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Behaviour 152 (2015) 1871–1881

Behaviour
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Liquid sand burrowing and mucus utilisation as novel adaptations to a structurally-simple environment in *Octopus kaurna* Stranks, 1990

Jasper Montana [a,*], **Julian K. Finn** [b] **and Mark D. Norman** [b]

[a] Department of Zoology, University of Melbourne, Parkville, VIC 3010, Australia
[b] Department of Sciences, Museum Victoria, GPO Box 666, Melbourne, VIC, Australia
*Corresponding author's e-mail address: jasper.montana@alumni.unimelb.edu.au

Accepted 11 August 2015; published online 11 September 2015

Abstract

Cephalopods are often celebrated as masters of camouflage, but their exploitation of the soft-sediment habitats that dominate the ocean floor has demanded other anti-predator strategies. Previous research has identified a small number of cephalopods capable of burying into sand and mud, but the need to directly access the water column for respiration has restricted them to superficial burying. Here, we report on the first known sub-surface burrowing in the cephalopods, by *Octopus kaurna*, a small benthic species that uses advanced sand-fluidisation and adhesive mucus for sediment manipulation. This burrowing strategy appears linked to easily fluidised sediments as shown in experimental trials in three size-grades of sediment. While the selective pressures that drove evolution of this behaviour are unknown, its identification enriches our understanding of the possible life-history traits and functional role of mucus in other benthic octopus species living in soft-sediment environments.

Keywords

Octopus, burrowing, burying, cephalopod, fluidisation, mucus.

1. Introduction

Camouflage has become an emblematic behaviour of cephalopods (Hanlon et al., 2009); however, this strategy for predator avoidance and escape is often less suited to the structurally-simple habitats that dominate the seafloor (Merilaita, 2003; Hanlon, 2007). Recent studies have revealed that the exploitation of soft-sediment environments has led to some remarkable anti-predator strategies in cephalopods, including mimicry in *Thaumoctopus*

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DOI 10.1163/1568539X-00003313

mimicus (mimic octopus; Norman & Hochberg, 2005) and the construction of mobile armour in *Amphioctopus marginatus* (Finn et al., 2009). Here, we report on the first known sub-surface burrow-formation in the cephalopods, by *Octopus kaurna* Stranks, 1990 (Stranks, 1990): a small, benthic and nocturnal octopus endemic to the sand plains of south-eastern Australia, which uses rapid sediment-fluidisation and adhesive mucus for sediment manipulation.

Burying and burrowing are common life strategies in soft-sediments (Trueman & Ansell, 1969). The two behaviours are considered distinct: burying being the superficial covering with sediment; and burrowing being the active movement through soft substrates, in which grains are displaced and the surrounding material structure is often altered (see Dorgan, 2015). Animals that dig, burrow and disturb sediments and soils are considered to be ecosystem engineers, whose burying and burrowing mechanisms are determined by both their morphology and the mechanical properties of the sediments on which they reside (Jones et al., 1997; Dorgan, 2015).

Despite the abundance of burying and burrowing species, only a small number of cephalopods exhibit these behaviours (Hanlon & Messenger, 1996; von Boletzky, 1996). To date, the most comprehensive study of burying in Cephalopoda identified a single burying mechanism for octopuses (von Boletzky, 1996): the manual displacement of sediment by sweeping movements of the arms and suckers (e.g. *Octopus berrima* Stranks and Norman, 1992, see Video 1 in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x). This mechanism has also been reported anecdotally in ethology studies of other octopuses (e.g. Huffard, 2007) and is commonly considered to be the sole means of movement into the sediment in octopuses (Watling & Thiel, 2013, p. 289). The current paper sets out to investigate and describe an alternative burrowing mechanism in octopuses.

Initial observations suggested that the Southern Sand Octopus, *O. kaurna*, burrows using a highly specialised sediment-fludisation technique, akin to quicksand formation. Sediment fluidisation has been reported in a number of burrowing animals (see Dorgan, 2015). This species also showed apparent sub-surface burrow formation, utilising mucus to manipulate the sediment. This is the first detailed account of this burrowing strategy in cephalopods, which were previously understood to be limited to superficial burying to maintain direct contact with the water column for respiration (von Boletzky, 1996). This behaviour also emphasises the functional role of mucus in the cephalopods, which has previously been considered of limited significance compared with other molluscs (Davies & Hawkins, 1998). Here, we describe this novel behaviour and use experimental burrowing trials in size-graded sediment to examine the favourable sedimentary conditions for this animal sediment relationship (Snelgrove & Butman, 1994).

2. Materials and methods

2.1. Burrowing mechanism

Live specimens of adult *O. kaurna* (12–53 g wet weight) were observed on SCUBA and collected from shallow-water (1–4 m) sand plains at Rye (38°22′S, 144°49′E), Australia between March and August 2008. Observations were made between 18:00 and 23:00 h and recorded on an HDV camcorder.

The burrowing mechanism of *O. kaurna* was documented in situ for 11 individuals following a standardised disturbance event: foraging octopuses were captured in plastic jars with mesh lids, held for 30 s, released and their behaviour observed. Including the above, a total of 24 octopuses were captured over the period of research and removed to the closed-circulation aquarium facilities at the University of Melbourne, Melbourne, Australia (14 ± 2°C, 36.1–43.0 ppt, pH 7.7–8.2). Octopuses were kept in holding tanks ($35 \times 18 \times 20$ cm) with washed and sieved (<2 mm) field-site sediment of 15 cm depth, fed live crabs daily and were acclimatised for a two-day period prior to the commencement of treatments.

To examine the subsurface behaviour, five of these captive octopuses were sequentially placed in a specially-designed ‘ant-farm’ aquarium ($45 \times 4 \times 30$ cm) in which the octopuses burrowed against the glass. Here, the subsurface behaviour was observed and the extent of mucus penetration into the sediment was roughly determined by directing flowing water at the sediment surface to dislodge loose sand, thus leaving only the sand captured by the mucus behind.

2.2. Burrowing rate

Sediment was collected from the field site, as well as from a nearby coarse sand beach (Mount Martha, 38°16′S, 145°00′E). These were divided by sieves into three sediment grades based on the Wentworth Scale (Wentworth, 1922): 'fine-to-medium' (<500 μm), 'coarse' (500–1000 μm) and 'very coarse' (1000–2000 μm).

Burrowing rate trials were carried out between 10:00 and 17:00 h in three experimental aquaria (45 × 20 × 25 cm). Each contained 20 cm of treatment sediment, which was stirred and allowed to settle five minutes prior to use to ensure saturation and aeration. Burrowing trials were recorded on videotape and burrowing time was determined to the nearest video frame for conversion to seconds. Burrowing was considered to begin when *O. kaurna* directed its siphon downwards and fully expanded its mantle in preparation for expelling the first jet of water into the sediment and ended when the apex of the mantle had reached the level of the sediment. The wet weights of the octopuses were recorded and their burrowing times standardised into the Burrowing Rate Index (BRI $= (\frac{\sqrt[3]{\text{Wet weight}}}{\text{Burrowing time}}) \times 10^2$; from Stanley, 1970; reviewed in Brown & Trueman, 1994). Of the 24 captive octopuses, twelve octopuses completed all three trials and each was trialled in one randomly assigned treatment per day. A Related-Samples Friedman's Two-Way Analysis of Variance by Ranks determined if there was a statistically significant difference in BRI across treatments and post-hoc pairwise comparisons, with Bonferroni correction for multiple comparisons, was used to identify significant differences.

3. Results

3.1. Burrowing mechanism

Burrowing in *O. kaurna* can be divided into four discrete stages (Figure 1; and Video 2 in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x):

1. The injection of water into the sediment by jetting action of the mantle and siphon. The octopus remains at the sediment surface with arms out-stretched, while the underlying sand grains become 'fluidised' (temporarily suspended in water; Figure 1a).

2. The octopus moves its arms into the fluidised sediment, while maintaining its mantle and siphon above the surface to continue the jetting action. Once the underlying sediment is sufficiently fluidised, the octopus pulls the remainder of its body under the surface (Figure 1b).

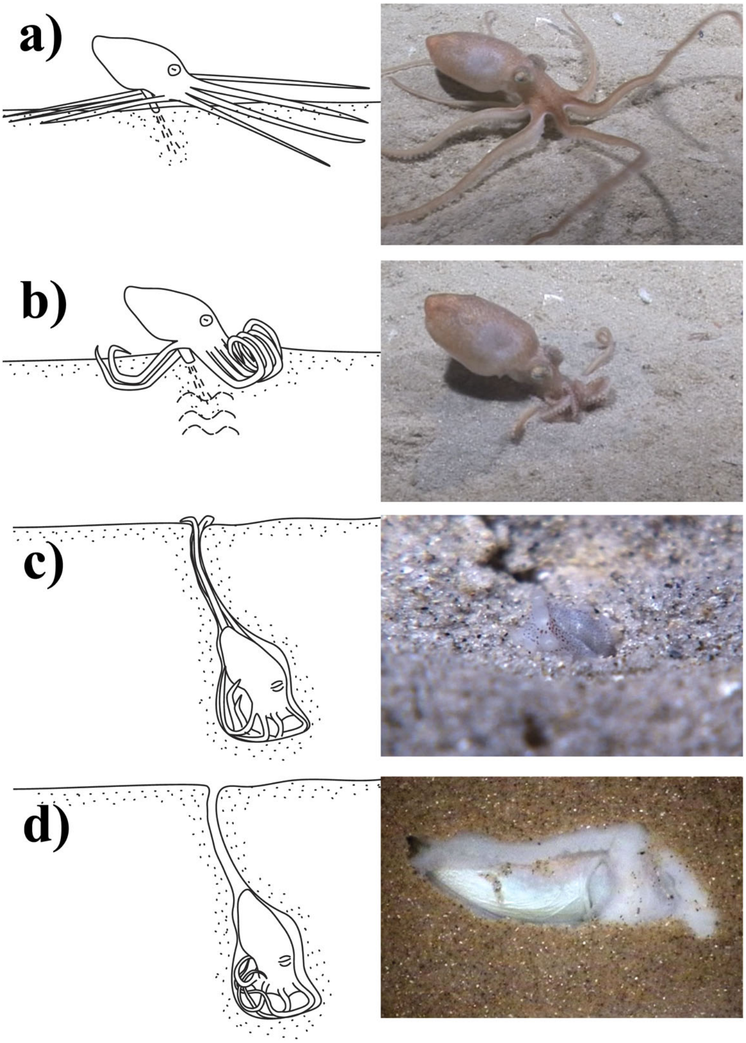

Figure 1. Drawings and photographs of burrowing in *O. kaurna*: (a) *Octopus kaurna* injects water into the sediment; (b) sediment becomes fluidised and the octopus moves downwards; (c) *O. kaurna* forms a mucus-lined sub-surface cavity and respiratory chimney; (d) arms are retracted and normal ventilation commences. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x.

3. Once under the surface, the octopus uses its arms and expanded mantle to push the surrounding sediment away from its body. The octopus then

extends two arms to the surface, in order to create a ventilation shaft or chimney, through which it can respire (Figure 1c).

4. Finally, the arms are retracted back into the burrow and a strong exhalation pushes any loose sand out of the chimney. The octopus begins to ventilate as usual (Figure 1d).

Burrows observed in the 'ant-farm' aquarium (3–11 cm deep at top of burrow, $N = 5$) typically had one chimney (in one case there were two) to the sediment surface and one burrow possessed a radiating mucus-lined shaft where the arm had been extended out into the surrounding sand. The dislodgement of loose sand by water from the aquarium outlet hose revealed mucus penetration up to 2 cm from the chimney and burrow walls.

Observations in the field found that even after considerable excavation, *O. kaurna* could remain concealed beneath the sediment and reconstruct its burrow and chimney some minutes later by pushing the tips of two arms up to the surface. On occasion, *O. kaurna* would encounter subsurface obstructions, such as shell rubble or seagrass root masses, which made sediment fluidisation difficult. In these cases, *O. kaurna* shifted to the mechanical burying mechanism reported previously in other octopuses (von Boletzky, 1996). *Octopus kaurna* was also observed to enter pre-existing burrows of infaunal worms, without the need to completely fluidise the sediment. Fluidising behaviour caused vertical mixing of the sediment, with darkened anoxic sand being brought to the surface during burrowing.

3.2. Burrowing Rate Index (BRI)

Under experimental conditions, Burrowing Rate Index values were statistically significantly different across the three sediment treatments ($\chi^2 = 12.667$, $p < 0.005$). Median BRI values are presented in Figure 2. BRI was statistically significantly different in the 'very coarse' (median = 4.72) sediment compared with both the 'coarse' (median = 9.52, $p < 0.05$) and 'fine-to-medium' (median = 14.89, $p < 0.005$) sediments, although there was no significant difference between the latter two ($p > 0.65$).

4. Discussion

4.1. Burrowing mechanism

The advanced fluidisation of sediment observed in *O. kaurna* is a novel burrowing strategy among cephalopods. In contrast to the sediment displace-

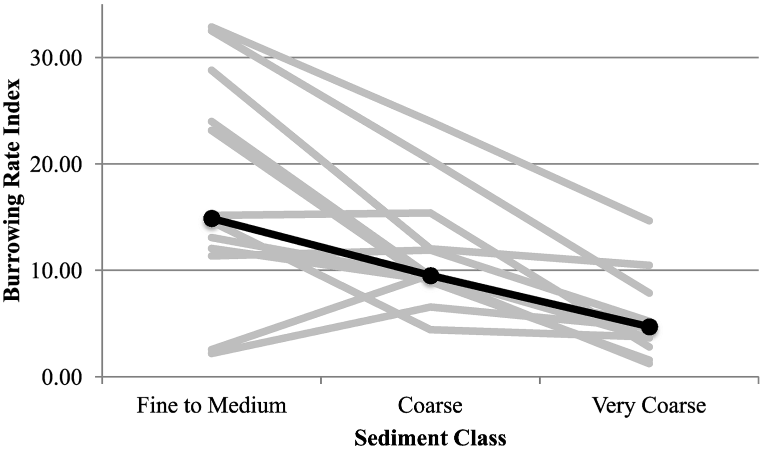

Figure 2. Burrowing Rate Index of *Octopus kaurna* showing medians (black line: 14.89, 9.52 and 4.72, respectively) and individual octopuses (grey lines) in three experimental sediment classes: 'fine-to-medium' (<500 μm); 'coarse' (500–1000 μm); and 'very coarse' (1000–2000 μm) sand.

ment reported in *Sepia* and *Sepiola* (von Boletzky & von Boletzky, 1970; Mather, 1986), which utilize water jets to excavate a depression at the sediment surface, *O. kaurna* injects water some centimetres into the sediment. Sediment fluidisation functions as a burrowing mechanism when the sand particles surrounding an organism are temporarily suspended following an increased pressure in the pore fluid (Dorgan, 2015). Following early reports in some bivalve species (e.g., Trueman, 1967), the metabolic implications of sediment fluidisation are more recently becoming apparent. The reduction of drag provided by sediment fluidisation has been shown to greatly increase burrowing effectiveness compared to that expected from muscle strength in the bivalve *Ensis directus* and to reduce the energy expenditure required for burrowing at depth (Winter et al., 2012, 2014). It is also likely to facilitate rapid burial and metabolic savings in *O. kaurna* under preferential sedimentary conditions.

Burrow formation in *O. kaurna*, which creates a sub-surface cavity in the sediment using mucus to line the burrow walls, can also be considered distinct from the superficial-burying and den-living previously reported in other cephalopods (Hanlon & Messenger, 1996; von Boletzky, 1996). Chimney-formation allows *O. kaurna* to attain a much greater depth in the sediment than burying cephalopods while still maintaining access to open water for respiration. Deeper burial has been known to reduce detection by predators (Snelgrove, 1999) and provide metabolic savings (von Boletzky, 1996) in other species. Although little research has been conducted on mucus use in cephalopods, observations of burrow-formation by *O. kaurna* and examples of functional mucus use in other orders of cephalopod (von Byern & Klepal, 2006; Liao & Lu, 2009) highlight the valuable, yet underappreciated role of mucus in this group. Histochemical and ultrastructure analysis might further reveal the type and location of mucous glands involved in this burrow-forming behaviour.

4.2. Burrowing rate

The efficiency of burrowing by sand-fluidisation observed for *Octopus kaurna* was limited by sedimentary conditions in captive experimental trials. *Octopus kaurna* burrowed slowest in the very coarse sediment because the larger grain size is likely to increase the fluid drag required to keep sand grains suspended, requiring more effort in fluidisation. The low shear strength of course sediment would also make it less suited to fluidisation and more suited to manual excavation. It is therefore likely that sedimentary conditions offering finer grained sediment that is more easily fluidised may favour the burrowing strategy shown by *O. kaurna*. While sediment grain size alone should not be seen as the categorical determinant of infaunal species distribution (Snelgrove & Butman, 1994), studying the sediment characteristics that facilitate burrowing by *O. kaurna* can assist our understanding of the environmental conditions that may have led to its selection.

While the original selective pressures of sub-surface burrowing behaviour in *O. kaurna* are unknown, considerations should extend beyond predation. The observation of *O. kaurna* entering pre-existing worm burrows, for example, might indicate the selective advantage of accessing a potentially abundant unexploited food source. As proposed in the evolution of elongation in snakes, selective pressures may also act concurrently on morphology (Gans, 1975).

Burrowing by sediment fluidisation and the formation of a mucus-lined subsurface burrow, shown in *O. kaurna*, broadens our understanding of the scope of life histories of benthic octopuses and provides insight into the possible ecology of other sand-associated species, whose burying or burrowing behaviour is as-yet unknown (e.g. Voight, 1994; Norman, 2001; Hanlon et al., 2008; Liao & Lu, 2009).

Acknowledgements

The authors thank John Ahern of the University of Melbourne for maintenance of the aquarium facilities and David L. MacMillan for comments on this research. This study was carried out under approval of the Animal Ethics Committee of the Faculties of Science and Land & Food Resources, The University of Melbourne. Animals collected under Permit of the State Government of Victoria. This research was financially supported by a Museum Victoria 1854 Student Scholarship.

Supplementary material

Video 1. Burying by manual excavation in *Octopus berrima*. *Octopus berrima* displays a more typical octopus burying mechanism using the sweeping motion of the arms and suckers. QuickTime Movie, Codec H.264.

Video 2. Burrowing by sediment fluidisation in *Octopus kaurna*. *Octopus kaurna* burrows by injecting water into the sediment to create a pocket of fluidised sand and a mucus lined burrow. QuickTime Movie, Codec H.264.

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