BEHAVIOUR OF JUVENILE CEPHALOPODS: PREFERENCE FOR TEXTURE AND BRIGHTNESS OF SUBSTRATA

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ABSTRACT

Behavioural preference for different types and levels of brightness of substrata was studied in cultured, juvenile sepiid cuttlefish, *Sepia pharaonis* and *Sepiella inermis*. Sand, muddy sand and mud represented types of benthic substratum. Both species of cuttlefish preferred sand to mud in long term studies (24 hrs). White, grey and black plastic plates were used as substrata representing high, medium and low levels of brightness. The cuttlefish preferred medium level of brightness during the early phase (up to 3 hrs) of the experiment. The degree of preference for substratum was higher in *Sepia*, living in the open sea, compared to the estuarine *Sepiella*. Cuttlefish selected substrata in relation to benefits, which we suggest are facilitated respiration, crypsis, and energy conservation. Cultured bigfin squid, *Sepioteuthis lessoniana*, was studied as an outgroup on preference for brightness of substrata. The bigfin squid preferred medium level of brightness during the first 6 hrs and then gradually changed to prefer low level of brightness at night. We suggest that preference for brightness is associated with visual discrimination of depth in the pelagic squid performing innate diurnal migration to greater depth. The present results should be applied to painting cephalopod culture tanks in order to reduce stress, promote growth and enhance the contrast of feed.

INTRODUCTION

The term "substratum" refers to structures to which organisms in question maintain, temporarily or permanently, a close contact and does not include aquatic or nutritive media (Gerlach 1972). Animals may move on, or attach to the surface of solid substrata called epilithion. The surface of soft substrata is referred to as epipsammon (on sand) or epipelos (on mud). An important property for the inhabiting animals is the suitability to move upon and to burrow in the soft substrata. Substrata affect metabolism and activity of the animals via their physico-chemical properties, particularly their resistance to animal activities such as locomotion, respiratory ventilation, and burrowing in benthic cephalopods. Exposure and water movement influences properties of soft substrata. Therefore, habitat selection of aquatic animals is essentially the relationship between behaviour and environment (Meadows & Campbell 1972). The animals are not able to differentiate between every variable they encounter in their environment. Meadows & Campbell (1972) commented that few animals are known to show lack of preferences. The preference may alter depending upon physiological state as well as previous experience and learning. The behavioural preference of individuals may vary in rather unexpected ways but preference of a species has a recognisable pattern. Closely related species living in the same environment have different habitat preferences but changes in physiology associated with preference are very little known.

Cephalopods lie, burrow and attach to substrata. Hence, the type of substratum affects the behaviour of cephalopods in several ways, *e.g.* in ritualised behaviour, in ambushing, and sheltering (crypsis, camouflage). Most cephalopods can adjust the appearance of their bodies to match different substrata. Female cephalopods attach their egg capsules to various types of substrata; sponge, coral, seaweed, and rock. The Thai pygmy squid, *Idiosepius thailandicus*, adheres to seaweed fronds in ritualised behaviour, in mating behaviour, and for transportation when the seaweed turns into a floating substratum (Nabhitabhata 1998).

The eyes of cephalopods are excellent and have a structure and function comparable to those of higher vertebrates, but cephalopods are colour-blind (Messenger *et al.* 1973, Roffe 1975, Messenger 1977, Flores *et al.* 1978). However, they able to distinguish the level of brightness (Messenger & Sanders 1972) and the plane of polarisation of the substrata and other objects (Wells 1966). The retina of the cephalopod eye may possess a system for detection of wavelength (Messenger *et al.* 1973). Different colours appear as different degrees of brightness contrasting against the surrounding background on a grey scale. Messenger & Sanders (1972) reported that the eyes of *Octopus vulgaris* were able to discriminate brightness better than orientation of shapes. Young (1968) found that octopus tended to attack black objects more often than white ones if appearing against a white background. Such difference was not present with a grey background. The appearance of the objects to the cephalopod eyes partly depends on the contrast of the object against the surrounding background.

MATERIALS AND METHODS

Soft substrata (texture)

The experimental cuttlefish were reared from hatchling to juvenile at the age of about 30 days. The average size of the pharaoh cuttlefish, *Sepia pharaonis*, was 3.68 ± 0.33 cm in mantle length and 8.67 ± 2.79 g in weight. The average size of the spineless cuttlefish, *Sepiella inermis*, was 3.22 ± 0.16 cm in length and 6.53 ± 0.85 g in weight. The rearing method followed Nabhitabhata (1978b) and Nabhitabhata *et al.* (1984) respectively. The animals were reared in concrete tanks and had no experience on encountering any types of soft substrata in order to avoid learning behaviour.

The bottom of each glass aquarium (35 x 60 x 36 cm) was divided into 3 sections. Each section was randomly spread with one of the three types of substrata, sand, sandy mud and mud. One glass aquarium represented one replicate. The soft substrata had been collected from Changwat Rayong, Thailand. The sandy mud was a mixture of collected sand and mud at the ratio of 1:1 by volume.

The trial consisted of 8 tests, each with 3 replicates (24 replicates in total) for spineless cuttlefish. For the pharaoh cuttlefish, the trial consisted of 2 tests, one test with 3 replicates and another with 2 replicates (5 replicates in total). Ten cuttlefish were released into one aquarium (replicate) and then the numbers of lying and burrowing cuttlefish on each type of substratum were counted and the behaviour were observed. The observation from initial time lapse was at 0.05, 0.15, 0.30, 0.45, 1.00, 1.30, 2.00, 3.00, 6.00, 9.00, 12.00, 18.00 and 24.00 hrs. The time interval was 5, 10, 15, 15, 30, 60, 180, 180, 360 and 360 min respectively. Significance was tested by chi-square test at 95 % ($P < 0.05$) and at 99 % ($P < 0.01$) for each observation of the test.

During the tests, water parameters were monitored every 6 hours. Water temperature, pH and salinity were determined by mercury thermometer, pH meter (HANNA electric paper) and refractometer (ATAGO S-10) respectively. Average temperature recorded was 28.2 ± 0.6 °C, average pH was 8.0 ± 0.1 and average salinity was 30 ± 2 ppt.

Solid substrata (brightness)

The experimental cephalopods were reared from hatchling to juvenile at the age of about 20 days. The rearing methods followed Nabhitabhata (1978a, 1996) for the bigfin squid, *Sepioteuthis lessoniana*, and as previously mentioned for the other two species of cuttlefish. The pelagic bigfin squids were tested as an outgroup for comparison. The rearing tanks were painted sky-blue considered as neutral brightness in order to avoid learning behaviour. The average size of the bigfin squids was 1.75 ± 0.15 cm in mantle length and 1.20 ± 0.34 g in weight. The average size of the pharaoh cuttlefish was 0.85 ± 0.06 cm in length and 0.45 ± 0.11 g in weight and of the spineless cuttlefish was 1.35 ± 0.18 cm, 0.85 ± 0.27 g.

The bottom of each glass aquarium (35 x 60 x 36 cm) was divided into 3 sections. Each section was randomly spread with one of the three plastic plates of white, grey or black in colour representing the high, medium and low level of brightness respectively. One aquarium represented one replicate for the cuttlefish test. The test for bigfin squid was held in circular concrete tanks of 1.50 m diameter with 60 cm water depth. One tank represented one replicate for the squid test. Plastic plates of the three colours were randomly and in equal numbers spread on the tank bottom.

The trial consisted of one test with 3 replicates for each species of cephalopod. In each replicate, the animals used were 24 pharao cuttlefish, 9 spineless cuttlefish, and 12 bigfin squids respectively. Other details as well as water quality were the same as mentioned for soft substrata.

RESULTS

TEXTURE OF SUBSTRATUM (SOFT SUBSTRATA)

Pharaoh cuttlefish, *Sepia pharaonis*

Preference for mud dominated significantly over other substrata (P < 0.05) in the first 15 minutes (Table 1) but after that the degree of preference decreased to non significant difference until 1.30 hrs (P > 0.10-0.35). The preference increased to significant difference again (P < 0.025) until 3.00 hrs. In nighttime through the daytime of the next day (24.00 hrs), the preference for sand and mud were not different (P > 0.05) but significantly different (P < 0.05) from sandy mud substratum. The highest degree of pref-

Table 1. Pharaoh cuttlefish, *Sepia pharaonis*. Test of preference on type of substratum. There were 50 individuals in this trial. N = the number of cuttlefish lying on substrata (swimming cuttlefish were excluded). *, ** indicate significant difference (P < 0.05, 0.01) respectively; a,b,c indicate significant range difference (P < 0.05) as different consonants; n = nighttime; ns = non significant difference (P > 0.05); x = maximum figure.

Time lapse (hr)	Time of day (hr)	Brightness preference ratio			N	chi^2	P
		high	medium	low			
00.05	12.05	1.0^a	0.5^a	2.5^b x	16	6.50 *	< 0.05
00.15	12.15	1.0^{ab}	0.3^b	1.6^a x	20	6.10 *	< 0.05
00.30	12.30	1.0	0.8	1.8 x	22	2.82 ns	> 0.10
00.45	12.45	1.0	0.5	1.4 x	23	3.22 ns	> 0.10
01.00	13.00	1.0	0.3	1.1 x	22	3.91 ns	> 0.10
01.30	13.30	1.0	0.4	1.2 x	24	2.25 ns	> 0.25
02.00	14.00	1.0^a	0.6^a	2.2^b x	31	8.98 *	< 0.025
03.00	15.00	1.0^{ab}	0.4^a	2.0^b x	37	13.35 **	< 0.005
06.00 n	18.00	1.0^a x	0.2^b	0.8^a	38	9.52 **	< 0.01
09.00 n	21.00	1.0^a x	0.1^b	0.7^a	20	7.89 *	< 0.025
12.00 n	00.00	1.0^a x	0.2^b	0.7^a	34	10.32 **	< 0.01
18.00 n	06.00	1.0	0.5	1.1 x	26	2.39 ns	> 0.025
24.00	12.00	1.0^a x	0.2^b	0.8^a	42	9.58 **	< 0.01

Table 2. Spineless cuttlefish, *Sepiella inermis*. Test of preference on type of substratum. There were 240 individuals in this trial. N = the number of cuttlefish lying on substrata (swimming cuttlefish were excluded). ** indicate significant difference (P < 0.05); a,b indicate significant range difference (P < 0.05) as different consonants; n = nighttime; ns = non significant difference (P > 0.05); x = maximum figure.

Time lapse (hr)	Time of day (hr)	Brightness preference ratio high	medium	low	N	chi^2	P
00.05	12.05	1.0	1.1 x	0.8	195	3.82 ns	> 0.10
00.15	12.15	1.0	1.1 x	1.0	208	0.30 ns	> 0.75
00.30	12.30	1.0 x	1.0 x	0.9	214	0.24 ns	> 0.75
00.45	12.45	1.0 x	0.9	1.0 x	219	0.73 ns	> 0.50
01.00	13.00	1.0 x	0.9	1.0 x	222	0.66 ns	> 0.50
01.30	13.30	1.0 x	0.8	0.9	219	1.34 ns	> 0.50
02.00	14.00	1.0 x	0.8	0.9	226	2.46 ns	> 0.25
03.00	15.00	1.0 x	0.8	0.9	225	1.78 ns	> 0.25
06.00 n	18.00	1.0 x	0.9	0.8	145	1.25 ns	> 0.50
09.00 n	21.00	1.0 x	0.6	0.7	179	7.12 ns	> 0.025
12.00 n	00.00	1.0 x	0.7	0.8	187	5.46 ns	> 0.05
18.00 n	06.00	1.0 x	0.7	0.8	213	4.42 ns	> 0.10
24.00	12.00	1.0^a x	0.5^b	0.5^b	231	31.28 **	< 0.005

Table 3. Pharaoh cuttlefish, *Sepia pharaonis*. Test of preference on type of substratum. There were 72 individuals in this trial. N = the number of cuttlefish lying on substrata (swimming cuttlefish were excluded). ** indicate significant difference (P < 0.05); a,b indicate significant range difference (P < 0.05) as different consonants; n = nighttime; ns = non significant difference (P > 0.05); x = maximum figure.

Time lapse (hr)	Time of day (hr)	Brightness preference ratio high	medium	low	N	chi^2	P
00.05	12.05	1.0^a	2.0^a x	1.4^a	22	1.73 ns	> 0.25
00.15	12.15	1.0^a	1.2^a x	0.9^a	28	0.50 ns	> 0.75
00.30	12.30	1.0^a	1.6^a x	1.0^a	39	1.85 ns	> 0.25
00.45	12.45	1.0^a	1.4^a x	0.7^a	37	3.30 ns	> 0.10
01.00	13.00	1.0^a	1.6^a x	0.9^a	39	2.92 ns	> 0.10
01.30	13.30	1.0^a	3.3^b x	0.4^a	43	26.56 **	< 0.005
02.00	14.00	1.0 x	1.2^a x	0.8^a	58	1.69 ns	> 0.25
03.00	15.00	1.0^a x	0.9^a	0.8^a	50	0.40 ns	> 0.75
06.00 n	18.00	1.0^a	1.2^a x	0.4^b	63	9.24 **	< 0.01
09.00 n	21.00	1.0^a x	1.0^a x	0.2^b	42	10.71 **	< 0.005
12.00 n	00.00	1.0^a	1.2^a x	0.7^a	50	2.44 ns	> 0.25
18.00 n	06.00	1.0^a	1.1	0.6^a	56	3.68 ns	> 0.10
24.00	12.00	1.0^a	1.4^a x	0.4^b	59	12.44 **	< 0.005

Table 4. Spineless cuttlefish, *Sepiella inermis*. Test of preference on brightness of substratum. There were 27 individuals in this trial. N = the number of cuttlefish lying on substrata (swimming cuttlefish were excluded). n = nighttime; ns = non significant difference (P > 0.05); x = maximum figure.

Time lapse (hr)	Time of day (hr)	Brightness preference ratio			N	chi²	P
		high	medium	low			
00.05	12.05	1.0	1.5 x	0.0	5	1.12 ns	> 0.50
00.15	12.15	1.0	2.5 x	0.5	8	3.25 ns	> 0.10
00.30	12.30	1.0 x	1.2 x	0.5	16	1.63 ns	> 0.25
00.45	12.45	1.0	1.0 x	0.5	15	1.20 ns	> 0.50
01.00	13.00	1.0	1.8 x	1.0	15	1.20 ns	> 0.50
01.30	13.30	1.0	1.6 x	0.8	17	1.53 ns	> 0.25
02.00	14.00	1.0	1.6 x	1.0	18	1.00 ns	> 0.50
03.00	15.00	1.0 x	1.0 x	0.6	13	0.62 ns	> 0.50
06.00 n	18.00	1.0 x	1.0 x	0.4	19	2.63 ns	> 0.25
09.00 n	21.00	1.0	1.3 x	1.0	20	0.40 ns	> 0.75
12.00 n	00.00	1.0	1.1 x	0.8	21	0.29 ns	> 0.75
18.00 n	06.00	1.0	1.5 x	0.7	19	2.00 ns	> 0.25
24.00	12.00	1.0 x	0.9	0.6	20	0.70 ns	> 0.50

Table 5. Bigfin squid, *Sepioteuthis lessoniana*. Test of preference on brightness of substratum. There were 36 individuals in this trial. N = the number of squid lying on substrata (swimming squid were excluded). a,b indicate significant range difference (P < 0.05) as different consonants; n = nighttime; ns = non significant difference (P > 0.05); x = maximum figure.

Time lapse (hr)	Time of day (hr)	Brightness preference ratio			N	chi²	P
		high	medium	low			
00.05	12.05	1.0[a]	11.0[b] x	6.0[b]	36	16.67**	< 0.01
00.15	12.15	1.0[a]	12.0[b] x	5.0[c]	36	20.67**	< 0.01
00.30	12.30	1.0[a]	26.0[b] x	9.0[c]	36	27.17**	< 0.01
00.45	12.45	1.0[a]	8.0[b] x	3.0[a]	36	19.50**	< 0.01
01.00	13.00	1.0[a]	4.6[b] x	1.8[a]	36	15.50**	< 0.01
01.30	13.30	1.0[a]	8.7[b] x	2.3[a]	36	25.17**	< 0.01
02.00	14.00	1.0[a]	4.8[b] x	1.4[a]	36	18.17**	< 0.01
03.00	15.00	1.0[a]	5.4[b] x	0.8[a]	36	28.17**	< 0.01
06.00 n	18.00	1.0[a]	4.0[b]	7.0[b] x	36	13.50**	< 0.01
09.00 n	21.00	1.0[a]	1.4[a]	1.6[a] x	36	1.17 ns	> 0.50
12.00 n	00.00	1.0[a] x	0.9[a]	0.6[a]	36	1.17 ns	> 0.50
18.00 n	06.00	1.0[a]	1.4[a]	2.1[a] x	36	3.50 ns	< 0.10
24.00	12.00	1.0[a]	1.7[a]	9.3[a] x	36	32.17**	< 0.01

erence during that period had shifted from mud to sand type. The exception was at dawn (18.00 hrs) where the preference was not significantly different (P > 0.025) and the highest preference shifted to mud type. The degree of preference for sand and mud was higher than for sandy mud throughout the test period.

Spineless cuttlefish, *Sepiella inermis*
The highest degree of preference shifted from sandy mud substrata at the first 30 min (0.30 hr) to sand type afterward (Table 2). However, at 0.45 and 1.00 hr the degree of sand preference was equal to of mud preference. The preference for type of substratum was not significantly different (P > 0.05). At 24.00 hrs, the preference for sand was significantly different from mud and sandy mud (P < 0.05). The preference for the latter two substrata was not different (P > 0.05). From 1.30 hrs, the degree of preference for mud was always higher than or equal to sandy mud except at dusk, 6.00 and 9.00 hrs.

Brightness of substratum (solid substrata)

Pharaoh cuttlefish, *Sepia pharaonis*
The degree of preference was highest for the medium level of brightness (Table 3). However, the difference was not significant (P > 0.05) most of the time except at 1.30 hrs, at dusk 6.00-9.00 hrs, and 24.00 hrs (P < 0.01). During the exception period, preference for the medium level of brightness was not different (P > 0.05) from preference for the high level but significantly different from low brightness (P < 0.05). The degree of preference was comparatively higher on the high than on the low brightness.

Spineless cuttlefish, *Sepiella inermis*
The preference for brightness was not significantly different (P > 0.10) at any experimental time (Table 4). The highest degree of difference was observed at preference for the medium level of brightness and equal to preference for high brightness (at 3.00 and 6.00 hrs). The exception was at 24.00 hrs where the highest degree of preference was for high brightness. The degree of preference was comparatively higher for high brightness than for low brightness.

Bigfin squid, *Sepioteuthis lessoniana*
The degree of preference for the medium level of brightness was highest with high significance after the first 5 min (0.05 hr, P < 0.01) to 3.00 hr (Table 5). Medium to low brightness were not differently preferred (P > 0.05) by the squids at 0.05 hr and at 6.00 hrs (at dusk). At night, the highest degree of preference shifted from medium to low level of brightness except at 12.00 hrs but the difference was not significant (P > 0.10). The change to low brightness preference at the highest degree was at 24.00 hrs with high significance (P < 0.01). The preference for high and medium brightness was not different at that time (P > 0.05).

DISCUSSION

Brightness of substratum
Young (1968) reported that octopus, *Octopus vulgaris*, showed preference for black or white objects (low and high brightness). The preference varied with the brightness level of the objects as well as the colour of background. In a white tank (high brightness background), the octopus attacked black objects (low brightness) more often than the white. The mean was 44 % for white preference. In grey tanks (medium brightness background), the preference for white was 56 %. The efficiency of discriminability was related to the level of contrast between the object and background. The probability of an attack on a particular shape might be further influenced by its brightness insofar as brightness affected the discriminability of the shape from the background (Sanders 1975). Bradley & Messenger (1977) reported that *Octopus vulgaris* made significant attacks on rectangular test objects whose

brightness contrasted with that of the background. They suggested that the octopus did not have an intrinsic brightness preference and the so-called preference was merely a function of the background or substratum brightness.

The present study revealed a tendency of intrinsic preference for brightness of substratum in both species of cuttlefish, considered from overall consistency or stability of the preference through the experimental period. The pelagic bigfin squid tended to react in a different pattern. The preference for low brightness after dark was interpreted as innate migration to greater depth. Hence, the brightness of substrata might be associated with visual discrimination of depth by the squid. Moreover, the degree of preference for low brightness that lowered to non-significant difference ($P > 0.10$-0.50) during the nighttime and might relate to photopositive taxis behaviour in low light environment, i.e. diurnal migration (Nabhitabhata 1978b). Moynihan & Rodaniche (1985) also reported the same pattern of behaviour in the juvenile Caribbean bigfin squid, *Sepioteuthis sepioidea*. The squids congregate in shallow water during the daytime and migrate offshore to deeper water at night. They also came to ship lights at night apparently rising from below (photopositive taxis in low light environment).

From the view of level of brightness, the sand, sandy mud and mud types of substratum should, at a certain level, be comparable to high, medium and low level of brightness respectively. The sand preference of the cuttlefish should have been interpreted as high brightness preference, but the medium brightness was preferred during the brightness tests. Therefore, the preference behaviour should vary as a function of physical properties of the substrata as well as the brightness. Holmes (1955) and Boucaud-Camou & Boismery (1991) reported that sand substratum was the usual habitat of *Sepia officinalis*. The sand substratum was preferred obviously on basis of burrowing capability since mud should make the surrounding water turbid during burrowing, which involved rapid movement of both fins and strong water jetting. The muddy turbid water also disturbed respiration since the cephalopod gills lack cilia for removing sediment. Sand was well and continuously oxygenated by current (Bacescu 1972) being a good habitat for respiration-ventilation during burrowing. Although the sand substratum might have excess brightness in primary sensing by the cuttlefish, the situation should have turned into the advantage of excess reflection to interspecific vision (either predator or prey) after complete burrowing.

Preference for medium brightness of the spineless cuttlefish, *Sepiella inermis*, was not prominent since the preference was without significance ($P > 0.10$-0.75). Preference was also low compared to pharaoh cuttlefish. This pattern might reveal another view on lesser dependence on benthic substrata of this species compared to the open sea *Sepia pharaonis*. The preference for substrata of the spineless cuttlefish should be added as a character of this species to behaviour reported by Nabhitabhata (1997). The habitat of this species is estuaries where current velocity and water turbidity are high. The level of underwater brightness in turbid water is comparatively lower than in clearer water of the open sea. So the preference could obviously not be an advantage in terms of camouflaging or crypsis. On the other hand, the preference for sand of this species seemed to be opposite to its natural habitat, muddy estuary. Nabhitabhata & Polkhan (1983) and Nabhitabhata (1997) suggested that there might be two groups (populations?) of the spineless cuttlefish. One group was an estuarine population (with mud preference) and another group was an open sea population (with sand preference).

Physiology

Habitat selection is essentially the relationship between behaviour and environment (Meadows & Campbell 1972). In an overall view, the selection sequences of preference for substratum of the cuttlefish, on basis of the physical properties of the substrata, should fall in four categories: lying capability, burrowing capability, respiration, and energy conservation. On soft substrata (sand and mud), both types of substratum offered capability for lying and burrowing. The sand was the better choice because of being compact for lying and creating less turbidity disturbing the respiration-ventilation while burrowing. On the other hand, burrowing in softer mud consumed less energy compared to burrowing in sand. In the meantime, higher turbidity disturbed the respiration. The preference for sand revealed that the selection depended on respiration capability hence, physiology. This would be in accordance with Meadows & Campbell (1972) who stated that habitat preference of aquatic invertebrates depended upon physiological benefits.

Crypsis

On solid substrata where burrowing could not be performed, colour selection should reveal capability for crypsis or camouflaging instead. The capabilities for lying and respiration were not different. For the cuttlefish, the possibility for crypsis on low brightness of substratum would have been the best choice. Cuttlefish would obtain the lowest degree of exposure by the lowest contrast between the animals and background.

In the meantime, crypsis is a process, which certainly consumes energy, and may cause chronic stress and lower growth. Nabhitabhata & Nilaphat (1999) suggested that no substratum was necessary for normal growth of cultured *Sepia pharaonis* but lack of proper substrata might be involved, to some degree, in a small final size of the cuttlefish. The cryptic process involves a chronic body-patterning component that is a combination of chromatic texture, posture and locomotion elements. The chromatic element consumes energy since the cephalopod chromatophores are complex "organs" and part of the muscular systems. Each chromatophore organ comprises an elastic sacculus containing pigment granules surrounded by a series of about 15-25 radial muscles (Hanlon & Messenger 1996). In cryptic dark patterning on low brightness substratum, the muscles contract and the sacculus is greatly expanded. In white patterning on high brightness substratum, the muscles relax and the energy stored in the sacculus cause it to retract.

Overall, the cryptic activities consume energy in an order reversed to the level of brightness: The higher the brightness the lesser the energy consumed. The preference by cuttlefish for sand with high brightness should also be reasonable seen from this point of view. Since the preference outcome was medium brightness, there might have been a compromise involved between risk of exposure and energy consumption in the cryptic process as well as the consequent stress.

In conclusion, the strategy of preference for substrata should depend on physical properties of the substrata at the first encounter. On soft substrata where burrowing, and nearly complete crypsis was feasible, the uninterrupted respiration was the point for the selection. On solid substrata where burrowing was not feasible and the life was at risk because of a higher degree of exposure, there should be a compromise between capability of crypsis and chronic energy consumption required by the cryptic component.

Application in aquaculture

The present study should be applied in aquaculture for colouring or painting of concrete culture tanks. The purposes are to promote growth as well as to reduce stress. This will yield good health of the animals in the long term. La Roe (1971) suggested that culture tanks of the Caribbean bigfin squids, *Sepioteuthis sepioidea*, should be coloured dark and substrata should be present, such as grass, coral, rocks or rubble, which exerted a calming influence. Matsumoto & Shimada (1980) painted a net pattern in black on the tank walls in order to reduce skin damage after tank collision of the squid, *Doryteuthis bleekeri*. Hulet *et al.* (1979) and Hanlon *et al.* (1983) also used tanks with various colour patterns on the walls made with black paint in order to increase contrast and make the walls more visible to the squids, *Loligo plei*, *L. pealei* and *Lolliguncula brevis*. The contrast and visibility of tank walls seemed to be more necessary to pelagic fast moving squids than benthic cuttlefish. However, these mentioned techniques might not be suitable for tanks used for various purposes.

Flores *et al.* (1978), Sirisaksophon *et al.* (1995) studied the response behaviour of the squid, *Todarodes pacificus*, to different brightness of objects and substrata in order to apply the findings for squid fishing where contrast of the jig (feed-prey) against the surrounding water was required. They reported that the contrast threshold of the squids was far better than of fish. Yang *et al.* (1983) used tanks painted black to enhance detection of the translucent prey organisms for the squid, *Loligo opalescens*. Feeding the cultured cephalopods with dead and artificial feed increased the importance of proper painting of the culture tanks by enhancing the contrast for visual attraction in the same manner. Both objectives, stress reduction with growth promotion and enhancing contrast and visibility could be achieved through painting the tanks in appropriate colour. On basis of the present study, the appropriate colour for cuttlefish should be medium brightness on a grey scale.

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