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Tri-axial accelerometers tease apart discrete behaviours in the common cuttlefish *Sepia officinalis*

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Acceleration data loggers can be used to construct time–energy budgets or identify specific behaviours in free living animals. Within a marine context such devices have been largely deployed on vertebrates with comparatively little attention paid to commercially important invertebrates such as cephalopod molluscs. Here we tested the utility of tri-axial accelerometers to tease apart six discrete behaviours in the common cuttlefish Sepia officinalis. *By considering depth profiles in conjunction with body pitch and roll and overall dynamic body acceleration we were able to make distinctions between resting at the seabed, active swimming, mating, post-coital panting and active manoeuvring along the seabed.*

Keywords: cephalopod, tri-axial accelerometer, behaviour, overall dynamic body acceleration, data loggers

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Cuttlefish are attracting increased attention from commercial fisheries and ecologists alike (Piatkowski *et al.*, 2001) yet behavioural studies are largely limited to direct observation and captive trials with almost no data available for free-ranging animals (Aitken *et al.*, 2005). Acceleration data loggers are emerging as an important tool in answering previously intractable questions, providing data on body orientation (e.g. Shepard *et al.*, 2008) and overall dynamic body acceleration (ODBA) (Wilson *et al.*, 2006), which can be used to identify behaviours (e.g. Shepard *et al.*, 2008) and allude to energy expenditure (Halsey *et al.*, 2009), respectively, in free-living animals. A recent study by Payne *et al.* (2011) was the first to use accelerometers to explore the behaviour of cephalopods, providing valuable insights into energy expenditure patterns of giant Australian cuttlefish (*Sepia apama*) during the breeding season. This study did not identify specific behaviours, however, because the accelerometers measured in two, of the three dimensional, axes and transmitted the data acoustically, requiring substantial data compression and precluding the high resolution acceleration data necessary for the identification of specific behaviours. Accelerometers that store, rather than transmit, data are not bound by such technological constraints but necessitate that devices be recovered. Recovery in the marine environment is challenging but not unrealistic and has been successfully completed in a range of species (e.g. Houghton *et al.*, 2009; Gleiss *et al.*, 2011). Working from this premise, we tested the extent to which high resolution acceleration data from storage tags could be used to identify discrete behaviours in the common cuttlefish *Sepia officinalis*.

For the pilot study wild cuttlefish (N = 10) were collected off the coast of Piran, Slovenia (45°31′41″N 13°34′05″W) via SCUBA on 5 November 2009. Post-capture, individuals were placed into flow-through tanks with either a sand or gravel bottom and left in an undisturbed laboratory with windows (allowing for normal photoperiod). The cuttlefish were allowed to acclimatize for four days and fasted for 72 hours prior to anaesthesia and data logger attachment.

Prior to experimentation, the acceleration data-loggers (G6A, CEFAS Technologies Limited, Lowestoft, UK) were programmed to record depth and acceleration at 30 Hz. The three orthogonal acceleration channels corresponded to the dorsal–ventral (up and down = heave), anterior–posterior (backwards and forwards = surge) and lateral (side to side = sway) axes. Temperature was recorded at the same frequency to minimize temperature-dependent energetic expenditure (Einstipp *et al.*, 2011). At the start of the experiment, the largest cuttlefish (13.5 cm mantle length, 282 g) was lifted from the holding tank into a transfer tank and anaesthetized in a freshly prepared 0.2 mol l^{-1} $MgCl_2$ solution (Messenger *et al.*, 1985) at 15°C for 110 seconds (until the collar flaps stopped moving and there was no response to touch). The other animals were subsequently excluded from having data loggers attached because of their small size, although they were used to investigate interactions between individuals.

Post-anaesthesia, the cuttlefish was removed from the water and two small incisions made in the skin overlying the dorsal anterior area of the cuttlebone. Following on from the methods of Aitken *et al.* (2005) the skin was held back with tweezers and the surface of the bone washed with 70% ethanol before two holes were drilled down to ~4 mm

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using a Dremel tool with a 0.9 mm drill bit. The acceleration data-logger was attached to the cuttlebone using two steel BZP self-tapper screws (No. 0 × 9.5 mm with long pozi head) and secured using a single drop of superglue in each screw hole. When the animal had been checked and appeared to be unaffected by the anaesthetic or the presence of the device, it was transferred to the aquarium. At the aquarium, underwater video monitoring was carried out concurrently to allow accelerometer readings to be attributed to specific behaviours.

During the tank trials we focused on six key behaviours: (1) active swimming; (2) resting (i.e. sitting on the bottom, but not buried); (3) mating (involving an initial contact grasp/lock with another cuttlefish and a small associated manoeuvring until separation); (4) post-coital panting (again resting on the bottom but this time with an increased rate of mantle ventilation); (5) burying in sand; and (6) manoeuvring across rocky substrate. Four separate deployments (D1–D4) over four days yielded >25 hours tri-axial accelerometry, temperature and depth data and >17 hours video footage. Attachment periods varied between a minimum of 270 minutes (D2) and a maximum of 495 minutes (D1). Data-logger runs did not commence prior to 13:45 nor continue past 22:00 local time to minimize circadian influences.

For the analysis we selected 30 seconds periods from the raw acceleration data that were indicative of the six pre-defined behaviours (validated against time-stamped video footage) and so that water temperature remained constant across all behaviours (i.e. total variance $<0.03^{\circ}$C). Burying behaviour only occurred in very short bursts lasting 3–7 seconds which required several events to be combined to make up the 30 seconds period. For the other five behaviours, the accelerometer traces were sub-sampled using a random number generator from more protracted behaviours that exceeded 30 seconds.

Analysis followed Houghton *et al.* (2000, 2002) who noted that two dimensional dive profiles in sea turtles (depth versus time) can sometimes lead to the incorrect classification of particular behaviours (e.g. foraging or resting at the seabed). Similarly, the fine-scale manoeuvrability and buoyancy regulation of cuttlefish leads to a scenario whereby (1) 'hovering' in the water column, (2) actively swimming in a horizontal plane or (3) resting on the seabed could theoretically generate similar 'flat-bottomed' dive profiles that suggest a minimal change in depth over time. To test whether accelerometry could aid the identification and discrimination of such behaviours, we considered changes in depth, pitch and roll for active swimming and resting at the seabed (Figure 1). Pitch and roll data were calculated through arcsine transformation of the static surge and heave acceleration signals (obtained by fitting a running mean of 2 seconds through the raw acceleration traces (Shepard *et al.*, 2008)). Kolmogorov–Smirnov tests revealed that depth and body orientation data were not normally distributed ($P > 0.05$; prior to, and following, transformation) so non-parametric tests were adopted. Wilcoxon signed rank tests revealed no significant differences in Δ depth (cm.sec^{-1}) ($P > 0.05$) between the two behaviours whilst the pitch and roll data ($^{\circ}$) revealed marked dissimilarities (test score (pitch) = 5.69, $P < 0.001$, N = 900; test score (roll) = 25.99, $P < 0.001$, N = 900).

Next, ODBA was calculated for the six sub-sampled behaviours using methods described in Qasem *et al.* (2012). Briefly, the residual variation at any moment in time between static acceleration and total acceleration (i.e. the raw value recorded by the accelerometer) is the 'dynamic' component of acceleration. OBDA is simply the sum total of dynamic acceleration in all three axes. Differences in ODBA between the six behaviours were again tested using non-parametric tests (related samples Friedman's test) following attempts at transformation (Figure 1). This analysis revealed that the distribution of acceleration readings varied significantly across the six categories of activity ($\chi^2 = 1691.34$, $P < 0.0001$, df = 5, N = 900). The highest ODBA value returned (0.965 *g*) was for mating (M) (median = 0.063 *g*; interquartile range (IQR) = 0.036–0.125 *g*). Active manoeuvring (AM) (0.822 *g*), active swimming (AS) (0.828 *g*) and to a lesser extent burying (B) (0.410 *g*) also returned relatively high maximum ODBA values (AM: median = 0.121 *g*, IQR = 0.077–0.204 *g*; AS: median = 0.096 *g*, IQR = 0.058–0.151; B: median =0.163 *g*, IQR = 0.102–0.264 *g*). The lowest maximum values of ODBA were recorded for post-coital panting (PCP) (0.209 *g*) and resting (R) (0.127 *g*) (PCP: median = 0.039 *g*, IQR = 0.026–0.054 *g*; R: median = 0.028 *g*, IQR = 0.022–0.041 *g*). *Post-hoc* pair-wise comparisons of ODBA revealed that behaviours were significantly different from each other ($P < 0.05$) with the exception of post-coital panting and resting at the seabed ($P > 0.05$).

This pilot study highlights the potential of accelerometers to tease apart subtly different behaviours in cuttlefish. The next step is to calibrate OBDA values for different behaviours against VO_2 using laboratory-based respirometry so that estimates of energy expenditure can be gathered remotely. Payne *et al.* (2011) made significant advances towards this goal in their study of *Sepia apama* by using summarized acceleration readings (validated against oxygen consumption) to construct accurate time and energy budgets for free-living individuals. Here, we propose that data storage tags might complement this existing approach, as high resolution acceleration readings (30 Hz) might enable specific costs to be attributed to temporally discrete behaviours. This should be facilitated by the universally linear relationship between ODBA and VO_2 (e.g. Halsey *et al.*, 2008, 2011) including in marine species (e.g. Gleiss *et al.*, 2011; Robson *et al.*, 2011). The need to retrieve the device from the animal post-deployment remains a challenge yet this pilot study provides some insight into how accelerometers might enhance the quality of field data that we can gather over the years to come.

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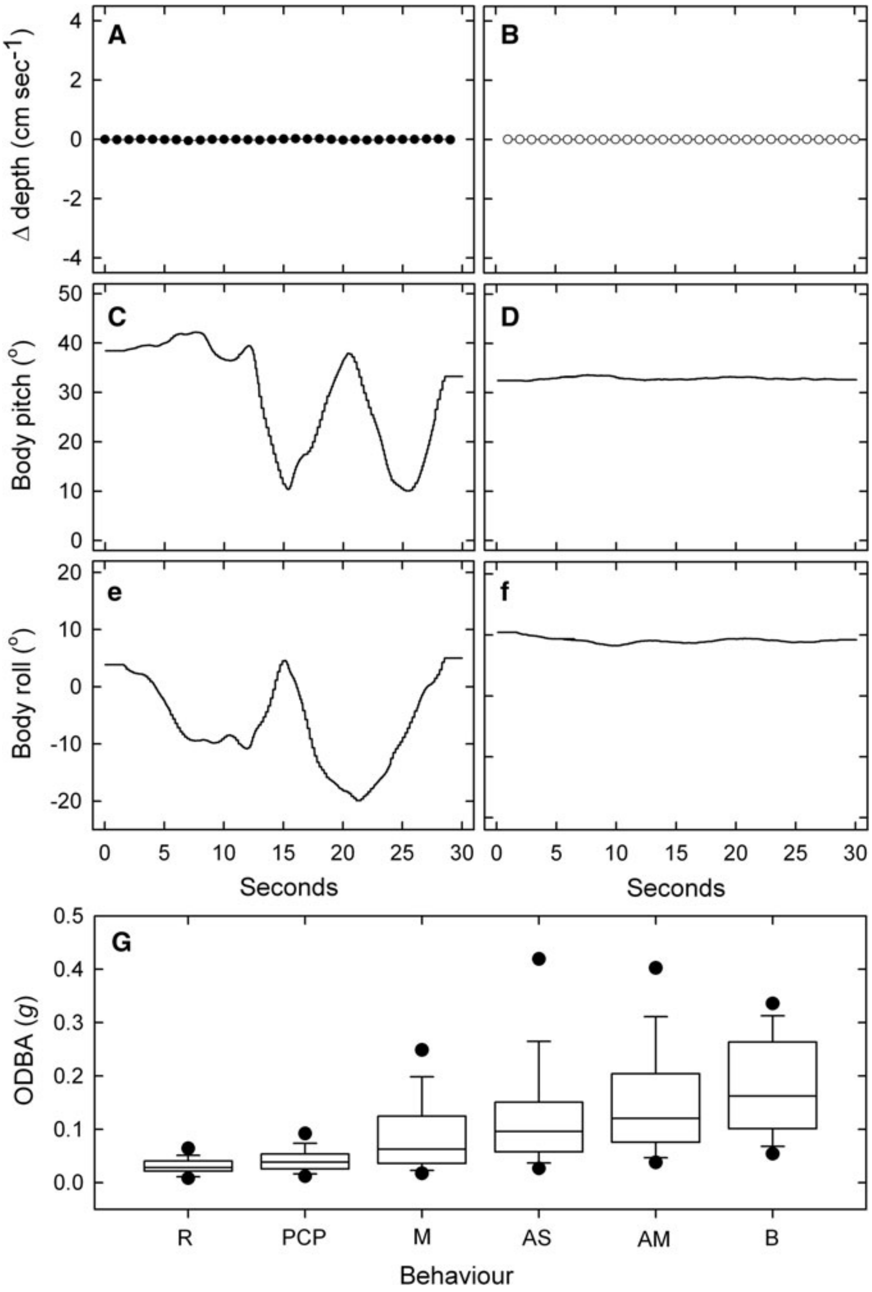

Fig. 1. Comparison of depth and acceleration readings between active horizontal swimming (A, C, E) and resting at the seabed (B, D, F). The two behaviours appear similar when considered in terms of depth alone (A, B) yet tease apart when body pitch (C, D) and roll (E, F) derived from static acceleration are taken into consideration; (G) reinforces how active swimming and resting can be further discrimated using overall dynamic body acceleration (OBDA). R, resting; PCP, post-coital panting; M, mating; AS, active swimming; AM, active manoeuvring (following contours of seabed); B, burying.

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