

RESEARCH NOTE

DISTANCE CHEMORECEPTION AND THE DETECTION OF
CONSPECIFICS IN *OCTOPUS BIMACULOIDES*MATTHEW D. WALDERON¹, KEVIN J. NOLT¹, ROBERT E. HAAS¹, KRISTA
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Octopuses are solitary predators that typically use their arms to grope into crevices to find food. They detect odours on contact using chemosensory cells on their lips and suckers (Budelmann, 1996). They can also detect water-borne odours (distance chemoreception) using receptors in their olfactory organs (olfactory pits; Budelmann, Schipp & Boletzky, 1997). Behavioural experiments have demonstrated increased arousal (Boyle, 1983), activity (Boyle, 1986) and attraction (Chase & Wells, 1986; Lee, 1992) in response to food-related odours, but responses to odours of conspecifics have not previously been investigated. Chemical communication has been demonstrated previously in cephalopods, such as *Nautilus* (Basil *et al.*, 2002), *Loligo* (Gilly & Lucero, 1992; Buresch *et al.*, 2003; King, Adamo, & Hanlon, 2003) and *Sepia* (Boal, 1997; Boal & Marsh, 1998; Boal *et al.*, 2010). Here we investigate behavioural evidence for detection and attraction to conspecific odours by *Octopus*.

Subjects were wild-caught *Octopus bimaculoides* Pickford & McConnaughey, 1949 (30–90 g) of unknown age and maturity (not correlated with physical size within this size range), trapped off the coast of Southern California and shipped overnight to Millersville, PA, USA. Differences in physical size did not explain any patterns in experimental results and will not be discussed; sex was confirmed by necropsy. Housing and experimental tanks were interconnected within a 57,000-l marine system of recirculating artificial seawater (ASW) made from Instant Ocean™ brand salts (salinity 34 ± 2 ppt, temperature $17.5 \pm 1.5^\circ\text{C}$) (see Hvorecny *et al.*, 2007). Trials were conducted between 2004 and 2009. All subjects were acclimated to the laboratory for 3 weeks to 6 months prior to being used in experiments. Subjects were fed thawed frozen shrimp or live fiddler crabs (*Uca* spp.) each day after experimental trials.

Odour samples were collected as follows. Crab odour samples were collected from a holding tank (*c.* 1,000 l) housing 80–100 fiddler crabs (*Uca pugnator*). Shrimp odour samples were obtained by thawing one tail segment of a large (*c.* 9.5 g) frozen shrimp in 10 ml of ASW; seaweed odours were obtained by soaking 3 g of dried seaweed (*Porphyra yezoensis*; Julian Sprung's Sea Veggies™) in 800 ml of ASW for 10 min. For both shrimp and seaweed, the particulate was removed and the remaining liquid served as the odour source.

Conspecific odour and intact egg odour samples were collected by placing an octopus or clutch of about 50 eggs (63 ± 1 days postlaying) into a small aquarium filled with ASW to a total volume of 2 l, with aeration (air stone), for 20 min (initial trials) or 30 min (later trials). After removal of the octopus or eggs, the remaining liquid was used as the odour source. For egg extracts, clutches of conspecific eggs of unknown age (not recently laid) were collected from newly wild-caught females (in California), frozen on dry ice, shipped to Texas and stored

at -80°C until extraction. Eggs were individually extracted (2004) or extracted in batches of 10 eggs (2005), centrifuged and purified using separate C18 Sep-Pak cartridges (Waters Corp., Milford, MA, USA) as described previously (Buresch *et al.*, 2003); C18 Sep-Pak purification primarily retains small molecules, peptides and small proteins. Small molecules such as these have been demonstrated to serve as signal molecules in other molluscs (Susswein & Nagle, 2004). The resulting pellets were dissolved in ASW to a concentration of 1.5 eggs/15 ml for use in trials.

To assess distance odour detection, we measured changes in the ventilation rate of octopuses in response to odours of seawater (negative control), food (positive control), conspecifics (same and opposite sex) and conspecific eggs (intact and purified extracts).

Ventilation-rate trials were conducted in the octopus's individual housing tanks ($38.1 \times 19.6 \times 26.7$ cm, 18.5 l) that were provided with flow rates of 91 ± 4 ml/s (Boal & Golden, 1999). The ventilation rate of an individual octopus was measured by counting ventilation cycles (inspiration and expiration) for the first 20 s (food, seawater) or 30 s (conspecifics, conspecific eggs) of each min. Ten minutes after the start of observations (baseline), a 5 ml (food, seawater, purified egg extracts) or 500 ml (conspecific, conspecific eggs) odour sample was added to the water supply line and ventilation cycles were counted for another 15 min. For conspecific odours, each octopus was exposed to the odour of each of the other octopuses once. For all other trials, each octopus received each odour treatment twice and responses were averaged. Resting ventilation rates (cycles min^{-1}) differed substantially among octopuses ($23\text{--}42$ cycles min^{-1}). Consequently, the change in ventilation rate for each octopus was used to evaluate response: peak ventilation rate (maximum recorded ventilation rate in the 5 min immediately after odour addition) minus mean prestimulus ventilation rate (5 min immediately prior to odour addition). Peak response was used because responses tended to be short-lived.

Octopuses increased their ventilation rates significantly in response to water-borne odours from foods (Kruskal–Wallis one-way analysis of variance by ranks, $KW = 14.32$, $n = 30$, $df = 2$, $P < 0.01$; Fig. 1A). A pair-wise comparison showed that the responses to crab and shrimp odours were significantly different from the responses to the control ($P < 0.05$), but the two food odours were not different from each other. Octopuses also increased their ventilation rates significantly in response to water-borne odours from conspecifics (Fig. 1B). The sex of the odour-donor had a significant effect on responses (Wilcoxon signed-ranks test, same *vs* opposite sex, $z = 2.11$, $n = 17$, $P < 0.05$). Females increased their ventilation rates significantly in response to odours from males but not to odours from other females (Friedman two-way analysis of variance by ranks, $F_r =$

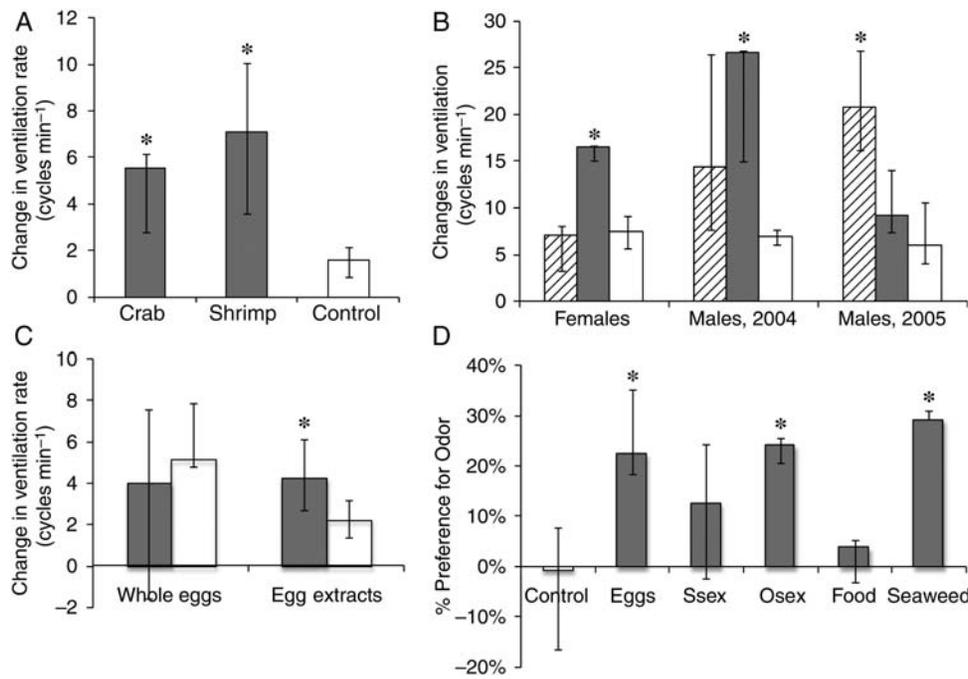


Figure 1. Responses of octopuses to odours. **A–C.** Median changes in ventilations per minute in response to odours. **A.** Response to food items (shaded) and control (clear) ($n = 30$). **B.** Response to conspecific females (hatched), conspecific males (shaded) and control (clear) (females: $n = 6$; males, 2004: $n = 5$; males, 2005: $n = 8$). **C.** Response to conspecific eggs (shaded) and control (clear) (intact eggs: $n = 7$; C18 Sep-Pak-purified egg extracts: $n = 12$). **D.** Median increase in percentages of time spent on the odour side of the γ -maze (during odour infusion as compared to before odour infusion) for odours of intact conspecific eggs, same-sex conspecifics (Ssex), opposite-sex conspecifics (Osex), food, seaweed and control ($n = 8$ for food trials; $n = 6$ for all other trials). Asterisks indicate statistical significance compared to control at $P = 0.05$; error bars indicate first and third quartiles.

7.00, $n = 6$, $k = 5$, $P \leq 0.05$). Males in the first group tested (2004) increased their ventilation rate significantly in response to odours of males but not to odours of females ($F_r = 7.60$, $n = 5$, $k = 3$, $P < 0.05$), while males in the second group (2005) responded to odours of females but not to odours of males ($F_r = 12.00$, $n = 8$, $k = 3$, $P < 0.01$; Fig. 1B).

Octopuses did not increase their ventilation rates significantly in response to odours from intact eggs (2005; Wilcoxon signed-ranks test, $T^+ = 13$, $n = 6$, $P > 0.05$); however, octopuses did increase their ventilation rates significantly in response to odours from C18 Sep-Pak-purified egg extracts ($T^+ = 68.5$, $n = 12$, $P < 0.01$; Fig. 1C).

To assess the biological significance of odour detection, we measured orientation behaviour in a γ -maze. The γ -maze base compartment ($49 \times 63 \times 20$ cm) was connected to two arms ($24.5 \times 70 \times 20$ cm each) separated by a 60° angle and filled to a depth of 15 cm (see Boal & Marsh, 1998). Water flowed from the ends of the arms to a drain at the end of the base of the maze. Laminar flow and flow rates (125 ml/min) were confirmed using red dye (food colouring). Water flow was continuous (24 h/day), trials were typically separated by many hours, and the location of odours added to the maze (left/right) was balanced within and across subjects. Odour solutions were dripped into water inflow at a fixed rate using intravenous solution bags (1 drop/s) suspended over each arm of the maze. Subjects were confined to a centre region ('animal compartment') of the base by plastic grates and were visually isolated with an opaque curtain around the maze.

Each octopus was placed individually into the animal compartment and allowed to acclimate. Trials began with a 5-min *Before Odour Period*. Immediately following, the odour was administered for a total of 5 min. The 5-min *During Odour Period* began at min 9 when the odour covered the entire side of the animal compartment. Each octopus was tested

a maximum of twice daily with an odour presented in one arm of the maze and seawater in the other arm of the maze. Octopuses received two to four trials with each odour type, in random order: each odour type was presented to each octopus on both the left and the right. All trials were recorded for later analysis using a video camera suspended overhead. γ -maze behaviour was scored by noting where the octopus was located within the animal compartment (right/left) every 10 s. A 10-s interval was chosen because it was long enough easily to encompass changes of side but not so long as to miss movements (preliminary trials; Boal & Fenwick, 2007). Trials in which the octopus never moved were discarded; the median response of remaining replicate trials was used in analyses.

Octopuses oriented within the γ -maze with respect to some odours but not others. No attraction to food odours was found (2008; Wilcoxon signed-ranks test, $T^+ = 27$, $n = 9$, $P > 0.05$). Nonfood odours did significantly affect orientation, however (2009; Friedman two-way analysis of variance by ranks, $F_r = 10.35$, $n = 6$, $k = 5$, $P < 0.05$). Paired comparisons revealed that octopuses spent significantly more time on the side of the maze with odours of eggs, same-sex conspecifics and seaweed, but did not prefer the side of the maze with odours of opposite-sex conspecifics (Fig. 1D). For the 2 years combined (2008, 2009), 10 out of 11 individuals preferred the side of the maze with the odours from intact eggs; the 11th individual spent equal time on the two sides of the maze.

Octopuses respond to odours from conspecifics; they detect a wide range of odours, including those of food (crabs, shrimp), nonfood (seaweed) and conspecifics (adults and eggs), and they approach some (eggs, same sex conspecifics and seaweed) but not all (food) of these odours. These results are consistent with findings noted above in other cephalopods. Small sample sizes and high behavioural variability combined to limit our ability to detect differences in responses, and suggest that our

findings could be conservative. For octopuses, the ability to identify the sex and reproductive status of a conspecific at a distance could be useful in facilitating reproduction and avoiding confrontation (Boal, 2006).

Octopuses did not respond equally to all odours. In ventilation-rate experiments, the median increase in ventilation rate to conspecifics was nearly twice as great as that to food (11.47 cycles min^{-1} to conspecifics, 6.07 cycles min^{-1} to food), indicating that this social information is highly salient even to this presumably solitary animal. The difference in the responses of males from the two different years (Fig. 1B) suggests that responses to odours from conspecifics could depend on the octopus's age or developmental stage. The clearer response to egg extracts, as compared to the odours of intact but old egg clusters, suggests that active factor(s) could have been degraded (Shimizu, 1985) or were unable to penetrate the hardened external egg capsule of the intact eggs (Boletzky, 1983). In the γ -maze, both male and female octopuses approached odours of intact conspecific eggs, as well as seaweed, a nonfood item, but did not approach the odours of food or opposite-sex conspecifics. These latter counter-intuitive results are similar to those obtained with cuttlefish (conspecifics, Boal & Marsh, 1998; food, Boal *et al.*, 2010). Future experiments providing direct choices between odours could clarify the significance of particular chemical cues to octopuses.

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REFERENCES

- BASIL, J.A., LAZENBY, G.B., NAKANUKU, L. & HANLON, R.T. 2002. Female nautilus are attracted to male conspecific odor. *Bulletin of Marine Science*, **70**: 217–225.
- BOAL, J.G. 1997. Female choice of males in cuttlefish (Mollusca: Cephalopoda). *Behaviour*, **134**: 13–14.
- BOAL, J.G. 2006. Social recognition: a top-down view of cephalopod behaviour. *Vie et Milieu*, **56**: 69–79.
- BOAL, J.G. & FENWICK, J.W. 2007. Laterality in octopus eye use? *Animal Behaviour Forum*, **74**: e1–e2.
- BOAL, J.G. & GOLDEN, D.K. 1999. Distance chemoreception in the common cuttlefish, *Sepia officinalis*. *Journal of Experimental Marine Biology and Ecology*, **235**: 307–317.
- BOAL, J.G. & MARSH, S.E. 1998. Social recognition using chemical cues in cuttlefish (*Sepia officinalis* Linnaeus, 1758). *Journal of Experimental Marine Biology and Ecology*, **230**: 183–192.
- BOAL, J.G., PROSSER, K.N., HOLM, J.B., SIMMONS, T.L., HAAS, R.E. & NAGLE, G.T. 2010. Sexually mature cuttlefish are attracted to the eggs of conspecifics. *Journal of Chemical Ecology*, **36**: 834–836.
- BOLETZKY, S.V. 1983. *Sepia officinalis*. In: *Cephalopod life cycles*. Vol. I: *Species accounts* (P.R. Boyle, ed.), pp. 31–52. Academic Press, New York.
- BOYLE, P.R. 1983. Ventilation rate and arousal in the octopus. *Journal of Experimental Marine Biology and Ecology*, **69**: 129–136.
- BOYLE, P.R. 1986. Responses to water-borne chemicals by the octopus *Eledone cirrhosa* (Lamarck, 1798). *Journal of Experimental Marine Biology and Ecology*, **104**: 23–30.
- BUDELMANN, B.U. 1996. Active marine predators: the sensory world of cephalopods. *Marine and Freshwater Behaviour and Physiology*, **27**: 59–75.
- BUDELMANN, B.U., SCHIPP, R. & BOLETZKY, S.V. 1997. Cephalopoda. In: *Microscopic Anatomy of Invertebrates, Vol. 6A. Mollusca II* (F.W. Harrison & A.J. Kohn, eds), pp. 119–414. John Wiley and Sons, New York.
- BURESCH, K.C., BOAL, J.G., KNOWLES, J., DEBOSE, J., NICHOLS, A., ERWIN, A., PAINTER, S.D., NAGLE, G.T. & HANLON, R.T. 2003. Contact chemosensory cues in egg bundles elicit male-male agonistic conflicts in the squid *Loligo pealeii*. *Journal of Chemical Ecology*, **29**: 547–560.
- CHASE, R. & WELLS, M.J. 1986. Chemotactic behaviour in *Octopus*. *Journal of Comparative Physiology A*, **158**: 375–381.
- GILLY, W.F. & LUCERO, M.T. 1992. Behavioural responses to chemical stimulation of the olfactory organ in the squid *Loligo opalescens*. *Journal of Experimental Biology*, **162**: 209–229.
- HVORECNY, L.M., GRUDOWSKI, J.L., BLAKESLEE, C.J., SIMMONS, T.L., ROY, P.R., BROOKS, J.A., HANNER, R.M., BEIGEL, M.E., KARSON, M.A., NICHOLS, R.H., HOLM, J.B. & BOAL, J.G. 2007. Octopuses (*Octopus bimaculoides*) and cuttlefishes (*Sepia pharaonis*, *S. officinalis*) can conditionally discriminate. *Animal Cognition*, **10**: 449–459.
- KING, A.J., ADAMO, S.A. & HANLON, R.T. 2003. Squid egg mops provide sensory cues for increased agonistic behaviour between male squid. *Animal Behaviour*, **66**: 49–58.
- LEE, P.G. 1992. Chemotaxis by *Octopus maya* Voss et Solis in a Y-maze. *Journal of Experimental Marine Biology and Ecology*, **153**: 53–67.
- PICKFORD, G.E. & MCCONNAUGHEY, B.H. 1949. The *Octopus bimaculatus* problem: a study in sibling species. *Bulletin of the Bingham Oceanographic Collection*, **12**: 1–66.
- SHIMIZU, Y. 1985. Bioactive marine natural products with emphasis on handling of water-soluble compounds. *Journal of Natural Products*, **48**: 223–235.
- SUSSWEIN, A.J. & NAGLE, G.T. 2004. Peptide pheromones in mollusks. *Peptides*, **25**: 1523–1530.