were associated with one deep, rapid dive. All ascents greater than 1.4 m s^{-1} were shown only by PAT 11/08B. Maximum vertical velocities previously recorded with PAT tags in the Gulf of California reached ±2 m s^{-1}, but these events were exceedingly rare (Gilly et al., 2012). The rarity and idiosyncratic nature of such fast movements thus complicates this comparison based on our limited data set. Although we cannot rule out some impairment of fast jetting by the Crittercam, it is clear that the squid is not seriously hindered for the vast majority of the deployment time.

Dynamic chromatophore displays: flashing and flickering

'Flashing' is characterized by a rapid change between pale (white) to dark (red) and back to pale that occurs over the entire body (Fig. 3A; supplementary material Movie 2). This dynamic display generally involved an episode of several cycles (Fig. 3B), but single flashes were occasionally observed. The amplitude of individual flashes varied widely (Fig. 3B,C, see later results), although in most cases the difference between pale and dark was large, and the color change was highly coordinated across the surface of the head (Fig. 3C) and over the rest of the body (secondary squid in Fig. 3A). The change from light to dark in a cycle of flashing as observed in secondary squid appeared to originate in the head and was followed within 1–2 frames by activity on the mantle and fins (not illustrated), but delays and timing of the spread of activity cannot be resolved with the frame–capture rate of the Crittercam (17 Hz). Observations of flashing made at 30 Hz during daylight by a diver with SCUBA in the study area in 2002 also indicate a delay of <2 frames between head and fins (our unpublished data).

Six distinct flashing episodes by the primary squid were observed during deployment 2, and 44 events occurred in deployment 3. Four flashing episodes in deployment 2 (67%) and 40 in deployment 3 (91%) occurred in the presence of at least one secondary squid that was clearly visible during the time in which the primary squid was flashing. For the six episodes that occurred without another squid in frame, the time preceding (or following) flashing and imaging of a secondary squid ranged from 0.5 s to 21 s. Two of these cases (gaps of 8 s and 21 s) occurred at depths with very dim lighting, making it impossible to detect a secondary squid that was not extremely close to the camera, and it is reasonable to assume that an out-of-frame encounter occurred in these cases as well. Thus, most, if not all, flashing occurred in conjunction with intraspecific encounters. An encounter in itself did not guarantee flashing, because one or more secondary squid were often seen without any flashing, and flashing by secondary squid but not the primary was also common.

'Flickering' is a qualitatively different type of dynamic display that is generally evident whenever flashing is not occurring, both in the presence and absence of conspecifics. It is characterized by irregular chromatophore activity in neighboring patches of skin and has a noisy wave-like appearance (initial portion of the record in Fig. 3B and supplementary material Movie 3). Variation in skin color during flickering is of much lower amplitude and not so spatially coherent and temporally synchronized as that during flashing (Fig. 3D).

Flashing frequency

Instantaneous frequency of flashing was measured as the inverse of the cycle period between successive paling minima in the calculated time-course of chromatophore activity. The overall mean of instantaneous frequencies during an episode of flashing by the primary squid varied between 2 and 4 Hz (Fig. 4A). A Wilcoxon rank-sum test showed a significant difference (P=0.0048) in mean frequencies between deployments 2 and 3, 3.5±0.3 Hz versus 2.8±0.2 Hz, respectively. Instantaneous frequency often changed

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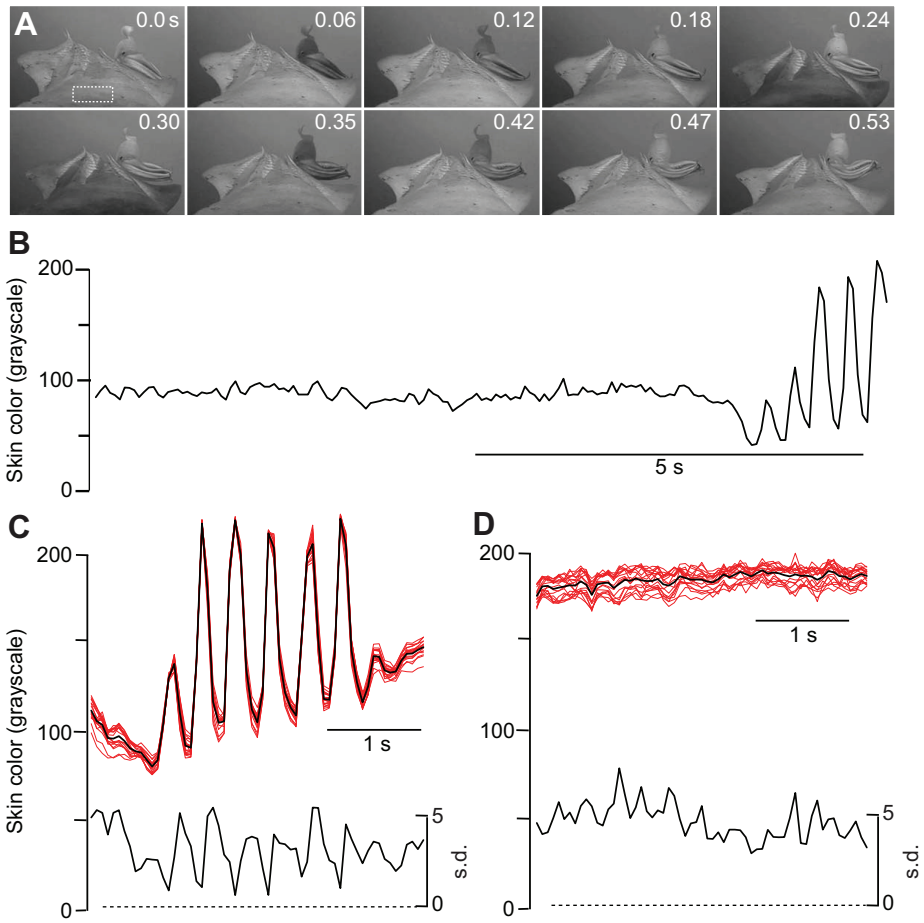

Fig. 3. Flashing and flickering behaviour in *Dosidicus gigas*. (A) Sequential frames from video show out-of-phase flashing between the primary squid and a conspecific. The white box on the primary squid indicates the approximate area over which skin color was quantified. The projected straight-line distance between the base of the outer-most arms is ~15 cm. (B) Time course of change in skin color averaged over the entire boxed area before and during an episode of flashing. (C) Flashing is highly synchronized over the dorsal surface of the primary squid's head. The light red traces represent skin color measured in 18 10×10 pixel boxes in an array of 3 rows by 6 columns. The heavy black trace is the mean. The s.d. was computed from these 18 individual measurements. Minima in the standard deviation trace tend to occur during the peak dark periods and indicate a high degree of spatial synchronization. (D) Flickering was quantified in an analogous way in the same squid at a different time in the video clip. Spatial coherence of flickering as revealed by the s.d. does not show the periodic minima associated with the dark peaks during flashing.

during an individual flashing episode, and Fig. 4B indicates the maximum difference between mean frequency and an individual cycle in a given episode. There was a suggestion that mean frequency during a flashing episode was lower when direct physical contact was made with another squid, but this difference was not significant (Fig. 4C). Change of frequency during a flashing episode, as indicated by the standard deviation of instantaneous frequency during a single flashing episode, was significantly less in cases that involved physical contact between the primary and secondary squid (Fig. 4D).

Timing of flashing by multiple squid during interactions

Because flashing is a sinusoidal-like global oscillation, the color of two squid that are simultaneously flashing tends to come in and out of phase if their frequencies are not identical. In the case illustrated in Fig. 5A the primary squid (black trace) displayed an average frequency (2.2±0.1 Hz; n=7) that was lower than that of the secondary squid (2.8±0.2 Hz; n=9) for the first 3 s of the record (primary cycles 1–7). During this time, flashing frequency (inverse cycle period) was fairly constant in both squid (Fig. 5B), with darkening of the two squid drifting in an out of phase (Fig. 5C). The cycle period of the primary squid then began to increase and diverge from that of the secondary, thereby changing the pattern of the phase relationship for darkening of the two squid as illustrated in Fig. 5C. Modulation of flashing during interactions through frequency change was seen for both secondary and primary squid in deployment 3.

Another way to acutely alter the phase relationship between two flashing squid is indicated in Fig. 6A. In this case, a secondary squid (red trace) flashed at a regular frequency for 1.6 s. A single flash by the primary squid (black trace at 1 s) was followed by a prolonged darkening that lasted for about 1 s. The secondary squid then also displayed an extended dark period (cycle 5) that began in the middle of the primary squid's dark period, and this behavior led to a large increase in cycle period (red circles in Fig. 6B) before flashing resumed at the previous frequency (cycles 6–10). The primary squid then began flashing at 3 s, which was more or less in phase with the secondary squid.

During the time when both primary and secondary squid were in their extended dark period (centered on the 2 s mark in Fig. 6A) another secondary squid (blue trace) flashed in phase with the original secondary squid (onset of cycle 5). This third squid continued flashing at a constant frequency but out of phase with the other two squid during cycles 6–9. At cycle 10, all three squid flashed in phase.

Extending a dark period in this way thus provides another mechanism for rapid, punctuated control of flash timing, and another example of prolonged darkening (by a secondary squid) is evident at the end of the record in Fig. 5A. This behavior suddenly alters the phase relationship between animals and produces a syncopation-like effect between the individual flashing rhythms. The apparent high degree of control over the timing, frequency and phase of flashing strongly suggests that this behavior is fundamentally important to intra-specific signaling in *D. gigas*. How the structure (i.e. syntax) of such flashing varies with specific interactions such as mating or aggression remains unknown.

Temporal control of flickering

About half of the flashing episodes in squid 3 were immediately preceded by a significant diminution of flickering activity across the

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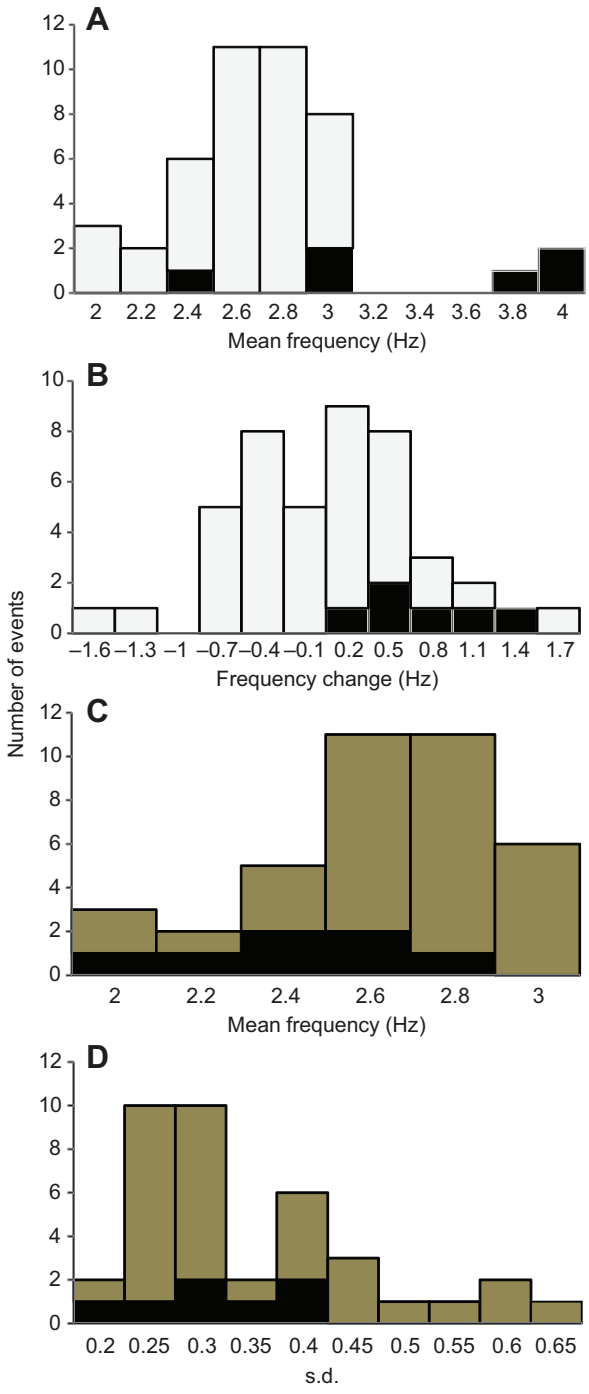

Fig. 4. Flashing frequency in *Dosidicus gigas*. (A) Mean flashing frequency for individual flashing episodes was significantly higher for primary squid 2 (black bars) than for primary squid 3 (white bars). (B) Both primary squid 2 (black bars) and primary squid 3 (white bars) showed substantial changes in instantaneous frequency during individual flashing episodes. (C) In primary squid 3, flashing episodes that coincided with direct physical contact with a secondary squid (black bars) had a lower average frequency than those that did not involve direct physical contact (brown bars). This difference was not significant. (D) Instantaneous frequency during a flashing episode showed significantly less variability (s.d.) if direct physical contact with a secondary squid was made (black bars vs brown bars; P=0.0401).

entire head, during which time the skin uniformly paled (arrows in Fig. 7A). This 'pausing' behavior could be detected by a large decrease in moving variance of the intensity signal (lower trace in Fig. 7A), and the duration of such pauses varied greatly, ranging from only a few frames to several seconds (Fig. 7B). Although this activity was often observed immediately before a flashing episode, similar pauses in flickering sometimes occurred with no apparent association with flashing (not illustrated). In one case, a pause of several seconds was followed by a return to flickering before the onset of flashing (Fig. 7C). Global pausing of flickering is thus another component of chromogenic behavior in *D. gigas*.

Flickering does not show the high degree of synchronization of flashing, but in some cases flickering appeared to smoothly change into a flashing-like behavior of low amplitude. Such coordination is evident in Fig. 7D with four cycles of low-amplitude activity (0.7–2.0 s) that show the temporal characteristics (and spatial synchronization, not illustrated) of flashing occurring before the brief pause that precedes the stronger flashing episode at the end of the record. These observations suggest that flickering can be coordinated to a considerable extent into a muted flashing-like mode. We do not know the spatial extent of this type of activity, because we can only observe such details on the head of the primary squid. This type of subdued flashing activity did not appear to be preceded by well-defined pauses.

Spectral analysis of flickering and flashing

Spectral analysis was undertaken to investigate underlying component frequencies during both flickering and flashing. Time-series data for ~30 s of uninterrupted flickering (Fig. 8A, top panel) and flashing (Fig. 8B, top panel) were filtered with a high-pass filter (0.5 Hz cut-off frequency; Fig. 8A and Fig. 8B, bottom panels) and then processed with a Fast Fourier Transform. Flashing amplitude covered a large range, but power spectra were dominated by strong, discrete peaks, in this case at 2.2 Hz, 2.6 Hz and 2.75 Hz (blue bars, Fig. 8C). Flickering was of much weaker amplitude and showed a series of weaker peaks between 1 and 4 Hz (open red bars). Flickering thus does have some spectral structure and is not simply random 'white' noise that would have a flat spectrum at all frequencies.

To an observer, flickering resembles the variations of light seen on the bottom of a pool caused by fluctuations of light in the water column due to the focusing and defocusing of incident sunlight after refraction by moving ripples at the surface (Darecki et al., 2011). The Crittercam recorded reflections of fluctuations of down-welled natural sunlight from the dorsal surface of the squid in daytime deployments 2 and 3 before the animals were released (supplementary material Movie 4). Analysis of sunlight fluctuations was carried out using identical procedures to those used for flickering activity at depth. In each case, low frequency spectral content was evident in sunlight fluctuations that varied from episode to episode (Fig. 9A), and these spectra were qualitatively similar to those derived from flickering at depth over similar periods of time (Fig. 9B).

Although down-welled sunlight is much brighter than chromogenic flickering as shown by the large difference in amplitude of the respective Fourier spectra, focusing of down-welled sunlight attenuates exponentially with depth in the upper water column, decreasing about 10-fold in 10 m under conditions similar to those in this study (Darecki et al., 2011). At depths of ≥50 m, at which most of the flickering was analyzed, focused irradiance of sunlight would thus be negligible. However, between the surface and 50 m depth, focused sunlight would be a significant signal, and at some point, the amplitude of sunlight variations and flickering would be comparable. Similarity in spectral characteristics suggests that flickering may imitate features of the dynamic natural-light field in this intermediate

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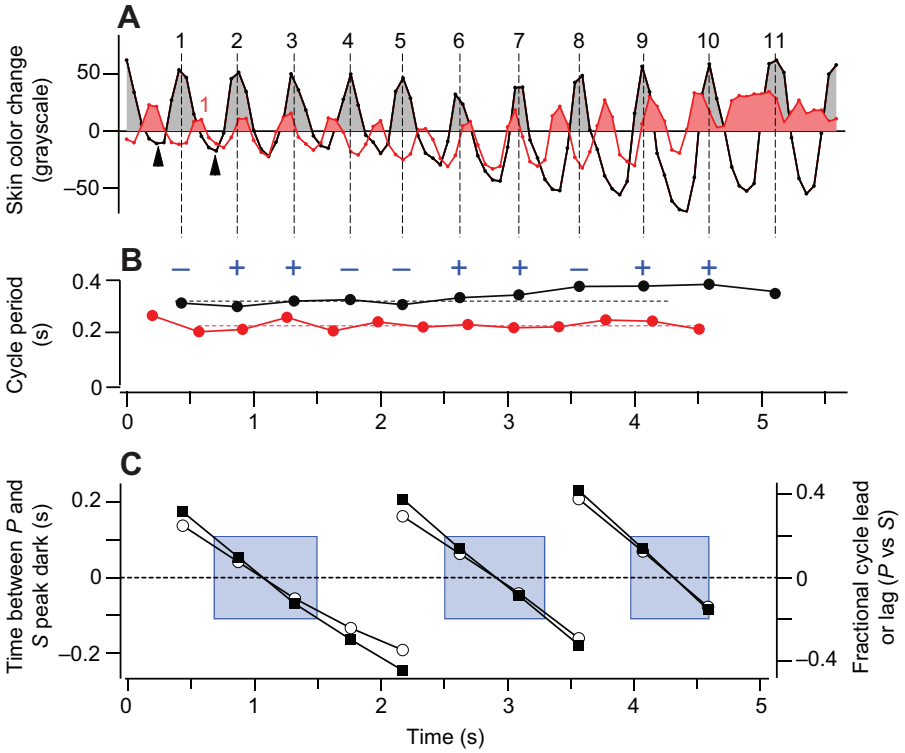

Fig. 5. Alterations in the phase relationship between two flashing squid. (A) Change in skin color of the primary (black trace) and a secondary (red trace) squid relative to the individual mean values for the entire time course are superimposed to facilitate comparison. Times with skin color darker than the mean value (positive values) are shaded. Numbered cycles 1–11 refer to the timing of maximal darkness of the primary squid as indicated by vertical dashed lines. Timing was estimated by interpolation of the point when the derivative of color change crossed zero (not illustrated). (B) Changes in cycle period during the flashing episode depicted in A. Cycle period was measured as the time between paling minima (indicated by arrowheads for cycle 1 of primary squid) as estimated from the derivative of color change (not illustrated). Dotted lines indicate mean frequencies computed for the first 3 s. (C) Time course of the phase relationship for flashing of the two squid depicted in A. Phase was calculated for each cycle 1–10 as the time between peak darkness of the primary squid and the secondary squid (open circles) and as this value divided by the relevant cycle period for the primary squid (fractional cycle period, black squares). Shaded boxes indicate times when dark cycles are in phase (<20% cycle period lag or lead).

region of the water column. Furthermore, in the only Crittercam deployment using artificial light that provided constant and stable illumination, flickering was not observed, unlike in deployments 2 and 3 when flickering commenced shortly after the squid was released. These factors suggest that fluctuations in external light cues in the upper water column at depths of tens of meters may influence flickering behavior.

DISCUSSION

In this paper, we describe behaviors of Humboldt squid filmed with an animal-borne video package under natural lighting in the Gulf of California. This is the first study of natural chromogenic behavior of any cephalopod using this approach. Three basic types of chromatophore display are common. First, static patterns that are maintained for several seconds are similar to those seen in many other species of squid. Second, a dynamic flashing occurs in which the entire body oscillates between pale and dark, at frequencies of 2–4 Hz. This type of display also occurs in *Sthenoteuthis oualaniensis* (our unpublished data), another oceanic ommastrephid squid, but it has not been described for species in any other family of squid (or any other cephalopods) to our knowledge. Third, a more subtle flickering with irregular wave-like characteristics occurs in the absence of flashing. Both flickering and flashing can be quickly and globally turned on and off, and thus both types of behavior must be under strong neural control. Direct transitions between flashing, flickering and pausing are demonstrated in this paper.

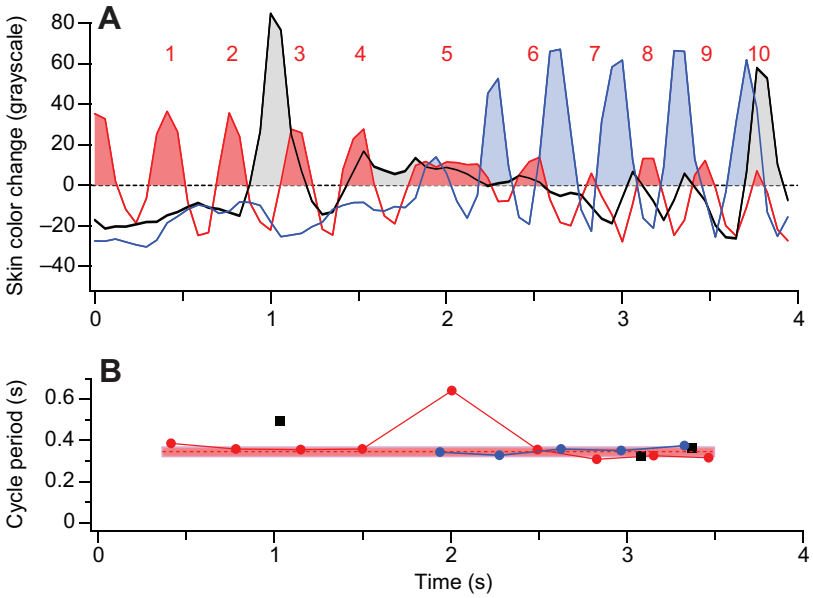

Fig. 6. Alteration of the phase relationship between flashing squid by extension of the dark period of a flashing cycle. (A) Flashing records for a primary (black trace) and two secondary (red and blue traces). The primary squid displays an extended dark time (1.5−2.5 s) followed by a similar behavior in one of the secondary squid (red, cycle 5). The other secondary squid commenced flashing during this time. (B) Cycle periods for the primary (black squares) and secondary squid (red and blue circles) corresponding to the records in panel A. Timing of the points is determined by the time of maximum darkness, and cycle period was measured between minimum white values as described above. The extended dark time in cycle 5 corresponds to a large increase in cycle period for that secondary squid and also alters the phase relation with the other secondary squid. The dotted red line indicates mean frequency of the first secondary squid omitting cycle 5; the width of the pink stripe indicates the corresponding s.d.

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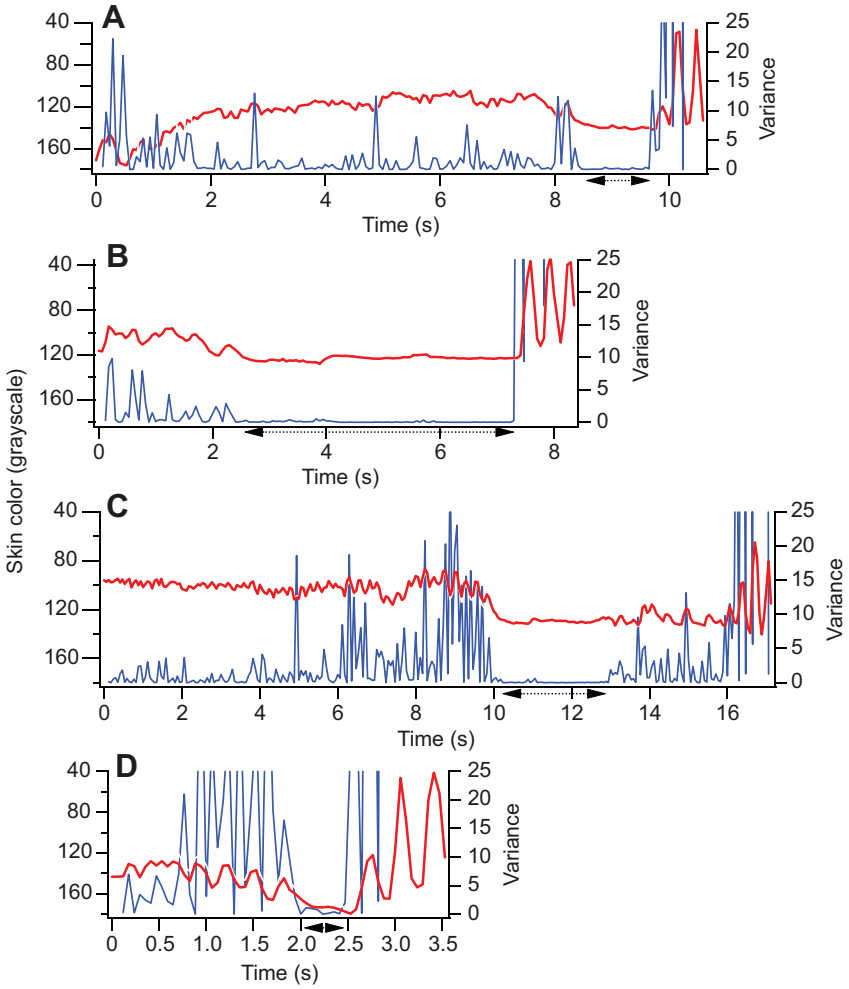

Fig. 7. Pausing of flickering before a flashing episode. (A) Flickering (red trace) during the first 8.5 s of the record is followed by reduced level of fluctuation and overall paling immediately before the onset of a flashing episode. The pause in flickering (time between arrowheads) corresponds to a large reduction in the rolling s.d. (blue trace) of flickering. (B) An example of an unusually long pause in flickering before a flashing episode. (C) Flickering can be interrupted by a pause without an immediate progression to flashing. (D) Flickering can be coordinated into a low-amplitude form of flashing, in this case followed by a pause before an episode of strong flashing commences.

Two major features of these chromatophore displays facilitate behavioral analysis. First, the single-color chromatophore system eliminates the color dimension. Second, dynamic global control of flashing and pausing removes the spatial aspects of these displays. Thus, these chromogenic behaviors of *D. gigas* can be represented as one-dimensional time series, a major simplification in analysis of intra-specific communication (syntax, etc.). This feature also permits spectral analyses to relate specific behaviors (e.g. flickering) to relevant environmental cues (e.g. light fluctuations in the water column).

Mating behavior in *Dosidicus gigas*

Putative mating attempts were captured by Crittercam during both deployments 2 and 3 based on the unambiguous appearance of spermatophores in the midst of the splayed arms of the primary squid. These observations suggest brief, arms-to-arms mating attempts. Mating encounters of this sort have been reported in laboratory observations of two other ommastrephids, *Todarodes pacificus* (Sakurai et al., 2003) and *Illex illecbrosus* (O'Dor and Dawe, 2013). Mating of ommastrephids in the wild has rarely been observed, but putative arms-to-arms encounters in *D. gigas* have been described as being of much longer duration than reported here – from 1 min (Nigmatullin et al., 2001) to more than 10 min (Gilly et al., 2006). Both of these reports were based on observations made from the deck of a vessel at night using artificial illumination. This situation may lead to a different type of mating behavior than that observed by Crittercam. More than one type of mating behavior has been observed in *Illex illecebrosus* in captivity (O'Dor and Dawe, 2013).

Mating near the thermocline depth as observed with Crittercam is not unexpected. The only egg mass reported thus far for *D. gigas* was suspended at the thermocline in the Gulf of California (Staaf et al., 2008), similar to the depth at which mating attempts were observed by Crittercam. If mating and egg-laying both took place near the thermocline (which is generally equivalent to the pycnocline or maximum density gradient), then embryonic development at this depth might be guaranteed by simply equilibrating the density of the pelagic egg mass with the surrounding seawater (O'Dor and Dawe, 2013). Natural mating and egg-laying have not been observed in the wild, so the timing between these events remains unclear.

Flashing and intra-specific interactions

Flashing by the primary squid was essentially never observed in the absence of a conspecific. Direct physical contact between the primary and secondary squid was always associated with flashing, but flashing often began before physical contact was made, and many flashing exchanges did not involve any physical contact. These observations strongly suggest that flashing represents intraspecific signaling and that visual contact is a key driver of this behavior. But flashing is clearly not a fixed-action-pattern type of behavior that is simply triggered whenever another squid comes into view. What circumstances trigger flashing, what behavioral context may modulate the behaviour and what sort of information is transmitted between squid remain unknown.

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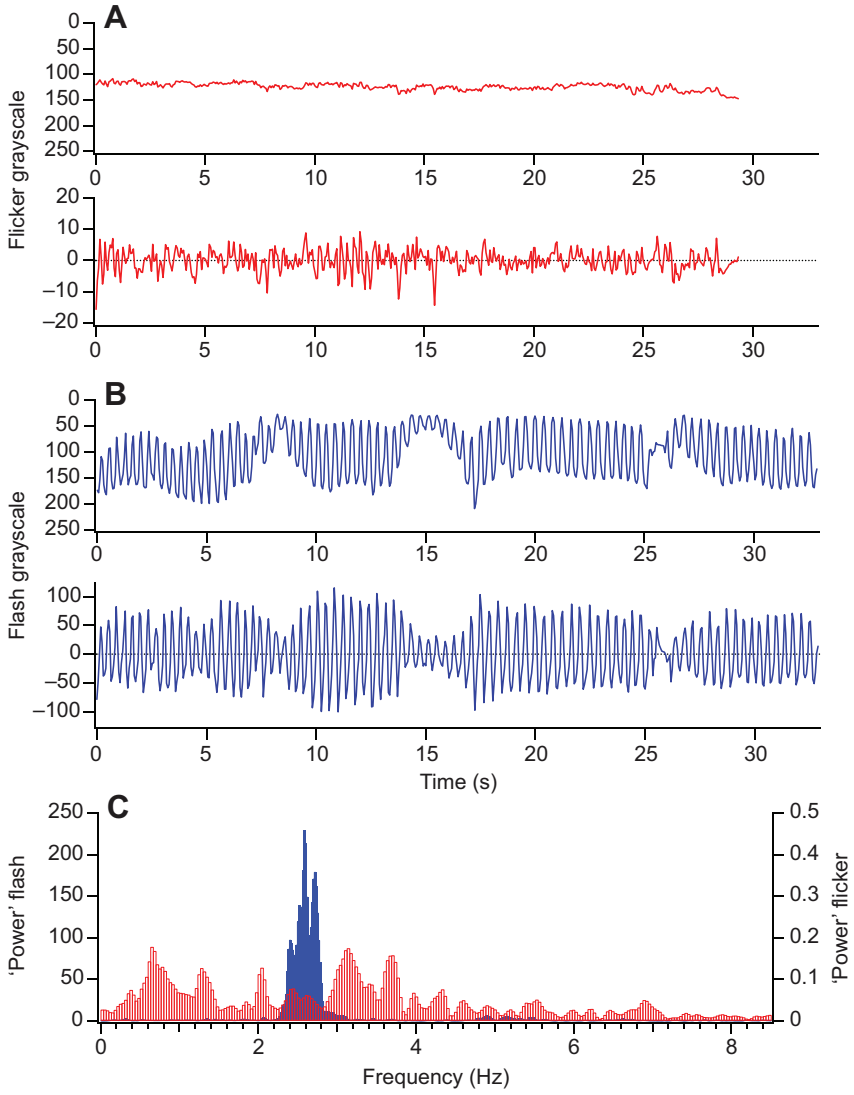

Fig. 8. Spectral analysis of flickering and flashing. (A) Thirty seconds of continuous flickering activity measured over a 20×20 pixel square (top) was processed with a high-pass filter with a 0.5 Hz cut-off to reveal fluctuations (bottom). (B) Data for continuous flashing were processed in the same manner as for flickering. (C) Amplitude of the real part of the Fast-Fourier power spectrum for flashing (blue bars) features strong peaks centered around 2.6 Hz. Flickering is characterized by much weaker peaks in the 1–4 Hz range (open red bars). The 0–0.5 Hz range has been omitted from these data because of the high-pass filtering, and the upper frequency limit is set by the Nyquist frequency (8.5 Hz) for the video sampling rate used.

Several observations indicate that flashing can be controlled to a high degree. Flashing amplitude, frequency and phase can all be adjusted by an individual squid, but the motivation for and the effects of these changes are unclear. Regardless, such a high degree of control over flashing dynamics is consistent with the proposal that this behavior is a fundamental form of intraspecific signaling in *D. gigas*. Unfortunately, because of the limited range of depth at which flashing behavior could be resolved under natural lighting conditions, the effects of abiotic factors such as temperature and oxygen concentration on this behavior could not be ascertained. Future Crittercam deployments in locations with different relationships between these parameters and depth would provide insight into this question.

What sets the range of 2–4 Hz for flashing is a complex question that involves (at a minimum) the physiological limits of the chromatophore neuromuscular system, synchronization capability of the central nervous system and flicker-fusion frequency of the visual system. Unfortunately, we know little of these processes in *D. gigas*. Additionally, if flashing serves as some sort of predator deterrence, the question would also have a significant co-evolutionary component involving vertebrate predators.

Flickering and crypsis

Flickering was always observed whenever adequate natural light was present to image satisfactorily, and it would appear to represent a basal level of chromatophore activity that occurs in the absence of flashing. Although flickering might be considered analogous to muscle tone in a conventional motor system, the underlying spectral composition suggests that it is not simply random noise in the chromatophore musculature or neuronal activation pathway. We propose that chromogenic flickering provides dynamic crypsis for this oceanic squid and provides a function analogous to the camouflage associated with coastal loliginid squid that mimic static benthic features (Hanlon and Messenger, 1996). Living in an oceanic environment, *D. gigas* might have little need for feature patterning that resembles objects, but dynamic patterning that mimics downwelled sunlight striking its body might be of some value particularly since this squid often makes excursions into shallow, better-illuminated depths, regardless of time of day (Gilly et al., 2012). How flickering would be perceived by predators from various angles and depths remains to be established.

Chromogenic behaviors and control pathways in *Dosidicus gigas* versus other cephalopods

Flashing in *D. gigas* is likely to be rooted in the same neural-control system as the static patterns seen in loliginid species (Hanlon et al., 1994; Hanlon et al., 1999; Trueblood, 2010). These behaviors share characteristics such as speed of pattern generation and strict coordination of individual chromatophores, but flashing has an

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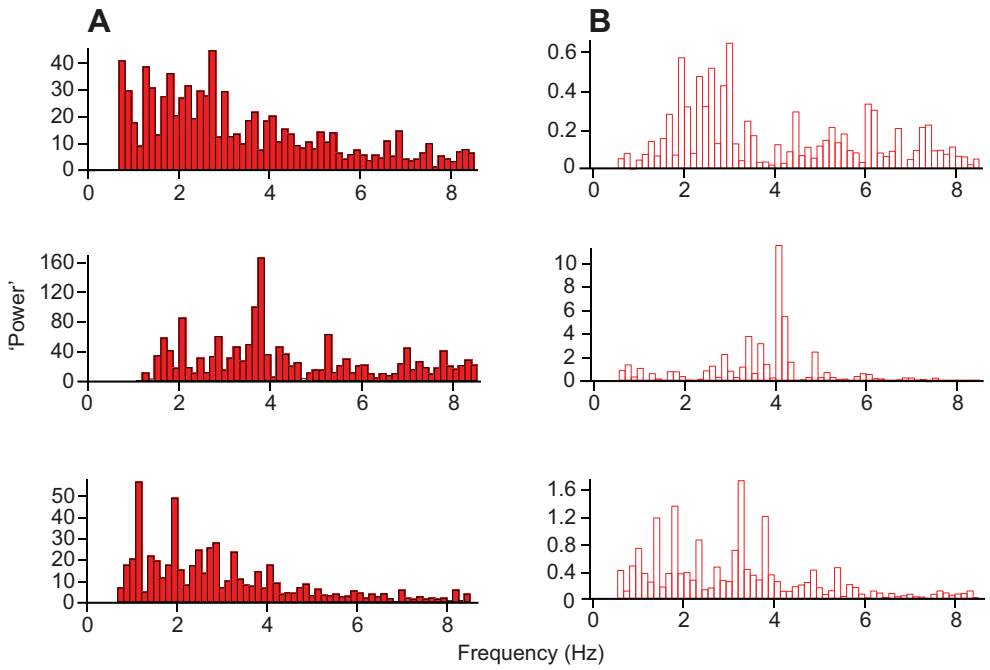

Fig. 9. Spectral analysis of fluctuations of downwelled sunlight versus chromogenic flickering viewed on the head of the primary squid. (A) Examples of spectra for fluctuations of reflected sunlight with the squid in the upper 1–2 m of the water column. Data were collected for 7.1 s (top), 16.5 s (middle) and 9.1 s (bottom). (B) Analogous data for periods of flickering at depth where sunlight-related fluctuations are of very low amplitude. Data were collected for 5.8 s in each case. Average depths for squid were 43.5 m (top), 47.2 m (middle) and 50.1 m (bottom).

accelerated and highly synchronized timescale and a lack of spatial patterning. Signaling in the time-domain thus appears to be of primary importance for *D. gigas*, whereas the spatial domain may be more significant for loliginid species.

The dynamic display we define as 'flickering' has not been described in other cephalopods as a distinct behavior, but an analogous process may occur in other species. Rapid oscillatory cycles of partial expansion/retraction in individual chromatophores (and small groups) have been described in the loliginid, *Sepiotuethis lessoniana* (Suzuki et al., 2011). These 'miniature oscillations' of 2–7 Hz are prominent in the feature areas of patterns, but they also occur in background areas of minimal chromatophore activity. In both aspects miniature oscillations thus show similarity to the low-amplitude, irregular flickering we describe in *D. gigas*, and both phenomena may represent related processes.

Flickering also shares some characteristics with spontaneous wave-like chromatophore activity in denervated *Octopus vulgaris* (Packard, 1992) and in denervated *Loligo vulgaris* and *Doryteuthis (Loligo) opalescence* (Packard, 1995a). Such activity in chronically denervated conditions does not depend on ordinary descending motor control, and thus some myogenic control, inherent to the radial muscle fibers themselves and to the networks to which they belong, must be operative (Packard, 1995b). Relevant mechanisms and pathways underlying myogenic control of cephalopod chromatophores are not well understood, but the irregular wave-like nature of flickering in *D. gigas* suggests that peripheral myogenic control could be an important factor.

Alternatively, flickering could represent asynchronous oscillatory activity in descending motor axons that project to relatively small patches of skin (i.e. several motor units). If so, it would seem that switching from asynchronous to synchronous motor activity (as must occur during flashing) would take place in the chromatophore lobes where the relevant motor neurons are subject to control from higher centers of the brain (Boycott, 1961). Asynchronous and synchronous motor inputs could be transmitted by the same axons or by parallel tracts.

Pausing is another important feature of chromogenic behavior in *D. gigas* that operates in a synchronous mode, because both flashing and flickering can be globally halted very rapidly. Control of this highly timed behavior undoubtedly involves active inhibitory control, but whether this inhibition occurs centrally or peripherally (or both) cannot be specified at this time. Although no direct inhibitory motor innervation has ever been confirmed in the cephalopod chromatophore system, the presence of serotonin (a potent inhibitor) in peripheral axonal processes in loliginid squids (Messenger et al., 1997; Messenger, 2001), strongly suggests inhibitory serotonergic control in the periphery. It is unclear whether this pathway involves axons originating in the chromatophore lobes, the stellate ganglion (Gonzalez-Bellido et al., 2014) or some unknown peripheral neural plexus. Currently, there are no data concerning either excitatory or inhibitory control mechanisms or pathways for ommastrephid squids.

Chromogenic displays in all coleoid cephalopods (squid, cuttlefish, octopus) include both static and dynamic patterning (Hanlon and Messenger, 1996). It is therefore likely that the basic neural mechanisms of chromatophore control appeared early in the evolutionary history of this group. To what degree specialized functions of this unique chromatophore system have diverged in the face of remarkable structural conservation remains to be determined. The relative importance of signaling in the temporal versus spatial domain may vary widely across taxa, but the underlying control mechanisms are likely to be fundamentally similar.

MATERIALS AND METHODS

Pop-up archival transmitting (PAT) tags

PAT tags (MK10, Wildlife Computers, Redmond, WA, USA) were deployed on two large Humboldt squid (77 and 79 cm mantle length) from the research vessel BIP XII during the night of 8 Nov. 2008 off the Baja California coast of the Guaymas Basin, Gulf of California (27.53°N, 112.32°W). These PAT tags [11/08A (PTT 64004) and 11/08B (PTT 83048)] were deployed in the same location and time of year as the Crittercam deployments, and the sampling rate (1 Hz) was identical in all cases. Physical recovery of the PAT tags after 2 week deployments yielded full archival records. Methodological details and data from these tags were described previously (Gilly et al., 2012), but the first 6000 s of recordings following release of the squid were re-analyzed in this study to provide a comparison of vertical movements for squid bearing PAT tags versus

Crittercam. Average sea-surface temperature recorded by the PAT tags was 23°C, and depth of the thermocline was 40–50 m. Temperature at 120 m depth was ~16.5°C. Corresponding data recorded by Crittercam were: surface temperature, 28.5°C; thermocline depth, 50–60 m; temperature at 120 m depth, ~17.5°C.

Crittercam

This instrument package records depth and temperature plus tri-axial accelerometer data at a rate of 1 Hz and captures standard-resolution (720×480 pixels) black-and-white video at 17 Hz. It can be used with or without artificial lighting, and shutter speed under dim light conditions such as those in this study, is 0.02 s. The package stores all data onboard. The Crittercam and in its syntactic-foam cradle detaches from a mounting platform on the camera-bearing animal at a programmed time, floats to the sea surface and transmits a VHF signal by which it can be located and recovered in the field to allow retrieval of the recorded data. Although sensor data from the Crittercam can resolve the vertical component of velocity and orientation of the squid, the horizontal component or axial velocity cannot be determined.

Large specimens of *Dosidicus gigas* (>80 cm mantle length) were caught using rod and reel with weighted luminescent jigs (51 cm length) aboard the charter vessel *Sandman* (deployments 1–3: Sept. 2009; 27.54°N, 112.3°W). For all deployments, the squid was held just under the surface by a team member positioned on the swim-step of the vessel, who was able to manipulate the squid using the jig that was still attached to the arms, and by a diver in the water. A stretchable, synthetic cloth sleeve, to which a thin plastic mounting-platform was attached, was slid over the fins and cinched securely around the mantle using cable ties at the posterior end. Two cable ties were also passed through the sleeve and the dorsal edge of the mantle at the anterior end in order to stabilize the sleeve assembly. A foam cradle with the attached Crittercam was then secured to the mounting platform. In all cases, the Crittercam was oriented parallel to the mantle axis with a forward view, i.e. towards the arms. Once the Crittercam was secured, the jig was carefully removed from the arms, and the squid was gently held beneath the ocean's surface by the diver to confirm its ability to jet vigorously before being released. Additional details are given elsewhere (Gilly et al., 2012).

Deployments 2 (6 Sept. 2009) and 3 (7 Sept. 2009) were carried out in late afternoon using no artificial lighting with the mission programmed to end shortly before sunset at 18:30 h local time. In both cases, the floating camera was recovered within 3 miles of the deployment site. Deployment 1 (5 Sept. 2009) was carried out after sunset and used red (700 nm wavelength) LEDs for illumination. This mission was prematurely terminated when several other squid (*D. gigas*) tore the camera package off the camera-bearing squid shortly after it was released (see Fig. 1). Video during this deployment shows numerous attacks made by other squid, even while the Crittercam was floating to the surface. The absence of such attacks without LED illumination strongly suggests that Humboldt squid can see red light and that illumination of this sort at night can lead to aggression and cannibalism, behaviors that are common for *D. gigas* (Ibáñez and Keyl, 2010).

Video analysis

Videos were split into image stacks using Virtualdub (v1.9.11, Avery Lee, Cambridge, MA, USA), and the resulting images were analyzed using the Matlab Image Processing Toolbox (v8.0 The MathWorks Inc., Natick, MA, USA). Start and end times of all visible chromogenic behaviors were logged and documented, along with any distinct physical postures. Note was also taken of the presence of other squid, any direct physical contact with another squid, or the presence of visible spermatophores over the course of each interaction. In this paper, the squid carrying the Crittercam is referred to as the primary squid and conspecifics that were encountered are secondary squid.

Matlab-based software and a graphical user-interface were developed to permit automated analysis of dynamic chromogenic behaviors. The dorsal surface of the head of the primary squid is always visible in the Crittercam video, and a selectable array of square areas on the imaged surface of the head was analyzed for intensity changes and corrected for movements of the squid's head (yawing to the left and right of <±30 deg) by tracking based on an unchanging anatomical feature that served as a landmark. The total grayscale value (0–255, with 0 being white) was summed for all the pixels in each area during each frame and plotted as a time-series of chromatophore activity over the spatial scale specified, generally 10×10 pixels (~0.1 cm^2 on imaged surface of head) or 20×20 pixels or a rectangular array of such squares.

Chromatophore density was measured on the dorsal surface of the head of a freshly sacrificed *Dosidicus gigas* (50 cm mantle length) under seawater using a high-definition video camera on a tripod. Patches of skin 0.5×0.05 cm in size were analyzed, and chromatophore density was 1332±155 cm^{-2} (mean ± s.d.; n=7). This value was similar to that obtained in the same way for the dorsal mantle (1480±174 cm^{-2}; n=6). An area of 10×10 pixels on the imaged head would correspond to ~0.1 cm^2 and contain at least 100 individual chromatophores.

Oceanic light fluctuations

Fluctuations of down-welled sunlight on the dorsal surface of the primary squid's head were recorded with Crittercam during the time when the squid was held beneath the sea surface (up to 2 m depth) prior to release. These data were analyzed with the identical procedures as those used for chromatophore activity. Sunlight fluctuations at these shallow depths are 10–100 times more intense than relevant chromatophore activity, and we assume that the chromatophore signal is negligible under these conditions and that the sunlight signal is negligible at depths where most flickering was analyzed (>50 m).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

H.R. analyzed all video data and prepared the manuscript. W.G. carried out the Crittercam deployments in the field, analyzed PAT tag data and chromatophore time-course data and assisted with manuscript preparation. K.A. carried out Crittercam deployments in the field and provided manuscript edits. L.B. carried out a preliminary analysis of video and sensor data from Crittercam in an undergraduate honors thesis and provided edits to the manuscript. G.M. provided technical and scientific oversight for the Crittercam deployments and provided edits to the manuscript.

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Supplementary material

Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.114157/-/DC1

References

Bazzino, G., Gilly, W. F., Markaida, U., Salinas-Zavala, C. A. and Ramos-Castillejos, J. (2010). Horizontal movements, vertical-habitat utilization and diet of the jumbo squid (*Dosidicus gigas*) in the Pacific Ocean off Baja California Sur, Mexico. *Prog. Oceanogr.* **86**, 59-71.

Benoit-Bird, K. J. and Gilly, W. F. (2012). Coordinated nocturnal behavior of foraging jumbo squid *Dosidicus gigas*. *Mar. Ecol. Prog. Ser.* **455**, 211-228.

Boycott, B. B. (1961). The functional organization of the brain of the cuttlefish *Sepia officinalis*. *Proc. R. Soc. B* **153**, 503-534.

Bullock, T. H. and Horridge, G. A. (1965). *Structure and Function in the Nervous System of Invertebrates*, Vol. II, pp. 1435. San Francisco, CA: W.H. Freeman and Company.

Bush, S. L., Robison, B. H. and Caldwell, R. L. (2009). Behaving in the dark: locomotor, chromatic, postural, and bioluminescent behaviors of the deep-sea squid *Octopoteuthis deletron* Young 1972. *Biol. Bull.* **216**, 7-22.
Darecki, M., Stramski, D. and Sokolski, M. (2011). Measurements of high-frequency light fluctuations induced by sea surface waves with and Underwater Porcupine Radiometer System. *J. Geophys. Res.* **C**, 116.
Davis R. W., Jaquet, N., Gendron, D., Markaida, U., Bazzino, G. and Gilly, W. (2007). Diving behavior of sperm whales in relation to behavior of a major prey species, the jumbo squid, in the Gulf of California, Mexico. *Mar. Ecol. Prog. Ser.* **333**, 291-302.
FAO (Food and Agriculture Organization) (2012). *Capture Production by Principal Species in 2012*. Available at: ftp://ftp.fao.org/fi/stat/summary/a1e.pdf
Field, J. C., Elliger, C., Baltz, K., Gillespie, G. E., Gilly, W. F., Ruiz-Cooley, R. I., Pearse, D., Stewart, J. S., Matsubu, W. and Walker, W. A. (2013). Foraging ecology and movement patterns of jumbo squid (*Dosidicus gigas*) in the California Current System. *Deep Sea Res.* **95**, 37-51.
Florey, E. (1969). Ultrastructure and function of cephalopod chromatophores. *Am. Zool.* **9**, 429-442.
Gilly, W. F., Elliger, C. A., Salinas, C. A., Camarilla-Coop, S., Bazzino, G. and Beman, M. (2006). Spawning by jumbo squid *Dosidicus gigas* in San Pedro Martir basin, Gulf of California, Mexico. *Mar. Ecol. Prog. Ser.* **313**, 125-133.
Gilly, W. F., Zeidberg, L. D., Booth, J. A. T., Stewart, J. S., Marshall, G., Abernathy, K., Bell, L. E. and Bell, L. E. (2012). Locomotion and behavior of Humboldt squid, *Dosidicus gigas*, in relation to natural hypoxia in the Gulf of California, Mexico. *J. Exp. Biol.* **215**, 3175-3190.
Gonzalez-Bellido, P. T., Wardill, T. J., Buresch, K. C., Ulmer, K. M. and Hanlon, R. T. (2014). Expression of squid iridescence depends on environmental luminance and peripheral ganglion control. *J. Exp. Biol.* **217**, 850-858.
Hanlon, R. T. and Messenger, J. B. (1996). *Cephalopod Behavior*, pp. 31-46. Cambridge: Cambridge University Press.
Hanlon, R. T., Smale, M. J. and Sauer, W. H. H. (1994). An ethogram of body patterning behavior in the squid *Loligo vulgaris reynaudii* on spawning grounds in South Africa. *Biol. Bull.* **85**, 163-167.
Hanlon, R. T., Maxwell, M. R., Shashar, N., Loew, E. R. and Boyle, K. L. (1999). An ethogram of body patterning behavior in the biomedically and commercially valuable squid *Loligo pealei* off Cape Cod, Massachusetts. *Biol. Bull.* **197**, 49-62.
Hunt, J. C. and Seibel, B. A. (2000). Life history of *Gonatus onyx* (Cephalopoda: Teuthoidea): ontogenetic changes in habitat, behavior and physiology. *Mar. Biol.* **136**, 543-552.
Ibáñez, C. M. and Keyl, F. (2010). Cannibalism in cephalopods. *Rev. Fish Biol. Fish.* **20**, 123-136.
Kubodera, T., Koyama, Y. and Mori, K. (2007). Observations of wild hunting behaviour and bioluminescence of a large deep-sea, eight-armed squid, *Taningia danae*. *Proc. Biol. Sci.* **274**, 1029-1034.
Markaida, U. and Sosa-Nishizaki, O. (2003). Food and feeding habits of jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae) from the Gulf of California, Mexico. *J. Mar. Biol. Assoc. UK* **83**, 507-522.
Marshall, G., Bakhtiari, M., Shepard, M., Tweedy, J., Rasch, D., Abernathy, K., Joliff, B., Carrier, J. C. and Heithaus, M. R. (2007). An advanced solid-state animal-borne video and environmental data-logging device ("Crittercam") for marine research. *Mar. Technol. Soc. J.* **41**, 31-38.
Messenger, J. B. (2001). Cephalopod chromatophores: neurobiology and natural history. *Biol. Rev. Camb. Philos. Soc.* **76**, 473-528.
Messenger, J., Cornwell, C. and Reed, C. (1997). L-glutamate and serotonin are endogenous in squid chromatophore nerves. *J. Exp. Biol.* **200**, 3043-3054.
Nigmatullin, C., Nesis, K. and Arkhipkin, A. (2001). A review of the biology of the jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae). *Fish. Res.* **54**, 9-19.
O'Dor, R. K. and Dawe, E. G. (2013). *Illex illecebrosus*, northern short-finned squid. In *Advances in Squid Biology, Ecology and Fisheries: Part II – Oegopsid Squids* (ed. R. Rosa, G. J. Pierce and R. O'Dor), pp. 73-108. New York, NY: Nova Science Publishers, Inc.
Packard, A. (1992). A note on dark waves ('wandering clouds') in the skin of *Octopus vulgaris*. *J. Physiol.* **446**, 164.
Packard, A. (1995a). Discrimination of coupled muscle fibre populations in the skin of squids (*Loligo vulgaris* and *L. opalescens*) following unilateral nerve section. *J. Physiol. (Lond.)* **489**, 134.
Packard, A. (1995b). Organization of cephalopod chromatophore systems: a neuromuscular image-generator. In *Cephalopod Neurobiology* (ed. N. J. Abbott, R. Williamson and L. Maddock), pp. 331-367. Oxford: Oxford University Press.
Packard, A. (2011). Squids old and young: scale-free design for a simple billboard. *Opt. Laser Technol.* **43**, 302-309.
Sakurai, Y., Kidokoro, H., Yamashita, N., Yamamoto, J., Uchikawa, K. and Takahara, H. (2003). *Todarodes pacificus*, Japanese common squid. In *Advances in Squid Biology, Ecology and Fisheries: Part II – Oegopsid Squids* (ed. R. Rosa, G. J. Pierce and R. O'Dor), pp. 249-272. New York, NY: Nova Science Publishers, Inc.
Staaf, D. J., Camarillo-Coop, S. and Haddock, S. H. D. (2008). Natural egg mass deposition by the Humboldt squid (*Dosidicus gigas*) in the Gulf of California and characteristics of hatchlings and paralarvae. *J. Mar. Biol. Assoc. UK* **88**, 759-770.
Stewart, J. S., Hazen, E. L., Foley, D. G., Bograd, S. J. and Gilly, W. F. (2012). Marine predator migration during range expansion: Humboldt squid *Dosidicus gigas* in the northern California Current System. *Mar. Ecol. Prog. Ser.* **471**, 135-150.
Stewart, J. S., Hazen, E. L., Bograd, S. J., Byrnes, J. E. K., Foley, D. G., Gilly, W. F., Robison, B. H. and Field, J. C. (2014). Combined climate- and prey-mediated range expansion of Humboldt squid (*Dosidicus gigas*), a large marine predator in the California Current System. *Global Chang. Biol.* **20**, 1832-1843.
Suzuki, M., Kimura, T., Ogawa, H., Hotta, K. and Oka, K. (2011). Chromatophore activity during natural pattern expression by the squid *Sepioteuthis lessoniana*: contributions of miniature oscillation. *PLoS ONE* **6**, e18244.
Trueblood, L.A. (2009). Physiology and behavior of the jumbo squid *Dosidicus gigas*. Ph.D. Thesis. University of Rhode Island.
Vechionne, M., Roper, F. E., Widder, E. A. and Frank, T. M. (2002). In situ observations on three species of large-finned deep-sea squids. *Bull. Mar. Sci.* **71**, 893-901.