

Video playback demonstrates episodic personality in the gloomy octopus

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SUMMARY

Coleoid cephalopods, including octopuses, cuttlefish and squid, rely mainly on visual signals when interacting with conspecifics, predators and prey. Presenting visual stimuli, such as models, photographs, mirrors and live conspecifics, can thus provide insight into cephalopod behaviour. These methods, however, have limitations – mirrors and live animals lack experimental control, whereas models and photographs sacrifice motion-based information. Video playback addresses these issues by presenting controlled, moving and realistic stimuli but, to date, video playback has not been used successfully with any cephalopod. Here, we developed a video playback technique for the gloomy octopus (*Octopus tetricus*) that incorporated recent advances in video technology. We then used this technique to test for personality, which we defined as behavioural differences between individuals that are consistent over time and across ecologically important contexts. We captured wild octopuses and tested them on 3 separate days over a 10 day period. On each test day, subjects were presented with videos of a food item, a novel object and a conspecific. These represented a foraging, novel and threatening context, respectively. A fourth video without a moving stimulus controlled for the playback monitor itself and potential artifacts associated with video playback. Experimental stimuli evoked unambiguous and biologically appropriate responses from the subjects. Furthermore, individuals' responses to the three experimental contexts were highly correlated within a given test day. However, within a given context, individuals behaved inconsistently across the 3 test days. The reordering of ranks suggests that rather than fulfilling the criteria for personality, gloomy octopus show temporal discontinuities, and hence display episodic personality.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/213/7/1035/DC1>

Key words: *Octopus tetricus*, cephalopod, video playback, visual signal.

INTRODUCTION

Visual signals and cues are critical in many species for tasks such as predator recognition (Blumstein et al., 2000; Tinbergen, 1951), predator avoidance (Otte, 1974), species recognition (Bradbury and Vehrencamp, 1998) and social communication (Ord et al., 2002). Presenting controlled visual stimuli is therefore important for determining the function of visual signals and cues in these species. Traditionally, visual stimuli have included models or inanimate objects (Hemmi and Zeil, 2003), pictures (Dawkins, 1996), live animals (Herzog and Burghardt, 1974), conspecifics (Evans and Marler, 1991) or mirrors (Povinelli et al., 1997). Each of these stimuli has limitations – mirrors and live animals offer little experimental control, whereas models and photographs sacrifice potentially salient motion-based information.

Video playback provides a unique method for presenting moving visual stimuli that are both controlled and realistic. Video stimuli can also be manipulated easily with video editing software and are unaffected by the behaviour of the subject being tested. But video playback is not without its challenges. For example, video was designed for the human visual system, so differences in the visual systems of other animals means they may perceive video differently from humans. Spatial and temporal resolution, the lack of a third dimension, and the representation of colour and brightness may all affect how video stimuli are perceived by animals (for reviews, see D'Eath, 1998; Fleishman et al., 1998; Zeil, 2000). Nevertheless, by addressing many of these issues, video playback has been used successfully with a variety of species, including arachnids,

crustaceans, fish, reptiles, amphibians, birds and primates (Ord et al., 2002).

Coleoid cephalopods, including octopuses, cuttlefish and squid, rely heavily on visual signals and cues when interacting with conspecifics, predators and prey (Hanlon and Messenger, 1996). Their skin can display brilliant colours and striking patterns that are used for signalling in a variety of contexts, including social communication, defence and prey capture (Langridge, 2009; Moynihan and Rodaniche, 1982; Warren et al., 1974). They also have large eyes with high visual acuity, focal lengths similar to fish (Hanlon and Messenger, 1996) and flicker fusion rates of up to 60 Hz (Hamasaki, 1968). They cannot discriminate between different hues but they can see polarized light (Hanlon and Messenger, 1996). By presenting them with visual stimuli such as inanimate objects (Sinn and Moltschanowskyj, 2005), live animals (Mather and Anderson, 1993) and mirrors (Adamo and Hanlon, 1996), previous research has gained considerable insight into cephalopod visual behaviour. No study, however, has elicited biologically appropriate responses from cephalopods using video playback.

In the present study, we developed a successful video playback technique for the gloomy octopus, *Octopus tetricus* Gould 1852, and then used this technique to test for the presence of personality, which is also known as behavioural syndromes, temperament and individuality. We defined 'personality' as a suite of intercorrelated personality traits (Kurvers et al., 2009) and 'personality traits' as interindividual differences in behaviour that are consistent over time and context (Biro and Stamps, 2008; Kurvers et al., 2009).

'Consistent' in this sense means that interindividual differences in the expression of a trait are maintained, but that the absolute level of expression can be variable (Réale et al., 2007). Such phenotypic variation, both between and within individuals, may result from intrinsic factors, such as experience, hormone levels and physiological state (e.g. Frost et al., 2006; Koolhaas et al., 1999). In dumpling squid (*Euprymna tasmanica*), for example, individual differences can be attributed to sex, body size, genetic composition and sexual maturity (Sinn and Moltschanivskyj, 2005; Sinn et al., 2006; Sinn et al., 2007). Of course, extrinsic factors, such as environmental conditions, food availability, predation risk and the social environment, can also affect the expression of personality traits (e.g. Biro and Stamps, 2008; Brown et al., 2007; McGuire et al., 1994).

We used video playback to present focal individuals with three types of experimental stimuli representing three different ecologically important contexts. We presented each stimulus to each subject on each of 3 days, and we recorded several response variables. Our objectives were to test (1) whether video playback evokes biologically appropriate responses from the gloomy octopus and (2) whether subjects respond consistently over time and across multiple contexts.

MATERIALS AND METHODS

Subjects

Thirty-one gloomy octopuses (*O. tetricus*) were caught by hand in bays within Sydney Harbour, Sydney, Australia (33°50'S, 151°22'E) between May and September 2008. They were transported to the Sydney Institute of Marine Science, Chowder Bay, Sydney. Before transferring them to holding tanks, they were weighed using a 1000 g Pesola spring scale (accuracy: ± 10 g) or, for those exceeding 1000 g, a 3000 g Scientech electronic balance (accuracy: ± 0.01 g). Sex could not be determined reliably, as the majority of subjects were small and would not have had a fully developed hectocotylus.

At the Sydney Institute of Marine Science, each octopus was housed in a separate opaque plastic tank (60 cm \times 36 cm \times 27 cm, 1 \times w \times h) that received a constant flow (approximately 1 litre min^{-1}) of filtered seawater from Chowder Bay. The floor of each tank was covered with pebbles, rocks and pieces of pipe, and a terracotta pot was provided as a shelter. The aquarium facility was maintained on a natural daylight cycle *via* skylights and fluorescent lights (approximately 10 h light per day during May, June, July; 11 h light per day during August; 13 h light per day during September). Octopuses were fed thawed, frozen prawn or squid every second day in the evening. Octopuses exceeding 400 g received two to three prawns or half a squid, whereas those weighing less than 400 g received one prawn or one quarter of a squid. On test days, feeding occurred in their home tank at least 30 min after testing was complete. Octopuses were kept for a minimum of 10 days for experimentation, after which they were released at sites not closer than 2 km to any collection site. The gloomy octopus is a sedentary species and individuals occupy permanent lairs (Norman and Reid, 2000). It is therefore unlikely that released individuals were subsequently recaptured.

Video stimuli

The video stimulus types included footage of a novel object (jar), a food item (crab, *Leptograpsus variegatus*), a conspecific and a control (water-filled aquarium). For each stimulus type, three different exemplars (i.e. 12 stimuli in total) were created to reduce pseudoreplication and ensure that behavioural responses to the stimuli reflected the stimulus type and not some idiosyncrasy of

a particular exemplar. Consistent with previous work, the novel object was used to test the subject's boldness and/or exploratory behaviour (Réale et al., 2007). The crab is a preferred food item of the gloomy octopus and was therefore used to simulate a foraging context. As octopuses are asocial, solitary, largely intolerant and, in some species, aggressive toward conspecifics (Hanlon and Messenger, 1996), the conspecific footage was used to simulate a threatening, aggressive encounter. Video of a water-filled aquarium served as a control, which accounted for the presence of an illuminated monitor and for potential motion artifacts associated with video playback.

Stimuli were video recorded in a glass aquarium (122 cm \times 35 cm \times 51 cm, 1 \times w \times h) filled with seawater. The aquarium was lit by four 60 W incandescent globes and by natural light from overhead skylights. The rear of the aquarium was covered with a white cloth, which formed the background of each clip (see supplementary material Table S1). The crab, however, was difficult to view while positioned flat on the aquarium bottom. We therefore placed a moveable plastic floor under the crab and tilted it slightly towards the camera (approximately 15 deg. above horizontal) to provide a more complete view of the stimulus (see supplementary material Table S1). Footage was shot with a Sony HDR-HC7 Handicam (format: HDV1080i50; shutter speed: 1/100 s) and was recorded digitally to the hard drive of an Apple computer (Mac mini 2.0 GHz Intel Core Duo) using QuickTime Pro software (v.7, Apple Inc., Cupertino, CA, USA). The camera's optical zoom was set so that a section of the middle of the aquarium, including the floor, occupied the entire field of view.

For each of the 12 exemplars, footage was edited using Final Cut Pro software (v.6, Apple Inc.) to make a 3 min sequence in which the stimulus made several alternating passes across the aquarium (see stimuli descriptions in supplementary material Table S1). Each stimulus moved off screen at the end of each pass and entered again from the side it exited to simulate a realistic movement. The 12, 3 min clips were then exported for playback as QuickTime video files (format: DVCPRO720p50).

Finally, we measured the size, brightness and movement of the nine experimental video stimuli (i.e. three crabs, three novel objects, three conspecifics) so that these properties, independent of stimulus type, could be related to the subjects' behavioural responses. Size was measured on the monitor used for playback. The size of each conspecific video stimulus was described by the lateral length from the eyes to the end of the mantle. The size of each novel object stimulus was described by its maximum width, while the size of each crab video stimulus was described by the maximum width of its carapace. Octopuses cannot discriminate between different hues (Hanlon and Messenger, 1996), so we measured brightness instead of colour. This was measured using Colour Picker Pro software (v.3.1.0, Critical Matter Inc., Salt Lake City, UT, USA) on the computer broadcasting the stimuli. Specifically, we measured brightness at 10 random locations on each novel object stimulus, 10 random locations on the mantle of each conspecific stimulus, and 10 random locations on the carapace of each crab stimulus. The average of each set of 10 brightness measurements was then used to approximate the brightness value of each of the nine video stimuli. Note that the contrast between the stimulus and its background was not measured because the background brightness was held constant across all stimulus videos. Finally, we measured movement by counting the number of alternating passes that each stimulus made across the playback monitor. Supplementary material Table S1 provides a complete description of the stimuli created.

Playback apparatus

During tests, subjects were placed in the same glass aquarium that was used to record video stimuli. It was filled with filtered seawater and was visually isolated from the surroundings by white cloth and black screens (see Fig. 1). To facilitate scoring, the aquarium was placed on top of a Cartesian coordinate grid that divided the floor into 32 equal squares (see Fig. 1). Stimuli were played using Final Cut Pro software (v.5, Apple Inc.) on a Macintosh computer (Mac mini 2.0 GHz Intel Core Duo), and were broadcast to subjects on a 43 cm BenQ LCD monitor (50 Hz refresh rate; 1280×1024 lines of resolution, 0.264 mm pixel pitch) that was placed firmly against one end of the aquarium (Fig. 1). This approach is similar to that used by Rosenthal and colleagues (Rosenthal et al., 1996), in which sword-tail fish responded realistically and appropriately to video stimuli played on a screen abutting the subject's tank. A Sony HDR-HC7 Handicam placed directly above the aquarium provided a live view of the aquarium to a remote laptop computer (MacBook 2.0 GHz Intel Core Duo), which recorded the subjects' responses using QuickTime Pro software (v.7, Apple Inc.; format: HDV1080i50).

Test procedure

Each octopus was tested on 3 separate days: the day after capture, 4 days later and 4 days later again. All testing occurred in the late afternoon so that it finished within 1 h of sunset [gloomy octopuses are crepuscular and nocturnal (Norman and Reid, 2000)].

Immediately prior to testing, the subject was caught with a hand net from its home tank and transferred to one of two water-filled plastic boxes that was fitted with a door on one side (8 cm×8 cm×19 cm, 1×w×h, with 8 cm diameter door for octopuses under 1 kg; 22 cm×22 cm×22 cm with 10 cm diameter door for subjects exceeding 1 kg). The box was then transferred into the start arena of the test aquarium (end of aquarium opposite monitor, Fig. 1) and, after

3 min of habituation, the door of the box was opened remotely. Octopuses were given a maximum of 15 min to emerge from the box into the surrounding test aquarium. If they did not emerge during this time, the box was slowly lifted out of the water until the subject exited through the door (the subject was never held out of water

for more than 5 s). Immediately following emergence, the box was replaced with a terracotta pot that provided the subject with refuge during subsequent stimulus presentations.

Following emergence from the box, the subject was given 3 min to habituate to the test aquarium. One exemplar (selected at random and without replacement) of each of the four stimulus types was then shown in a randomly determined sequence. The first of the four stimuli was presented when the subject entered the start arena. Each subsequent stimulus was then presented after a minimum of 3 min following the completion of the previous stimulus, at a time when the subject was again within the start arena. If, for any stimulus presentation, the subject did not move to the start arena after 10 min, the stimulus was presented regardless of the subject's position. If a subject had inked or produced any noticeable secretions whilst being tested, the water in the test aquarium was replaced before the next subject was tested.

Throughout the trial, except when broadcasting a video stimulus, the monitor displayed a still image of the control stimulus (i.e. a water-filled aquarium; see supplementary material Table S1). The transition to each stimulus clip was then softened using a 4-frame cross-dissolve transition to ensure that stimulus presentations did not begin or end with a sudden change in brightness on the monitor. To prevent interference from the observer, video playbacks were controlled remotely using Remote Desktop software (v.3, Apple Inc.) running on the same laptop computer that was used to record the subject's response. Subjects were returned to their home tank immediately after testing using a net and water-filled bucket.

Data scoring

Test footage was scored on a 30 in monitor using Final Cut Pro software (v.6, Apple Inc.) on a Macintosh computer. For each of the 3 test days, five response variables were measured during each of the four video presentations. These included latency, movement, number of stimulus touches, brightness change (range between lightest and darkest colour) and chromatic/textural change. Scoring was done blind with respect to subject identity and test day for all variables. In addition, brightness change and chromatic/textural change were scored blind with respect to stimulus type.

Latency was recorded as the time taken for the subject's eye to cross the first gridline (parallel to the playback monitor) between the subject and the monitor after a stimulus commenced. Reference to the eye was used in all measurements, as it is the most central, recognizable point on octopuses. Movement was measured by recording the subject's position relative to the Cartesian coordinate grid at 20 s intervals throughout the 3 min stimulus presentation. The distances moved between each 20 s interval were calculated using the Pythagoras theorem and were then summed to provide an estimate of the subject's total movement for that 3 min period. The number of times that the subject touched the end of the aquarium displaying the stimulus was also noted.

The brightness level corresponding to the majority of the body's surface area (excluding arms) was scored according to a seven-point ordinal scale. The lightest skin colour observed across all subjects was assigned a score of 1 and the darkest colour a score of 7 (scores 2–6 were distributed evenly between these two values). Brightness scores were assigned by comparing the brightness of the subject with that of seven still images that corresponded to the seven brightness values. The same images were used to score all trials, thereby ensuring that all measurements were made relative to the same standards. Chromatic/textural pattern, as defined previously (Hanlon and Messenger, 1996), was scored according to a four-point ordinal scale. A score of 1 indicated that textural patterns (i.e.

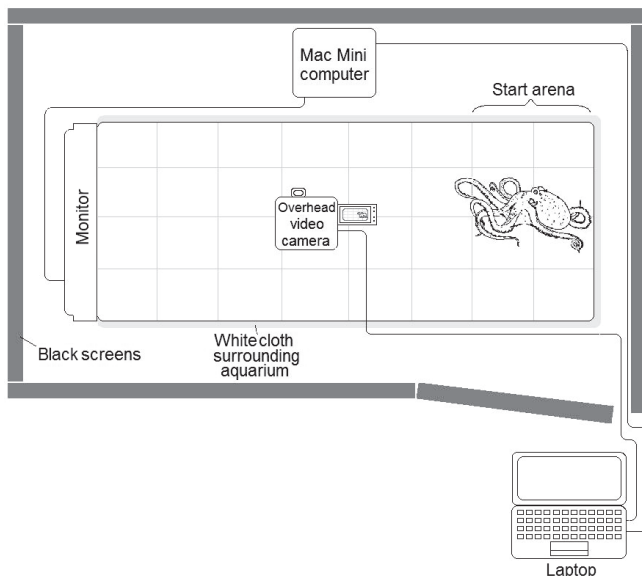


Fig. 1. Overhead schematic diagram of the test apparatus. Not to scale.

erect papillae) and/or colour patterns (i.e. chromatic arrangement) were absent from the entire body, whereas a score of 4 indicated that a texture and/or colour pattern was evident over the entire body. Scores of 2 and 3 indicated a colour pattern and/or textural pattern on one-third and two-thirds of the body, respectively. Brightness level and chromatic/textural pattern scores were recorded 10 s prior to each stimulus presentation. Maximum scores observed throughout each stimulus presentation were also noted. For each stimulus presentation, the pre-stimulus scores were subtracted from the maximum scores to obtain difference scores that reflected the subject's maximum change in brightness level and chromatic/textural pattern in response to that particular stimulus.

Data analysis

To control for putative differences in individuals' daily motivational states (e.g. hunger), the latency, movement, number of stimulus touches, brightness change and chromatic/textural pattern change were corrected using a baseline measure of each. To achieve this, each response measure exhibited during the control stimulus on a particular day was subtracted from that recorded for each experimental stimulus on that same test day. The behaviour expressed during the control is an ideal baseline measure, as the control stimulus is identical to the other stimulus types (i.e. video of a water-filled aquarium), except that it lacks a moving object. Principal components analysis (orthogonal rotation method: varimax) was then conducted on the five baseline-corrected response variables to reduce them to a smaller number of orthogonal factors (Table 1). The resulting factor scores were used in all data analyses.

General linear mixed models with repeated fixed factors were used to analyse the population-level responses and determine whether subjects responded differently to the three different stimulus types (i.e. conspecific, crab, novel object). Test day and stimulus type were included as repeated fixed factors, and subject weight as a covariate. Separate models were run on each of the orthogonal factors derived from the principal components analysis. Where an overall model was significant, *post hoc* pairwise comparisons were used to identify statistically significant differences. Overall type I error was controlled using the sequential Bonferroni method (Holm, 1979; Rice, 1989).

Pearson correlations were conducted on each principal component to test whether individuals responded consistently across the three experimental contexts on each day (i.e. conspecific, crab and novel object), and across the 3 test days within each experimental context. A measure of repeatability (*R*), as described by Lessells and Boag (Lessells and Boag, 1987), was also provided for each correlation matrix to describe the overall consistency of individuals' behaviour.

Finally, we used multiple linear regression to test for a possible relationship between stimulus characteristics, independent of

stimulus type, and subjects' behavioural responses. Independent variables included stimulus size, the number of passes that the stimulus made across the screen and stimulus brightness. A separate analysis was conducted for each of the nine experimental trials (i.e. excluding the three control trials) and for each of the derived principal components.

For all analyses, data complied with the parametric assumptions of normality and homogeneity of variance, as determined by Kolmogorov–Smirnov tests (all $P > 0.098$) and inspection of histograms. All tests were conducted in SPSS 16.0 (SPSS Inc., Chicago, IL, USA), were two-tailed, and had an α -level of 0.05.

RESULTS

The principal components analysis generated two principal components that together accounted for 68.10% of the variance contained in the original five response variables. Movement, latency and the number of stimulus touches were highly intercorrelated, as all of them loaded heavily onto the first principal component (Table 1). Movement and the number of stimulus touches correlated positively with each other and negatively with latency. Chromatic/textural pattern change loaded heavily onto the second principal component, and brightness change loaded approximately equally onto the two principal components.

At the population level, there was a significant difference in subjects' responses to the three different stimulus types (general linear mixed model, PC1: $F_{2,170}=11.583$, $P < 0.0001$, Fig. 2; PC2: $F_{2,165}=5.661$, $P=0.004$, Fig. 3). During the food item presentation, subjects approached significantly faster, moved greater distances and touched the stimulus more often, compared with presentations of the conspecific and novel object (Fig. 2, supplementary material Movie 1). Approaches to the conspecific stimulus were the slowest and there was less movement and fewer stimulus touches than in response to the food item and novel object stimuli (Fig. 2, supplementary material Movie 2). Subjects also exhibited a chromatic/texture change over more of their body during presentation of the food item stimulus than during presentation of either a novel object or the conspecific (Fig. 3). Finally, we detected no interaction between the stimulus type and test day for PC1 (general linear mixed model, $F_{4,123}=0.179$, $P=0.949$) or PC2 ($F_{4,131}=0.154$, $P=0.961$), suggesting that subjects habituated to the four stimulus types at approximately the same rate throughout the experiment.

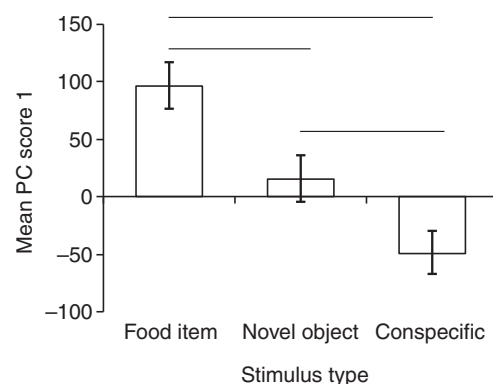


Fig. 2. Mean PC1 responses (\pm s.e.m.) of 31 *Octopus tetricus* to three types of experimental stimuli, averaged across the 3 test days. Bars indicate a significant difference between treatments (overall type I error controlled using the sequential Bonferroni method).

Table 1. Factor loadings for the principle components derived from a principal components analysis conducted on five response variables

Variable	PC1	PC2
Movement	0.897	0.010
Latency	-0.866	-0.068
Stimulus touches	0.754	-0.097
Brightness change	0.477	0.411
Chromatic/texture change	-0.105	0.928
% of variance explained	47.35	20.75

Orthogonal rotation method: varimax.

Table 2. Pearson correlations for test day 1 for PC1 (values below and left of diagonal) and PC2 (values above and right of diagonal)

Stimulus type	Food item	Novel object	Conspecific
Food item		0.511**	0.544**
Novel object	0.415*		0.476**
Conspecific	0.577**	0.522**	

* $P < 0.05$, ** $P < 0.001$ (two-tailed).

Repeatability measure for PC1 across the three contexts on day 1, $R = 0.60$.

Repeatability measure for PC2 across the three contexts on day 1, $R = 0.49$.

Table 3. Pearson correlations for test day 2 for PC1 (values below and left of diagonal) and PC2 (values above and right of diagonal)

Stimulus type	Food item	Novel object	Conspecific
Food item		0.496**	0.408*
Novel object	0.600**		0.577**
Conspecific	0.606**	0.612**	

* $P < 0.05$, ** $P < 0.001$ (two-tailed).

Repeatability measure for PC1 across the three contexts on day 2, $R = 0.49$.

Repeatability measure for PC2 across the three contexts on day 2, $R = 0.43$.

Table 4. Pearson correlations for test day 3 for PC1 (values below and left of diagonal) and PC2 (values above and right of diagonal)

Stimulus type	Food item	Novel object	Conspecific
Food item		0.628**	0.508**
Novel object	0.483**		0.707**
Conspecific	0.495**	0.653**	

* $P < 0.05$, ** $P < 0.001$ (two-tailed).

Repeatability measure for PC1 across the three contexts on day 3, $R = 0.42$.

Repeatability measure for PC2 across the three contexts on day 3, $R = 0.36$.

Table 5. Pearson correlations and repeatability values (R) between each test day for PC1 and PC2 within each stimulus type

	Between days	Food item	Novel object	Conspecific
PC1	1 and 2	-0.127	0.088	0.034
	1 and 3	-0.205	-0.094	-0.127
	2 and 3	0.231	-0.209	-0.194
		$R = 0.000$	$R = 0.044$	$R = 0.000$
PC2	1 and 2	-0.162	0.037	-0.016
	1 and 3	-0.008	0.062	-0.226
	2 and 3	0.210	-0.074	-0.021
		$R = 0.044$	$R = 0.012$	$R = 0.074$

* $P < 0.05$, ** $P < 0.001$ (two-tailed).

On any given day, subjects' responses to video presentations of the food item, conspecific and novel object were highly intercorrelated (all $R > 0.36$, Tables 2–4). PC1 and PC2 were both significantly and positively correlated among the three contexts within each of the 3 test days (Tables 2–4). Therefore, individual octopuses behaved consistently with respect to their activity and body pattern within a given test day.

In marked contrast, gloomy octopus behaviours were inconsistent from one day to the next in response to any particular stimulus type (all $R < 0.07$). The principal component scores were not correlated over the 3 test days for any stimulus type (Table 5). As an example of this inconsistency, subject 1 responded to the conspecific stimulus on test day 1 by immediately approaching it, touching it repeatedly with its arms extended, erecting its papillae and flashing dark colouration. In contrast, on test day 2 that same subject avoided the conspecific stimulus, remained motionless behind the pot, and only erected its papillae on a small section of skin.

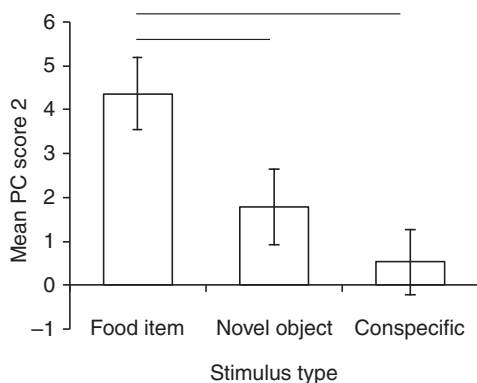


Fig. 3. Mean PC2 responses (\pm s.e.m.) of 31 *O. tetricus* to three types of experimental stimuli, averaged across the 3 test days. Bars indicate a significant difference between treatments (overall type I error controlled using the sequential Bonferroni method).

Of the 18 multiple linear regression analyses conducted (two response variables; nine tests involving experimental stimuli), there were only two significant linear relationships between subjects' responses and the properties of the stimuli shown to them. Stimulus size and stimulus brightness together explained a significant amount of the variation in both PC1 [multiple linear regression analysis, $F_{3,25} = 8.12$, $P = 0.001$, $R^2_{adj} = 0.703$; size: $t = -2.342$, $P = 0.027$; colour (brightness): $t = -2.091$, $P = 0.047$] and PC2 ($F_{3,25} = 13.21$, $P < 0.02$, $R^2_{adj} = 0.369$; size: $t = -2.116$, $P = 0.044$) during the fifth video stimulus presentation. No other significant linear relationships existed between PC1 or PC2 and the size, colour and movement of the stimuli (multiple linear regressions, all $P > 0.063$).

DISCUSSION

Video responses

Gloomy octopuses reacted differently to the three different video stimuli (Figs 2, 3). Their responses to the stimuli were also biologically appropriate. For example, subjects rapidly approached the crab stimulus – often using jet propulsion (supplementary material Movie 1) – and repeatedly touched the stimulus while simultaneously displaying dark colouration and pronounced body patterns. This sequence of behaviours is qualitatively similar to the crab attack sequence described for free-living octopuses (Maldonado, 1964) and Packard (Packard, 1963). In response to the conspecific stimulus, most subjects reduced their activity and avoided the stimulus, often cowering at the far end of the tank or seeking refuge behind or inside the terracotta pot (Fig. 2, supplementary material Movie 2). In the wild, octopuses are solitary animals that actively avoid conspecifics (Byrne et al., 2004; Hanlon and Messenger, 1996). Aggressive encounters with conspecifics are also costly and can result in severe damage, such as loss of arms (e.g. Aronson, 1986). Our subjects' seemingly adaptive responses to simulated conspecifics were therefore consistent with responses to live conspecifics in the wild. This study thus provides the first demonstration that video playback can evoke stimulus-specific and biologically appropriate responses from a cephalopod.

Prior to conducting this experiment, there were no published accounts in which video playback had been presented to cephalopods. We therefore optimized every parameter that has been shown to be important in previous studies with other taxa to increase the probability that subjects would respond appropriately to video stimuli (for reviews, see D'Eath, 1998; Fleishman and Endler, 2000; Zeil, 2000). This included using high-definition stimuli broadcast at 50 frames s⁻¹. As cephalopods have excellent spatial acuity (Hanlon and Messenger, 1996) and high flicker fusion rates of approximately 60 Hz (Hamasaki, 1968), these parameters were probably critical for creating realistic stimuli. In addition, we broadcast stimuli on an LCD monitor. Unlike conventional CRT monitors, the pixels on LCD monitors do not flash on and off between screen refreshes but, rather, update themselves without turning off, which prevents known problems associated with refresh-induced flicker (D'Eath, 1998). However, LCD monitors use polarizing filters that cause each pixel to be polarized in a different way. Although octopuses use polarized light patterns when interacting with predators, prey and conspecifics (Shashar and Cronin, 1996; Shashar et al., 2000), potential polarization artifacts associated with our LCD monitor did not prevent subjects from responding appropriately to video stimuli. Nevertheless, polarization is an important factor to consider in future video playback studies with cephalopods. Finally, we avoided artifacts associated with interlaced video (e.g. ghosting or jagged and distorted images) by converting our stimuli to progressive scan video before presenting them to subjects.

Personality

Individuals behaved consistently across the three experimental contexts (conspecific, foraging, novel object) on any given test day, which was reflected by strong correlation coefficients and high measures of repeatability (Tables 2–4). For example, an individual that aggressively attacked the crab stimulus on day 1 (i.e. quick approach, high levels of movement, touched the stimulus many times) was also relatively aggressive towards the conspecific and the novel object on day 1. The gloomy octopus therefore satisfies part of our definition of personality, which is that interindividual differences in behaviour are consistent across multiple contexts (Biro and Stamps, 2008; Kurvers et al., 2009).

In marked contrast, none of the personality traits were repeatable over time (Table 5). An individual that was bold, aggressive and exploratory on day 1 was just as likely to have been shy, submissive and stationary in the same context on day 2. Although personality traits can change with time or experience, the relative ranks of individuals should remain the same (Réale et al., 2007). As subjects did not meet this requirement, they violated a key requirement of personality, which is that personality traits are consistent over time. This suggests that the gloomy octopus does not have personality but, rather, that it has an 'episodic' personality.

The negative results obtained from the repeatability analysis were not simply a result of insufficient statistical power. Indeed, power analysis revealed that a sample of only 24 individuals would be necessary to detect a moderate effect (correlation coefficient of 0.7) with a power of 0.8 (two-way test, $\alpha=0.05$). Furthermore, our comparisons of behaviour across contexts within the same day involved precisely the same sample of subjects, yet all of the 18 correlations in that analysis were statistically significant (Tables 2–4). In comparison, none of the 18 correlations in the repeatability analysis were statistically significant (Table 5), suggesting that octopus behaviours were genuinely inconsistent over multiple test days.

Low repeatability also cannot be attributed readily to our experimental design. For example, a possible explanation for episodic personality is that subjects' motivational states varied from one day to the next, perhaps as a function of varying levels of hunger. This seems unlikely, however, because behaviours expressed in each experimental context on any given day were corrected for baseline behaviour observed in the control treatment on that same day. Finally, low repeatability can occur when the environment in which the repeated measures are obtained is highly variable (Réale et al., 2007). Again, this seems unlikely, as our test apparatus and experimental stimuli were stringently controlled.

Some previous studies of personality have shown that individuals' behaviours are correlated only in certain contexts and only over certain periods of time. For example, it has been shown that octopus' behaviour in response to alerting, threatening and feeding tests could be reduced into principal components that represented broader dimensions of personality but, as in our study, these were not consistent over the 2 week test period (Mather and Anderson, 1993). Similarly, adult dumpling squid displayed context-specific personality traits during feeding and threat tests, but these traits were repeatable only in the threat tests (Sinn and Moltshaniwskyj, 2005). Furthermore, over the entire lifetime of the dumpling squid, personality traits were consistent both before (up to 9 weeks of age) and after sexual maturation (after 16 weeks of age) (Sinn et al., 2007). But, during sexual maturation, from 9 to 12 weeks of age, the degree of consistency varied significantly among different personality types (Sinn et al., 2007). Finally, in stickleback (*Gasterosteus aculeatus*), personality type also decouples over time. Similar to the gloomy octopus, the stickleback may be aggressive, bold and active at one point in time but later in life the same individual may express a completely different personality (Bell and Stamps, 2004). Thus, some species show stability of personality over time, whereas others show no stability over time, or stability only in certain life phases.

There are a variety of mechanisms that could underlie the short-term behavioural correlations observed in our study. For example, octopuses have an advanced neural system, which is reflected by their capacity for associative learning and their long-term memory in both visual and tactile tasks (Hanlon and Messenger, 1996; Hochner, 2008). This large neuronal capacity may afford octopuses considerable behavioural flexibility that allows them to change their behaviour adaptively over time. Alternatively, short-term behavioural correlations may result from a physiological process, such as the release of hormones that affect multiple behaviours (Ketterson and Nolan, 1999). Activational effects of hormones are generally short term, as they are more easily altered by modifications of hormonal pathways (Elekonich and Robinson, 2000). Consequently, behavioural correlations that have an endocrine basis can also have a short duration (Sih et al., 2004). For example, a chemosensory cue – most likely a pheromone – elicits strong aggressive behaviour in longfin shore squid (*Loligo pealeii*) (Buresch et al., 2003).

Gloomy octopuses live in highly heterogeneous environments that fluctuate over both spatial and temporal scales (Anderson, 1997). According to the adaptive plasticity hypothesis, these conditions may select for behavioural flexibility, as individuals could then optimize their behaviour in a variety of typical environmental conditions (Van Buskirk, 2002). For example, behaving shyly might be an individual's optimal response when it is being threatened by a predator, whereas behaving boldly might be that same individual's optimal response in a foraging context a short time later. However, complete behavioural plasticity can be costly because of the

energetic costs associated with the requisite sensory and regulatory mechanisms (for a review, see DeWitt et al., 1998). A species' degree of behavioural flexibility may hence reflect a trade-off between the associated costs and benefits, which, in turn, may be affected by the volatility of that species' environmental conditions. For the gloomy octopus, it may be advantageous to forgo plasticity over short periods of time in which environmental conditions are relatively constant. In the lab, this period of time might correspond to the brief duration of our trials on any particular test day, which might explain why behaviours were correlated across contexts but not across test days.

Conclusion

Our study provides the first evidence that video playback can elicit biologically appropriate responses from a cephalopod. Given the importance and the complexity of visual signalling in this group, we believe that video playback will be a valuable tool for studying a wide range of cephalopod behaviours, including visual communication, learning, and social and reproductive behaviour. In addition, we showed that subjects' responses to one functionally important class of stimuli predicted their responses to other important classes of stimuli on the same test day. However, subjects' responses during our relatively short test period were inconsistent from one day to the next, which suggests that the gloomy octopus has an episodic personality.

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REFERENCES

- Adamo, S. A. and Hanlon, R. T. (1996). Do cuttlefish (Cephalopoda) signal their intentions to conspecifics during agonistic encounters? *Anim. Behav.* **52**, 73-81.
- Anderson, T. J. (1997). Habitat selection and shelter use by *Octopus tetricus*. *Mar. Ecol. Prog. Ser.* **150**, 137-148.
- Aronson, R. B. (1986). Life history and den ecology of *Octopus briareus* Robson in a marine lake. *J. Exp. Mar. Biol. Ecol.* **95**, 37-56.
- Bell, A. M. and Stamps, J. A. (2004). Development of behavioural differences between individuals and populations of sticklebacks, *Gasterosteus aculeatus*. *Anim. Behav.* **68**, 1339-1348.
- Biro, P. A. and Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends Ecol. Evol.* **23**, 361-368.
- Blumstein, D. T., Daniel, J. C., Griffin, A. S. and Evans, C. S. (2000). Insular tamar wallabies (*Macropus eugenii*) respond to visual but not acoustic cues from predators. *Behav. Ecol.* **11**, 528-535.
- Bradbury, J. W. and Vehrencamp, S. L. (1998). *Animal Communication*. Sunderland: Sinauer Associates.
- Brown, C., Jones, F. and Braithwaite, V. A. (2007). Correlation between boldness and body mass in natural populations of the poeciliid *Brachyrhaphis episcopi*. *J. Fish Biol.* **71**, 1590-1601.
- Buresch, K. C., Boal, J. G., Knowles, J., Debose, J., Nichols, A., Erwin, A., Painter, S. D., Nagle, G. T. and Hanlon, R. T. (2003). Contact chemosensory cues in egg bundles elicit male-male agonistic conflicts in the squid *Loligo pealeii*. *J. Chem. Ecol.* **29**, 547-560.
- Byrne, R. A., Kuba, M. J. and Meisel, D. V. (2004). Lateralized eye use in *Octopus vulgaris* shows antisymmetrical distribution. *Anim. Behav.* **68**, 1107-1114.
- Dawkins, M. S. (1996). Distance and social recognition in hens: implications for the use of photographs as social stimuli. *Behaviour* **133**, 663-680.
- D'Eath, R. B. (1998). Can video images imitate real stimuli in animal behaviour experiments? *Biol. Rev.* **73**, 267-292.
- DeWitt, T. J., Sih, A. and Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* **13**, 77-81.
- Elekovich, M. M. and Robinson, G. E. (2000). Organizational and activational effects of hormones on insect behavior. *J. Insect Physiol.* **46**, 1509-1515.
- Evans, C. S. and Marler, P. (1991). On the use of video images as social stimuli in birds: audience effects on alarm calling. *Anim. Behav.* **41**, 17-26.
- Fleishman, L. J. and Endler, J. A. (2000). Some comments on visual perception and the use of video playback in animal behavior studies. *Acta Ethol.* **3**, 15-27.
- Fleishman, L. J., McClintock, W. J., D'Eath, R. B., Brainard, D. H. and Endler, J. A. (1998). Colour perception and the use of video playback experiments in animal behaviour. *Anim. Behav.* **56**, 1035-1040.
- Frost, A. J., Winrow-Giffen, A., Ashley, P. J. and Sneddon, L. U. (2006). Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proc. R. Soc. B.* **274**, 333-339.
- Hamasaki, D. I. (1968). The electroretinogram of the intact anesthetized octopus. *Vision Res.* **8**, 247-258.
- Hanlon, R. T. and Messenger, J. B. (1996). *Cephalopod Behaviour*. Cambridge, UK: Cambridge University Press.
- Hemmi, J. M. and Zeil, J. (2003). Burrow surveillance in fiddler crabs. I. Description of behaviour. *J. Exp. Biol.* **206**, 3935-3950.
- Herzog, H. A. J. and Burghardt, G. M. (1974). Prey movement and predatory behaviour of juvenile western yellow-bellied racers *Coluber constrictor mormon*. *Herpetologica* **30**, 285-289.
- Hochner, B. (2008). Quick guide: octopuses. *Curr. Biol.* **18**, R897-R898.
- Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scan. J. Stat.* **6**, 65-70.
- Ketterson, E. D. and Nolan, V. Jr (1999). Adaptation, exaptation, and constraint: a hormonal perspective. *Amer. Nat.* **154**, S4-S25.
- Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., De Jong, I. C., Ruis, M. A. W. and Blokhuis, H. J. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neurosci. Biobehav. Rev.* **23**, 925-935.
- Kurvers, R. H. J. M., Eijkelenkamp, B., van Oers, K., van Lith, B., van Wieren, S. E., Ydenberg, R. C. and Prins, H. H. T. (2009). Personality differences explain leadership in barnacle geese. *Anim. Behav.* **78**, 447-453.
- Langridge, K. V. (2009). Cuttlefish use startle displays, but not against large predators. *Anim. Behav.* **77**, 847-856.
- Lessells, C. M. and Boag, P. T. (1987). Unrepeatable repeatabilities: a common mistake. *Auk* **104**, 116-121.
- Maldonado, H. (1964). The control of attack by *Octopus*. *J. Comp. Physiol. A* **47**, 656-674.
- Mather, J. A. and Anderson, R. C. (1993). Personalities of octopuses (*Octopus rubescens*). *J. Comp. Psychol.* **107**, 336-340.
- McGuire, M. T., Raleigh, M. J. and Pollack, D. B. (1994). Personality features in vervet monkeys: The effects of sex, age, social status, and group composition. *Am. J. Primatol.* **33**, 1-13.
- Moynihhan, M. and Rodaniche, A. F. (1982). The behavior and natural history of the Caribbean reef squid *Sepioteuthis sepioides*: with a consideration of social, signal and defensive patterns for difficult and dangerous environments. *Adv. Ethol.* **25**, 1-5.
- Norman, M. D. and Reid, A. (2000). *A Guide to the Squid, Cuttlefishes and Octopuses of Australasia*. Melbourne: CSIRO Publishing.
- Ord, T. J., Peters, R. A., Evans, C. S. and Taylor, A. J. (2002). Digital video playback and visual communication in lizards. *Anim. Behav.* **63**, 879-890.
- Otte, D. (1974). Effects and functions in the evolution of signaling systems. *Annu. Rev. Ecol. Syst.* **5**, 385-417.
- Packard, A. (1963). The behaviour of *Octopus vulgaris*. *Bull. Inst. Oceanogr. (Monaco)* **No. 1 D**, 35-49.
- Povinelli, D. J., Gallup, G. G. J., Eddy, T. J., Bierschwale, D. T., Engstrom, M. C., Perilloux, H. K. and Toxopeus, I. B. (1997). Chimpanzees recognize themselves in mirrors. *Anim. Behav.* **53**, 1083-1088.
- Réale, D., Reader, S. M., Sol, D., McDougall, P. T. and Dingemans, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biol. Rev.* **82**, 291-318.
- Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223-225.
- Rosenthal, G. G., Evans, C. S. and Miller, W. L. (1996). Female preference for dynamic traits in the green swordtail, *Xiphophorus helleri*. *Anim. Behav.* **51**, 811-820.
- Shashar, N. and Cronin, T. W. (1996). Polarization contrast vision in *Octopus*. *J. Exp. Biol.* **199**, 999-1004.
- Shashar, N., Hagan, R., Boal, J. G. and Hanlon, R. T. (2000). Cuttlefish use polarization sensitivity in predation on silvery fish. *Vision Res.* **40**, 71-75.
- Sih, A., Bell, A. M., Johnson, J. C. and Ziemba, R. E. (2004). Behavioral syndromes: an integrative overview. *Quart. Rev. Biol.* **79**, 241-277.
- Sinn, D. L. and Moltchanivskyj, N. A. (2005). Personality traits in dumping squid (*Euprymna tasmanica*): context-specific traits and their correlation with biological characteristics. *J. Comp. Psychol.* **119**, 99-110.
- Sinn, D. L., Apiolaza, L. A. and Moltchanivskyj, N. A. (2006). Heritability and fitness-related consequences of squid personality traits. *J. Evol. Biol.* **19**, 1437-1447.
- Sinn, D. L., Gosling, S. D. and Moltchanivskyj, N. A. (2007). Development of shy/bold behaviour in squid: context-specific phenotypes associated with developmental plasticity. *Anim. Behav.* **75**, 433-442.
- Tinbergen, N. (1951). *The Study of Instinct*. Oxford: Oxford University Press.
- Van Buskirk, J. (2002). A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. *Amer. Nat.* **160**, 87-102.
- Warren, L. R., Scheier, M. F. and Riley, D. A. (1974). Colour changes of *Octopus rubescens* during attacks on unconditioned and conditioned stimuli. *Anim. Behav.* **22**, 211-219.
- Zeil, J. (2000). Depth cues, behavioural context, and natural illumination: some potential limitations of video playback techniques. *Acta Ethol.* **3**, 39-48.