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BEHAVIORAL RESEARCH METHODS FOR OCTOPUSES AND CUTTLEFISHES

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CEPHALOPODA
LABORATORY
ENRICHMENT
COLEOID
CARE AND HANDLING

ABSTRACT. – Cephalopods provide excellent model species for behavioral research; their large and complex nervous systems, coupled with their phylogenetic distance from vertebrates, allow for particularly interesting comparative investigations. The literature on cephalopod behavior and neurobiology is substantial; fortunately, there are several excellent books for the interested reader. Starting a cephalopod laboratory can be daunting, nonetheless. Previously published reviews address marine system requirements and animal health and welfare. Here, some of the behavioral propensities of octopuses and cuttlefish that influence housing and experimental design are discussed, with particular reference to *Octopus bimaculoides* and *Sepia officinalis*.

INTRODUCTION

For those interested in starting behavioral research with cephalopods there is a very large literature addressing marine system requirements (e.g. Loi & Tublitz 1999, Budelmann 2010, Smith *et al*. 2011), and many publications that address nutrition, diseases, and welfare (e.g. Boucaud-Camou *et al*. 1985, Nixon 1987, Boyle 1991, Lee 1994, Oestmann *et al*. 1997, Anderson & Wood 2001, Scimeca 2006, Garcia Garcia & Cerezo Valverde 2006, Mather & Anderson 2007, Moltschaniwskyj *et al*. 2007). Few publications address behavioral propensities that influence experimental design, however (see Walker *et al*. 1970, Boyle 1981). The observations collected here come primarily from my experience working with captive reared *Sepia officinalis* and wild caught *Octopus bimaculoides*, but likely generalize to other similar species. For those interested in working with squids, see Hanlon *et al*. (1983), Hanlon (1990), Budelmann (2010), and Smith *et al*. (2011); for nautiluses, see Landman (2010). The cephalopod literature, and my own experience, is strongly biased towards laboratory studies; more field studies are needed. For the intrepid investigator planning such research, recent technology that is proving helpful includes tagging, generally (reviewed in Semmens *et al* 2007), and radio acoustic positioning technology (RAPT; O'Dor 2002, Jackson *et al*. 2004, Rigby & Sakurai 2005), in particular.

WORKING WITH CUTTLEFISH

Cuttlefish live 1-2 years, mature quickly (within 4-8 months in captivity; up to 11-14 months in the English Channel), and show a wide range of interesting behaviors worthy of investigation (Hanlon & Messenger 1996). Of the coleoid species (octopuses, cuttlefishes, and squids), they are among the most tractable for behavioral experiments. Cuttlefish spontaneously seek substrate to bury in, shelter from overhead exposure, and objects to camouflage with. They will use their arms to grab prey, and laboratory-reared cuttlefish will reach out to touch novel objects, but they do not use their arms to modify their environment. Cuttlefish are often described as sit-and-wait, or ambush, predators (Hanlon & Messenger 1996) but will actively pursue live fish, shrimp, and crabs. When attacking swimming prey such as shrimp or fish, they strike first with their tentacles and then envelop the prey with their arms (Messenger 1968); when attacking live crabs, they pounce on the crab from behind (Boal *et al*. 2000a). When hunting, pursuing, approaching something attractive, or calmly moving about, they swim forwards, mostly using their fins. When agitated or alarmed, they jet-swim quickly backwards, using their funnel.

Wounds heal poorly (Smith *et al*. 2011) and impact injuries (from backwards jetting) that break the cuttlebone can be lethal (Boletzky & Overath 1989). Most injuries occur as a result of conspecific aggression, unwanted mating attempts, or human-induced startle responses (Hanley *et al*. 1999). Abrasion injuries are also common as the cuttlefish rub along the sides of tanks. Cataracts are frequently seen in older cuttlefish, although whether they result from artificial lighting, abrasion with tank surfaces, intrinsic aging processes, or something else remains unclear (e.g. Sivak *et al*. 1994, Schaeffel *et al*. 1999). Attention to housing needs can ameliorate many of these hazards (Smith *et al*. 2011).

Cuttlefish are benthic and adapted for camouflage (Hanlon & Messenger 1996), yet they are often kept in bare-bottomed tanks for ease of maintenance. While adequate for mariculture, this type of housing appears to be stressful for the cuttlefish while inducing futile and potentially harmful attempts to seek shelter. If a thin layer of substrate is added (1-3 cm of gravel, pebbles, or small stones), the cuttlefish are more apt to settle firmly on the bottom and are less prone to impact and abrasion injuries; substrate deep enough to permit burying results in the calmest animals. Gravel substrate can be efficiently cleaned with a wide-mouthed siphon hose ("aquarium gravel vacuum"). Alternatively, sandy substrate can be used; it is attractive to cuttlefish but is more difficult to keep clean and well-oxygenated.

Artificial aquarium plants are highly attractive to cuttlefish, whether widely branching and weighted to the bottom or a mass floating on the surface. These plants reduce startling and jetting behavior and lower the level of ambient light in the tank. If the cuttlefish are housed in groups, visual barriers such as tall, anchored plants or partial partitions provide escapes from chases by conspecifics. Cushioning the inside tank surfaces may also reduce injuries (Hanley *et al.* 1999), but keeping the soft materials clean is maintenance-intensive.

Habitat enrichment, such as substrate, plants, etc., not only reduces stress, but also supports cognitive development (Dickel *et al.* 2000). Group housing is common and can serve as a form of enrichment (ibid.); however, sexually maturing and mature adults often show abrasions and inking from male-male aggression and unwelcome mating attempts by males.

Individual identification can be achieved through careful inspection of physical markings. Sexually mature cuttlefish have individually unique zebra banding patterns on the dorsal mantle (see Hanlon & Messenger 1988); males also have distinct light-and-dark patterning on the fourth arms. Although it is more difficult, immature individuals can be individually identified by small markings on the dorsal mantle (white landmark spots and white square papillae, Hanlon & Messenger 1988), the pattern of white spots (dots and dashes) along the fins immediately adjacent to the mantle, and the relative sizes of the head and mantle.

Behavior can also facilitate identification. When housed in a group, sexually mature males often show a zebra banding pattern, even when resting on the bottom, while female and immature cuttlefish are more apt to show uniform, mottle, or disruptive patterning (Hanlon & Messenger 1988). In addition, mature males tend to be more active than mature females, swimming rather than settling on the bottom, and challenging and chasing others. This latter behavior can lead to startling, jetting, inking, and impact injuries (Hanley *et al.* 1999); it is helpful to have more than one tank available so that aroused males can be separated from other cuttlefish.

Cuttlefish are strongly visual (Williamson 1995). They have laterally placed eyes and mainly monocular vision on either side, with a 360-degree visual field; when focusing binocularly, they have an anterior overlap of about 75-degrees (Budelmann *et al.* 1997). Although color-blind (e.g. Marshall & Messenger 1996), they can perceive the plane of light polarization (Shashar *et al.* 2000; "polarization vision," Cronin *et al.* 2003). Cuttlefish are sensitive to light levels and are averse to bright light. Recent experiments have demonstrated that significant visual learning occurs prior to hatching (e.g. Darmaillacq *et al.* 2008), providing one possible mechanism for behavioral differences between wild-caught and laboratory-reared cuttlefish. They are keenly alert to human observers; video cameras are essential tools for most behavioral experiments.

Cuttlefish also possess excellent chemoreceptive abilities (Budelmann *et al.* 1997). They are able to detect the odors of both prey and conspecifics (Boal & Golden 1999) and can use these odors in orientation (Boal *et al.* 2010) and learning (Guibé *et al.* 2010). Care must be taken to avoid inadvertent olfactory cuing within experimental apparatuses.

Cuttlefish are easier to work with if they are habituated to people. Glass-sided tanks facilitate this process. Washable fabric can be used to occlude a fraction of the aquarium's top and sides, allowing the cuttlefish to watch lab activities or retreat when desired. The experimenter can adjust the exposure for each cuttlefish as needed. With repeated exposure to humans, slow movements, both above and below the water, are eventually tolerated without alarm by most individuals.

With sufficient exposure to humans, mantle length can be measured by gently maneuvering a transparent ruler above a resting cuttlefish *in situ*. Individuals can also be gently herded from one place to another or captured and transported in a clear container such as a plastic box or bag, without evidence of stress. In time, many laboratory-reared cuttlefish become quite tame and pet-like, watching laboratory activities, approaching and inspecting new objects, and responding to and approaching individual people. Each cuttlefish has a distinct individual temperament (Packard 1991, Adamo *et al.* 2006); for example, some will squirt ink, mucus, or water through the air at particular humans – with very good aim – if they are hungry while others will startle at the slightest provocation even after months of regular handling. Such individual differences (personalities, temperaments, behavioral syndromes; e.g. Sinn & Moltschaniwskyj 2005, Sinn *et al.* 2010) can have a significant impact on experimental outcomes. It can be helpful to plan for modifications of the apparatus to accommodate individual differences (e.g. along the shy/bold continuum).

Cuttlefish can be trained to take food from a hopper (Fig. 1). In our apparatus, a single live crab is dropped into the funnel at the upper end of the feeding tube. The crab is washed down the tube with a volume of water greater than that in the tube itself and lands in a bowl that constrains it from running away and hiding. The tube must be completely smooth or the crab will not be washed all the way down. In pre-training, the tube and bowl can

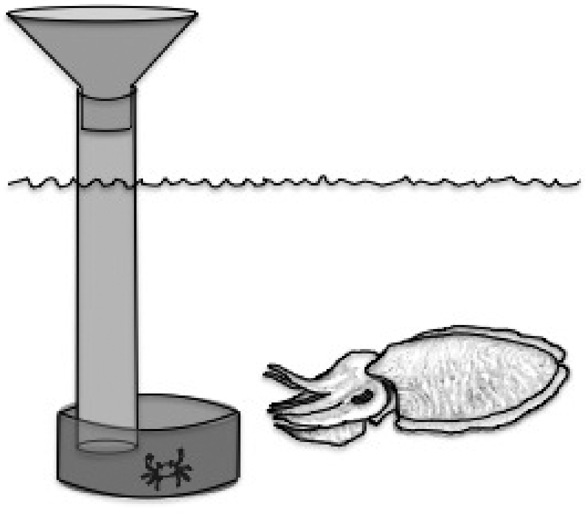

Fig. 1. – Food hopper for use with cuttlefish. A live prey item (crab) was washed down the funnel, through the tube (white PVC plastic pipe, 3 cm internal diameter x 75 cm long) to the bowl below. Opaque barriers (not shown) prevented the cuttlefish from seeing the experimenter.

both be transparent and additional water can be added to keep the crab moving within the bowl, so as to attract the cuttlefish's attention. As soon as the cuttlefish learns to associate the location (bowl) with the food reward (crab) (approx. 5-10 trials), it is best to substitute an opaque-sided tube and bowl so that the cuttlefish will not try to attack the crab through the tube or the side of the bowl.

Cuttlefish can be readily trained to attack one object rather than another (discrimination learning; Karson 2003). Evidence that cuttlefish can be trained to respond to video images of the discriminanda was obtained as follows. Cuttlefish were trained to attack a white object rather than a black object (or visa versa) presented inside the cuttlefish tank for a food reward (live crab, see above). Once the discrimination was mastered (approx. 20 trials), the objects were presented outside the glass tank rather than inside, and the learning transferred without disruption. Finally, the objects were presented on a standard CRT screen placed against the side of the tank, again with no transfer disruption. In the latter two conditions, the cuttlefish attacked the glass side of the tank immediately in front of the rewarded object or image, the object or image was removed, and the cuttlefish then turned and hunted for the crab in the food hopper. Behavior appeared quite similar to that seen in sign tracking (Purdy *et al.* 1999) and autoshaping (Cole & Adamo 2005).

Cuttlefish can learn to solve mazes (Karson 2003, Karson *et al.* 2003, Alves *et al.* 2007, Jozet-Alves *et al.* 2008). The maze must be sufficiently aversive (e.g. well lit or with no suitable substrate upon which to rest) that the cuttlefish does not simply settle on the bottom, but not so aversive that the cuttlefish startles or jets haphazardly backwards (see Karson *et al.* 2003). Successful flume designs are subject to similar constraints (see Boal *et al.* 2010). Hatchling cuttlefish (less than 3 months post-hatching) are strongly benthic and can adhere to the bottom using suction from the configuration of their ventral mantle (Nixon & Mangold 1998); they do not traverse a maze during daylight hours.

With the employment of these fundamental techniques and strategies, cuttlefish are excellent subjects for behavioral investigations of camouflage and visual communication (see Hanlon & Messenger 1996, Adamo & Hanlon 1996, Kelman *et al.* 2008, Palmer *et al.* 2006, Hanlon *et al.* 2007, Langridge 2008, Langridge *et al.* 2007), predatory behavior (e.g. Boal *et al.* 2000a, Shashar *et al.* 2000), reproductive strategies (e.g. Naud *et al.* 2004), behavioral development (e.g. Hanlon & Messenger 1988, Chichery & Chichery 1992, Dickel *et al.* 1997, Dickel *et al.* 1998, Darmaillacq *et al.* 2004, 2008), and learning (e.g. Cole & Adamo 2005, Hvorecny *et al.* 2007; see reviews in Hanlon & Messenger 1996), as well as camouflage, orientation, communication, individual differences (see above), and many other topics.

WORKING WITH OCTOPUSES

Octopuses are short-lived (typically < 1 year) and mature quickly. They show a wide range of interesting behaviors worthy of investigation (Hanlon & Messenger 1996). They are highly manipulative, touch or pounce on novel objects, and show extensive tactile exploratory behavior (Mather & Anderson 1999). Octopuses readily grab but do not readily let go. They are much stronger than they look and will rally all forces if the experimenter makes the mistake of engaging in a tug-of-war over some object. It is important to have extra pieces of all experimental apparatus; the octopus will eventually lose interest and let go and the object can be retrieved (Wells 1978).

Like cuttlefish, octopuses are typically benthic and adapted for camouflage and benefit from a habitat enriched with a layer of substrate, artificial aquarium plants weighted to the bottom, and plenty of loose shells and small stones (Anderson & Wood 2001, Beigel & Boal 2006). Here again, tank cleaning can be efficiently accomplished using a wide-mouthed siphon hose.

Octopuses use dens (shelters, burrows) to avoid predators (see review in Hanlon & Messenger 1996, Finn *et al.* 2009). In the laboratory; they prefer unfinished terra cotta to glazed pottery or glass, and they often barricade themselves into their dens with the shells and stones (Mather 1994). If no objects are available, they are apt to pull one or more of their own arms in front of their mouths, and highly stressed octopuses will engage in autophagy (pers obs, see Reimschuessel & Stoskopf 1990).

Even in optimal captive environments, octopuses of any age will cannibalize each other if the opportunity arises. Hatchlings and young octopuses can be housed in groups provided that dens or shelters are plentiful. Adult octopuses must be housed singly.

Housing tanks require secure, latching lids (Wood & Anderson 2004; not weighted lids: see Bitterman 1975) or a wide band of material that is resistant to sucker adhesion, such as Velcro™ . Light levels should be kept low, and newly caught wild octopuses do better when left entirely alone in a darkened tank for several days and then introduced to laboratory activity slowly. Since most octopus injuries occur as a result of abrasion with rough surfaces (pers obs), housing tanks should have no rough edges and all objects and surfaces should be allowed to accumulate a thin, natural slime layer before use.

Sexually mature males have enlarged proximal suckers on their right and left third and fourth arms (Voight 1991). They also tend to hold their third right, hectocotylized arm coiled. When relaxed, a groove can be distinguished that runs down the length of this arm for passing spermatophores to a receptive female. This characteristic is difficult to see in active animals but is obvious in animals that are anaesthetized or *post mortem*. Females and sexually immature males are difficult to distinguish (Voight 1994).

The following description of reproductive behavior is from my own experience; for further information, see reviews in Boyle (1987) and Hanlon & Messenger (1996). Female octopuses store sperm and wild-caught females may arrive in the laboratory already inseminated. They lay eggs over a period of a couple of days to a week or more and, once brooding, will no longer voluntarily leave their den (no lid is needed on the tank at this point), although they will continue to eat if food is presented to them. Egg hatching in the laboratory happens in the early evening and may be facilitated by the female pumping water over the eggs. Most eggs hatch in a single night, although some may hatch a day or two before and after. Young hatchlings are benthic during the day but move up into the water column at night. As a consequence, the aquarium must be well screened to prevent the hatchlings from being sucked into the water circulation system. The female typically dies within a few days of the eggs hatching (Arnold 1984), but we did have one female that lived for several months afterwards.

Octopuses, like cuttlefish, are easier to work with once they are habituated to people. We have found that a glass aquarium partially covered with fabric allows the octopus the opportunity to watch lab activities and also to retreat when desired. Octopuses can be gently herded from one place to another; however, the easiest way to move them is by herding them into their den and then picking up and moving the den with the octopus still in it (Walker *et al.* 1970, Papini & Bitterman 1991). If the octopus starts to climb out during transit, a light tapping with your fingers on the protruding arms is usually enough to cause retraction. If the den weight is known, the octopus's wet weight can be easily obtained in this way. Though movement is straightforward, transportation appears to be stressful for the octopus and should be minimized (Hvorecny

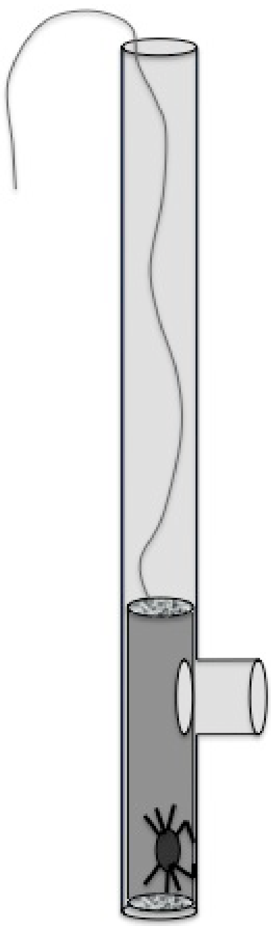

Fig. 2. – Food hopper for use with octopuses. A white PVC plastic pipe section (3 cm internal diameter x 75 cm long) was provided with an access hole (3 cm diameter) 12 cm from one end. A single crab was placed within a section of smaller PVC pipe (2.5 cm external diameter x 15 cm long) that was closed at each end with plastic screening and provided with an access hole (3 cm diameter) in the middle. To deliver the crab, the small pipe section was inserted into the larger tube, where it slid to the bottom. The opening in the side of the small pipe section exactly lined up with the opening in the larger pipe section, providing access for the octopus to reach down into the tube and take the hidden crab. The small pipe section was retrieved using a trailing piece of nylon filament that had been attached to one end of the small pipe section. Opaque barriers (not shown) prevented the octopus from seeing the experimenter.

et al. 2007). In time, octopuses can become quite tame and pet-like, watching laboratory activities with interest, approaching and reaching an arm towards particular people, and showing distinct individual temperaments (Mather & Anderson 1993, Sinn 2000, Pronk *et al.* 2010).

Octopuses have excellent vision (Budelmann 2010) and two non-overlapping visual fields allow them to optimally survey their surroundings (Mather 1991). They are keenly alert to human observers; thus, as with cuttlefish, video cameras are essential tools for most behavioral experiments. In an exciting development, Pronk and colleagues (2010) demonstrated that octopuses respond to video images. This discovery opens the door to new opportunities for controlled experimental investigations.

Octopuses possess excellent contact and distance chemoreception abilities (Wells *et al.* 1965, Budelmann *et al.* 1997). Experiments have demonstrated both arousal and taxis in response to a variety of biogenic chemicals (Boyle 1986, Lee 1992) and the odors of conspecifics (Walderon *et al.* 2011). Although we have no evidence that octopuses make or use chemical trails (Boal *et al.* 2000b), care must be taken to avoid inadvertent olfactory cuing within experimental apparatuses.

Octopuses feed by pouncing on a visually-detected prey or by chemotactile exploration using their arms

(Mather 1991). In addition, they are capable of visually-guided arm movements (Gutnick *et al*. 2011). We developed a food hopper (Fig. 2) based on these behaviors that we have used successfully in learning experiments. A white PVC plastic pipe section (3 cm internal diameter x 75 cm long) was provided with an access hole (3 cm diameter) 12 cm from one end. A single crab (*Uca* spp.) was placed within a section of smaller PVC pipe (2.5 cm external diameter x 15 cm long) that was closed at each end with plastic screening and provided with an access hole (3 cm diameter) in the middle of the pipe. To deliver the crab, the small pipe section was dropped into the larger tube, where it slid to the bottom. The opening in the side of the small pipe section exactly lined up with the opening in the larger pipe section, providing access for the octopus to reach down into the tubes and take the hidden crab. The small pipe section was retrieved using a trailing piece of nylon filament that had been attached to one end of the small pipe section (Fig. 2).

Pre-training was accomplished by leaving a crab in the food hopper overnight. During nocturnal exploration, the octopus discovered the crab and soon learned to associate the hopper with prey. Occasionally, a particularly timid octopus failed to discover the crab. In this case, the inner tube was modified by inserting a small rubber stopper into the end, allowing the crab to remain visible (just inside the tube opening) until the octopus approached the hopper for food. After several such trials, the octopus readily switched to using the standard food hopper.

Like cuttlefish, octopuses quickly learn discrimination tasks. They can be readily trained to attack one object rather than another (discrimination learning; e.g. Boal 1996). Octopuses can also learn to solve maze problems (e.g. Moriyama & Gunji 1997, Boal *et al*. 2000; see also Alves *et al*. 2008), including discriminating between mazes (Hvorecny *et al*. 2007). The maze itself must be sufficiently aversive that the octopus does not simply settle down in a corner somewhere (e.g. shallow, lit) but not so aversive that the octopus jets haphazardly (see Hvorecny *et al*. 2007). For successful designs, see Boal *et al*. (2000).

Octopuses are excellent subjects for behavioral investigations of learning, particularly instrumental conditioning (Byrne *et al*. 2006), exploratory learning (Boal *et al*. 2000), and problem solving (Fiorito *et al*. 1990, 1998, Anderson & Mather 2010), as well as individual differences in temperament (Mather & Anderson 1993, Sinn *et al*. 2001, Sinn & Moltschaniwskyj 2005, Pronk *et al*. 2010).

CONCLUSIONS

The literature on cephalopod behavior is extensive and I have made no attempt to review it thoroughly here; rather, I have provided some suggestions for maintenance and experimental strategies and some starting points for those interested in exploring recent papers on particular topics. Good general reviews of cephalopod behavior can be found in books by Hanlon & Messenger (1996), Nixon & Young (2003), and Boyle & Rodhouse (2005).

The rewards that come with working with this distinctive group of invertebrates are many, provided one can muster the means to provide for their physical needs. Certainly, a wider phylogeny of subjects is likely to shed light on many important and unresolved behavioral questions, such as the function of sleep (Duntley & Morrissey 2004, Brown *et al*. 2006); the embodiment of cognition (Laschi 2008); the evolution of problem-solving (Fiorito *et al*. 1990, 1998); the function of play (Mather & Anderson 1999); the role of sociality in learning (Fiorito & Scotto 1992), communication (Boal *et al*. 2004), and consciousness (Mather 2008); and many other topics. Best wishes to all new-comers!

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REFERENCES

Adamo SA, Ehgoetz K, Sangster C, Whitehorne I 2006. Signaling to the enemy? Body pattern expression and its response to external cues during hunting in the cuttlefish *Sepia officinalis* (Cephalopoda). *Biol Bull* 210: 192-200.

Adamo SA, Hanlon RT 1996. Do cuttlefish (Cephalopoda) signal their intentions to conspecifics during agonistic encounters? *Anim Behav* 52(1): 73-81. DOI:10.1006/anbe.1996.0153.

Alves C, Boal JG, Dickel L 2008. Short-distance navigation in cephalopods: a review and synthesis. *Cogn Process* 9(4): 239-247. DOI: 10.1007/s10339-007-0192-9.

Alves C, Chichery R, Boal JG, Dickel L 2007. Orientation in the cuttlefish *Sepia officinalis*: response versus place learning. *Anim Cogn*10(1): 29-36. DOI: 10.1007/s10071-006-0027-6.

Anderson RC, Wood JB 2001. Enrichment for giant Pacific octopuses: happy as a clam? *J Appl Anim Welf Sci* 4(2): 157-168. DOI: 10.1207/S15327604JAWS0402_10.

Anderson RC, Mather JA 2010. It's all in the cues: octopuses (*Enteroctopus dofleini*) learn to open jars. *Ferrantia* 59: 8-13.

Arnold JM 1984. Chapt 6: Cephalopods. *In* AS Tompa, NH Verdonk, JAM van den Biggelaar, The Mollusca, Vol 7: Reproduction. Orlando: Academic Press: 419-453.

Beigel M, Boal JG 2006. The effect of habitat enrichment on the mudflat octopus. *The Shape of Enrichment* 15(1): 3-6.

Bitterman ME 1975. Critical Commentary. *In* WC Corning, JA Dyal, AOD Willows, Invertebrate Learning, Vol. 3, Cephalopods and Echinoderms. New York and London: Plenum Press: 113-145.

Boal JG 1996. A review of simultaneous visual discrimination as a method of training octopuses. *Biol Rev* 71(2): 157-190. DOI: 10.1111/j.1469-185X.1996.tb00746.x

Boal JG, Dunham AW, Williams KT, Hanlon RT 2000. Experimental evidence for spatial learning in octopuses (*Octopus bimaculoides*). *J Compar Psychol* 114(3): 246-252. DOI: 10.1037/0735-7036.114.3.246

Boal JG, Golden DK 1999. Distance chemoreception in the common cuttlefish, *Sepia officinalis* (Mollusca, Cephalopoda). *J Exp Mar Biol Ecol* 235: 307-317. DOI: 10.1016/S0022-0981(98)00187-7

Boal JG, Prosser KN, Holm JB, Simmons TL, Haas RE, Nagle GT 2010. Sexually mature cuttlefish are attracted to the eggs of conspecifics. *J Chem Ecol* 36(8): 834-836. DOI: 10.1007/s10886-010-9816-0

Boal JG, Shashar N, Grable MM, Vaughan KH, Loew ER, Hanlon RT 2004. Behavioral evidence for intraspecific signaling with achromatic and polarized light by cuttlefish (Mollusca: Cephalopoda). *Behaviour* 141(7): 837-861.

Boal JG, Wittenberg K, Hanlon RT 2000a. Observational learning does not explain improvement in predation tactics by cuttlefish (Mollusca: Cephalopoda). *Behav Proc* 52(2-3): 141-153. DOI:10.1016/S0376-6357(00)00137-6

Boal JG, Dunham AW, Williams KT, Hanlon RT 2000b. Experimental evidence for spatial learning in octopuses (*Octopus bimaculoides*). *J Compar Psychol* 114: 246-252. DOI: 10.1037/0735-7036.114.3.246

Boletzky S, Overath H 1989. Shell fracture and repair in the cuttlefish *Sepia officinalis*. *In* E Boucaud-Camou Ed, La Seiche (The Cuttlefish). Caen: Centre du Publications, Université de Caen: 69-78.

Boucaud-Camou E, Yim M, Tresgot A 1985. Feeding and digestion of young *Sepia officinalis* L. (Mollusca: Cephalopoda) during post-hatching development. *Vie Milieu* 35(3-4): 263-266.

Boyle PR 1981. Methods for the aquarium maintenance of the common octopus of British waters, *Eledone cirrhosa*. *Labor Anim* 15: 327-331. DOI: 10.1258/002367781780952807

Boyle PR 1986. Responses to water-borne chemicals by the Octopus *Eledone cirrhosa* (Lamarck, 1795). *J Exp Mar Biol Ecol* 104 : 23-29.

Boyle PR (Ed)1987. Cephalopod Life Cycles, Vol. II, Comparative Reviews. London: Academic Press.

Boyle PR 1991. The UFAW Handbook on the Care and Management of Cephalopods in the Laboratory, Universities Federation for Animal Welfare, Ennisfield Print and Design, Longon.

Boyle PR, Rodhouse P 2005. Cephalopods: Ecology and Fisheries. Oxford, UK: Wiley-Blackwell.

Brown ER, Piscopo S, De Stefano R, Guiditta A 2006. Brain and behavioural evidence for rest-activity cycles in *Octopus vulgaris*. *Behav Brain Res* 172(2): 355-359. DOI:10.1016/j.bbr.2006.05.009?

Budelmann BU 2010. Cephalopoda. *In* R Hubrecht & J Kirkwood eds, The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals, Eighth Edition. Wiley-Blackwell, Oxford, UK. DOI: 10.1002/9781444318777.ch50?

Budelmann BU, Schipp R, Boletzky Sv 1997. Cephalopoda. *In* Harrison FW & Kohn A, Microscopic Anatomy of Invertebrates, Vol 6A, Mollusca II. New York: Wiley-Liss.

Byrne RA, Kuba MJ, Meisel DV, Griebel U, Mather JA 2006. Does *Octopus vulgaris* have preferred arms? *J Comp Psychol* 120(3): 198-204. DOI: 10.1037/0735-7036.120.3.198

Chichery R, Chichery MP 1992. Learning performances and aging in cuttlefish (*Sepia officinalis*). *Exp Gerontol* 27(2): 233-239. DOI: 10.1016/0531-5565(92)90047-4

Cole PD, Adamo SA 2005. Cuttlefish (*Sepia officinalis*: Cephalopoda) hunting behavior and associative learning. *Anim Cogn* 8(1): 27-30. DOI: 10.1007/s10071-004-0228-9

Cronin TW, Shashar N, Caldwell RL, Marshall J, Cheroske AG, Chiou T-H 2003. Polarization vision and its role in biological signaling. *Integr Comp Biol* 43: 549-558. DOI: 10.1093/icb/43.4.549.

Darmaillacq A-S, Chichery R, Poirier R, Dickel L 2004. Effect of early feeding experience on subsequent prey preference by cuttlefish, *Sepia officinalis*. *Dev Psychobiol* 45(4): 239-244. DOI: 10.1002/dev.20034.

Darmaillacq A-S, Lesimple C, Dickel L 2008. Embryonic visual learning in the cuttlefish, *Sepia officinalis*. *Anim Beh* 76: 131-134. DOI: 10.1016/j.anbehav.2008.02.006.

Dickel L, Boal JG, Budelmann BU 2000. The effect of early experience on learning and memory in cuttlefish. *Dev Psychobiol* 36: 101-110. DOI: 10.1002/(SICI)1098-2302(200003)36:2<101::AID-DEV2>3.0.CO;2-L.

Dickel L, Chichery M-P, Chichery R 1997. Postembryonic maturation of the vertical lobe complex and early development of predatory behavior in the cuttlefish (*Sepia officinalis*). Neurobiology of Learning and Memory 67: 150-160. DOI: 10.1006/nlme.1996.3754.

Dickel L, Chichery M-P, Chichery R 1998. Time differences in the emergence of short- and long-term memory during post-embryonic development in the cuttlefish, *Sepia*. *Behav Proc* 44(1): 81-86. DOI:10.1016/S0376-6357(98)00024-2.

Duntley SP, Morrissey MJ 2004. Sleep in the cuttlefish. Abstract. *Ann Neurol* 56, suppl 8: S68-S68.

Finn JK, Tregenza T, Norman MD 2009. Defensive tool use in a coconut-carrying octopus. *Curr Biol* 19(23): R1069-R1070. DOI:10.1016/j.cub.2009.10.052.

Fiorito G, Scotto P 1992. Observational learning in *Octopus vulgaris*. *Science* 256(5056): 545-547. DOI: 10.1126/science.256.5056.545.

Fiorito G, von Planta C, Scotto P 1990. Problem solving ability of *Octopus vulgaris* Lamarck (Mollsuca, Cephalopoda). *Behav Neur Biol* 53(2): 217-230. DOI:10.1016/0163-1047(90)90441-8.

Fiorito G, Biederman GB, Davey VA, Gherardi F 1998. The role of stimulus preexposure in problem solving by *Octopus vulgaris*. *Anim Cogn* 1(2): 107-112. DOI: 10.1007/s100710050015.

Garcia Garcia B, Cerezo Valverde J 2006. Optimal proportions of crabs and fish in the diet for common octopus (*Octopus vulgaris*) ongrowing. *Aquaculture* 253(1-4): 502-511. DOI:10.1016/j.aquaculture.2005.04.055

Guibé M, Boal JG, Dickel L 2010. Early exposure to odors changes later visual prey preferences in cuttlefish. *Dev Psychobiol*: 833-837. DOI: 10.1002/dev.20470

Gutnick T, Byrne RA, Hochner B, Kuba M 2011. *Octopus vulgaris* uses visual information to determine the location of its arm. *Cur Biol* 21: 460-462. DOI: 10.1016/j.cub.2011.01.052.

Hanley JS, Shashar N, Smolowitz R, Mebane W, Hanlon RT 1999. Soft-sided tanks improve long-term health of cultured cuttlefish. *Biol Bull* 197: 237-238.

Hanlon RT 1990. Maintenance, rearing and culture of teuthoid and sepioid squids. *In* Gilbert D, Adelman H, & Arnod JM, quid as Experimental Animals. New York: Plenum Press: 35-62.

Hanlon RT, Hixon RF, Hulet WH 1983. Survival, growth, and behavior of the loliginid squids *Loligo pleai, Lolito pealei,* and *Lolliguncula brevis* (Mollusca: Cephalopoda) in closed sea water systems. *Biol Bull* 165: 637-685.

Hanlon RT, Messenger JB 1988. Adaptive coloration in young cuttlefish (*Sepia officinalis* L.): the morphology and development of body patterns and their relation to behaviour. *Phil Trans Roy Soc London B Biol Sci* 320: 437-487.

Hanlon RT, Messenger JB 1996. Cephalopod Behaviour. Cambridge, UK: Cambridge University Press.

Hanlon RT, Naud M-J, Forsythe JW, Hall K, Watson AC, McKechnie J 2007. Adaptable night camouflage by cuttlefish. *Am Nat* 169(4): 543-551.

Hvorecny LM, Grudowski JL, Blakeslee CJ, Simmons TL, Roy PR, Brooks JA, Hanner RM, Beigel ME, Karson MA, Nichols RH, Holm JB, Boal JG 2007. Octopuses (*Octopus bimaculoides*) and cuttlefishes (*Sepia pharaonis, S. officinalis*) can conditionally discriminate. *Anim Cogn* 10(4): 449-459. DOI 10.1007/s10071-007-0085-4.

Jackson GD, O'Dor RK, Andrade Y 2004. First tests of hybrid acoustic/archival tags on squid and cuttlefish. *Mar Freshw Res* 56: 425-430. DOI: 10.1071/MF04248.

Jozet-Alves C, Modéran J, Dickel L 2008. Sex differences in spatial cognition in an invertebrate: the cuttlefish. *Proc Roy Soc B Biol Sc* 275(1646): 2049-2054. DOI: 10.1098/rspb.2008.0501

Karson MA 2003. Simultaneous discrimination learning and its neural correlates in the cuttlefish *Sepia officinalis* (Cephalopoda: Mollusca). Doctoral dissertation. Michigan State University, East Lansign, MI.

Karson MA, Boal JG, Hanlon RT 2003. Experimental evidence for spatial learning in cuttlefish (*Sepia officinalis*). *J Comp Psychol* 17(2): 149-155. DOI: 10.1037/0735-7036.117.2.149.

Kelman EJ, Osorio D, Baddeley RJ 2008. A review of cuttlefish camouflage and object recognition and evidence for depth perception. *J Exp Biol* 211: 1757-1763. DOI: 10.1242/jeb.015149.

Landman NH 2010. Nautilus. Topics in Geobiology, Vol 6. Netherlands: Springer: 585-594. DOI: 10.1007/978-90-481-3299-7_37.

Langridge KV 2008. Cuttlefish use startle displays, but not against large predators. *Anim Behav* 77(4): 847-856. DOI:10.1016/j.anbehav.2008.11.023.

Langridge KV, Broom M, Osorio D 2007. Selective signalling by cuttlefish to predators. *Curr Biol* 17(24): R1044-1045. DOI: 10.1016/j.cub.2007.10.028.

Laschi C 2008. Embodied intelligence research in Europe: the Octopus Project of the ICT-FET proactive initiative EMBODY. 2nd EU-Korea Cooperation Forum on ICT Research, December 1-2. Brussels, Belgium.

Lee PG 1992. Chemotaxis by *Octopus maya* Voss et Solis in a y-maze. *J Exp Mar Biol Ecol* 156: 53-67. DOI: 10.1016/0022-0981(92)90016-4.

Lee PG 1994. Nutrition of cephalopods: fueling the system. *Mar Fresh Behav Physiol* 25(1&3): 35-51.

Loi PK and Tublitz NJ 1999. Long term rearing of cuttlefish in a small scale facility. *Aquarium Sci Conserv* 2(3): 135-143. DOI: 10.1023/A:1009601200526.

Marshall NJ, Messenger JB 1996. Colour-blind camouflage. *Nature* 382: 408-409. DOI: doi:10.1038/382408b0.

Mather JA 1991. Foraging strategies and predation risk shape the natural history of juvenile *Octopus vulgaris*. *Bull Mar Sc* 49(1-2): 256-269.

Mather JA 2008. Cephalopod consciousness: behavioural evidence. *Consciousness Cogn* 17(1): 37-48. DOI:10.1016/j.concog.2006.11.006.

Mather JA 1994. 'Home' choice and modification by juvenile *Octopus vulgaris* (Mollusca: Cephalopoda): specialized intelligence and tool use? *J Zool* 233(3): 359-368. DOI: 10.1111/j.1469-7998.1994.tb05270.x.

Mather JA, Anderson RC 1993. Personalities of octopuses (*Octopus rubescens*). *J Compar Psychol* 107(3): 336-340. DOI: 10.1037/0735-7036.107.3.336.

Mather JA, Anderson RC 1999. Exploration, play and habituation in octopuses (*Octopus dofleini*). *J Compar Psychol* 113(3): 333-338. DOI: 10.1037/0735-7036.113.3.333.

Mather JA, Anderson RC 2007. Ethics and invertebrates: a cephalopods perspective. *Dis Aquat Organ* 75: 119-129.

Messenger JB 1968. The visual attack of the cuttlefish, *Sepia officinalis*. *Anim Behav* 16: 342-357. DOI: 10.1016/0003-3472(68)90020-1.

Moltschaniwskyj NA, Hall K, Lipinski MR, Marian JEAR, Nishiguchi M, Sakai M, Shulman DJ, Sinclair B, Sinn DL, Staudinger M, Van Gelderen R, Villanueva R, Warnke K 2007. Ethical and welfare considerations when using cephalopods as experimental animals. *Rev Fish Biol Fish* 17(2-3): 455-476. DOI: 10.1007/s11160-007-9056-8.

Moriyama T, Gunji YP 1997. Autonomous learning in maze solution by *Octopus*. *Ethology* 103(6): 499-513. DOI: 10.1111/j.1439-0310.1997.tb00163.x.

Naud MJ, Hanlon RT, Hall KC, Shaw PW, Havenhand JN 2004. Behavioural and genetic assessment of reproductive success in a spawning aggregation of the Australian giant cuttlefish, *Sepia apama*. *Anim Behav* 67(6): 1043-1050. DOI: 10.1016/j.anbehav.2003.10.005.

Nixon M 1987. Cephalopod diets. *In* Boyle PR ed, Cephalopod Life Cycles, Vol 2. London & New York: Academic Press: 201-219.

Nixon M, Mangold K 1998. The early life of *Sepia officinalis*, and the contrast with that of *Octopus vulgaris* (Cephalopoda). *J Zool* 245(4): 407-421. DOI: 10.1111/j.1469-7998.1998.tb00115.x.

Nixon M, Young JZ 2003. The Brains and Lives of Cephalopods. Oxford, UK: Oxford University Press.

O'Dor R 2002. Telemetered Cephalopod energetics: swimming, soaring, and blimping. *Integr Comp Biol* 42: 1065-1070. DOI: 10.1093/icb/42.5.1065.

Oestmann DJ, Scimeca JM, Forsythe J, Hanlon R, Lee P 1997. Special considerations for keeping cephalopods in laboratory facilities. AALAS Contemporary Issues in Laboratory *Anim Sci* 36(2): 89-93.

Packard A 1991. Cephalopods as individuals (Abstract). *Bull Mar Sci* 49: 666.

Palmer ME, Calvé MR, Adamo SA 2006. Response of female cuttlefish *Sepia officinalis* (Cephalopoda) to mirrors and conspecifics: evidence for signaling in female cuttlefish. *Anim Cogn* 9(2): 151-155. DOI: 10.1007/s10071-005-0009-0.

Papini MR, Bitterman ME 1991. Appetitive conditioning in *Octopus cyanea*. *J Comp Psychol* 105: 107-114. DOI: 10.1037/0735-7036.105.2.107.

Pronk R, Wilson DR, Harcourt R. 2010. Video playback demonstrates episodic personality in the gloomy octopus. *J Exp Biol* 213: 1035-1041. DOI: 10.1242/jeb.040675.

Purdy JE, Roberts AC, Garcia CA 1999. Sign tracking in cuttlefish (*Sepia officinalis*). *J Comp Psychol* 113(4): 443-449. DOI: 10.1037/0735-7036.113.4.443.

Reimschussel R, Stoskopf MK 1990. Octopus automutilation syndrome. *J Invertebr Path* 55(3): 394-400. DOI: 10.1016/0022-2011(90)90083-I.

Rigby PR, Sakurai Y 2005. Multidimensional tracking of giant pacific octopuses in northern Japan reveals unexpected foraging behavior. *Mar Tech Soc J* 39: 64-67. DOI: 10.4031/002533205787521730.

Schaeffel F, Murphy CJ, Howland HC 1999. Accommodation in the cuttlefish (*Sepia officinalis*). *J Exp Biol* 302: 3127-3134.

Scimeca JM 2006. Cephalopods. *In* Lewbart GA ed, Invertebrate Medicine. Ames, IA: Blackwell Publishing: 79-89.

Semmens J, Pecl G, Gillanders B, Waluda C, Shea E, Jouffre D, Ichii T, Zumholz K, Katugin O, Leporati S, Shaw P 2007. Approaches to resolving cephalopod movement patterns. *Rev Fish Biol Fish* 17: 401-423. DOI: 10.1007/s11160-007-9048-8.

Shashar N, Hagan R, Boal JG, Hanlon RT 2000. Cuttlefish use polarization sensitivity in predation on silvery fish. *Vision Res* 40(1): 71-75. DOI:10.1016/S0042-6989(99)00158-3.

Sinn D 2000. A longitudinal study of temperament in *Octopus bimaculoides*. MSc. Thesis, Portland State University, Portland, Oregon, USA.

Sinn DL, Moltschaniwskyj NA 2005. Personality traits in dumpling squid (*Euprymna tasmanica*): context-specific traits and their correlation with biological characteristics. *J Comp Psychol* 119(1): 99-110. DOI: 10.1037/0735-7036.119.1.99.

Sinn DL, Perrin NA, Mather JA, Anderson RC 2001. Early temperamental traits in an octopus (*Octopus bimaculoides*). *J Comp Psychol* 115(4): 351-364. DOI: 10.1037//0735-7036.115.4351.

Sinn DL, Moltschaniwskyj NA, Wapstra E, Dall SRX 2010. Are behavioral syndromes invariant? Spatiotemporal variation in shy/bold behavior in squid. *Behav Ecol Sociobiol* 64: 693-702. DOI 10.1007/s00265-009-0887-2.

Sivak JG, West JA, Campbell MC 1994. Growth and optical development of the ocular lens of the squid (*Sepioteuthis lessoniana*). *Vision Res* 34(17): 2177-2187. DOI: 10.1016/0042-6989(94)90100-7.

Smith SA, Scimeca JM, Mainous ME. 2011. Culture and maintenance of selected invertebrates in the laboratory and classroom. *ILAR J* 52(2): 153-164.

Voight JR 1991. Enlarged suckers as an indicator of male maturity in *Octopus*. *Bull Mar Sci* 49(1-2): 98-106.

Voight JR 1994. Morphological variation in shallow-water octopuses (Mollusca: Cephalopoda). *J Zool* 232: 491-504. DOI: 10.1111/j.1469-7998.1994.tb01590.x.

Walderon MD, Nolt KJ, Haas RE, Prosser KN, Holm JB, Nagle GT, Boal JG 2011. Distance Chemoreception and the detection on conspecificis in *Octopus bimaculoides*. *J Moll Stud* 77: 309-311. DOI: 10.1093/mollus/eyr009.

Walker JJ, Longo N, Bitterman ME 1970. The octopus in the laboratory. Handling, maintenance, training. *Behav Res Methods* 2(1): 15-18. DOI: 10.3758/BF03205718.

Wells MJ 1978. Octopus. Physiology and Behaviour of an Advanced Invertebrate. London: Chapman and Hall.

Wells MJ, Freeman NH, Ashburner M 1965. Some experiments on the chemotactile sense of octopuses. *J Exp Biol* 43: 553-563.

Williamson R 1995. A sensory basis for orientation in cephalopods. *J Mar Biol Ass U K* 75: 83-92. DOI: 10.1017/S0025315400015149.

Wood JB, Anderson RC 2004. Interspecific evaluation of octopus escape behavior. *J Appl Anim Welfare Sci* 7(2):95-106. DOI: 10.1207/s15327604jaws0702_2.

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