https://doi.org/10.24199/j.mmv.1983.44.17 30 June 1983

VISUAL DISCRIMINATION TESTING IN THE SQUID *TODARODES PACIFICUS*: EXPERIMENTAL EVIDENCE FOR LACK OF COLOR VISION

By Efren Ed. C. Flores

University of the Philippines in the Visayas, U.P., Diliman, Quezon City, 3004 Philippines

Abstract

Squids were taught by a reward and punishment paradigm to visually discriminate between lights of various intensities and colors using the simultaneous discrimination tests. The learning ability of the squid was established when it made discrimination between screens presented with lights of different intensities. For color discrimination test, the squid did not discriminate a green light (523.5 nm) from a blue light (450 nm) projected on separate screens.

The results of the present study which agrees with previous behavioral findings strongly suggest that *Todarodes pacificus* is color blind. With regards to squid fishing, the use of colored underwater lights does not seem practical in view of the findings in this study.

Introduction

In this study, squids were taught by a reward and punishment paradigm to visually discriminate between lights of various colors and intensities. The objective of the testing was to determine if *Todarodes pacificus* demonstrated a preference for squid jigs of specific colors, with the ultimate goal of designing a more attractive and efficient jig for commercial fisheries.

In squid fishing, a variety of small underwater lights of various intensities and colors are used for the attraction of squid to jigs. Recently, underwater chemical lights have been introduced in squid jig fishing. All these lights are used without any knowledge of the squid vision. The present study provides some information on the vision of the squid, *T. pacifica*, with regards to the color and intensity of light which could be applied to fishing. Also, this study provides information on the learning ability of the squid which is valuable for comparative study on animal behavior.

Among the cephalopods, the octopus has been widely used for learning experiments ranging from visual discrimination of orientation and form (Sutherland, 1957, 1960, 1960a, 1963; Sutherland & Muntz, 1959; Messenger & Sander, 1971) to monocular discrimination (Messenger & Sander, 1972), and visual preference (Messenger *et al.*, 1973). This area of study has been concentrated on the octopus because it is available and easy to maintain under laboratory conditions. During experimentation, the octopus can also withstand extensive manipulation or handling.

With regards to the hue discrimination using shapes painted red, green or blue, Messenger *et al.* (1973) presented evidence that strongly suggests that the octopus (*O. vulgaris*) lacks color vision. Roffe (1975) using colored lights reflected on a white disc also produced the same results. Again, Messenger (1977b) using colored shapes presented simultaneously showed further evidence that *Octopus* is color blind. Based on the above information and the similarity of eye construction in cephalopods it is likely that all cephalopods do not possess color vision. However, because of the wide ecological diversity within the class ranging from bottom dwellers to migratory pelagic species, a generalization on the absence of color vision may not be acceptable.

Outside of the octopus few other cephalopods have been used for learning experiments. Only recently have simple experiments of this sort been applied to *Sepia* (Messenger, 1977a). Messenger was able to train the cuttlefish not to attack a prawn inside a tube. After repeated unsuccessful attacks, the number of strikes decreased rapidly until the cuttlefish completely stopped attacking the prawn when it was presented.

Until now, no learning experiments have been performed on squid principally because laboratory handling and maintenance is difficult. Behavioral studies on squid in laboratory conditions started when Flores *et al.* (1976, 1977) developed an efficient handling and maintenance technique for *T. pacifica*. Flores *et al.* (1978) determined the absence of color vision by using colored objects (stripes) as shapes in discrimination by optomotor response. The disadvantage of this method was that there was no control over the brightness of the objects presented, and that the brightness differences between objects induced some amount of optomotor response. Here, the discrimination by learning with the use of colored lights allowed the observer to control brightness.

Materials and Methods

Experimental animals. The water off Hakodate, Japan, is a major fishing ground for the Japanese common squid, *Todarodes pacificus.* Over-night fishing operations were conducted in this fishing ground by 5 gross ton squid jigging vessels. These vessels were fitted with live hatches at midship. The automatic jigging machines mounted on the bulwark had slipways connecting them to the openings of the live hatches. Jigged squid would fall on to the slipway then into the live hatches.

The squid used in this study were taken from these vessels at the fish landings and transported to the Hakodate Fisheries Experimental Station in aerated 50 litre plastic pails. The handling and maintenance of squid are as described in Flores *et al.* (1976).

Experimental tanks. The wet laboratory of the Hakodate Fisheries Experimental Station had a row of five concrete tanks of identical size (230 cm × 180 cm × 120 cm). In two tanks the walls and bottom were black while the other three were white. The two black tanks were used in this study while the other tanks were used as holding tanks for other studies. One black tank was used to receive and hold newly caught squid while the other tank was used as test tank for discrimination experiment.

Simultaneous discrimination test. This was conducted in October, 1978. Ten newly caught squid were secured from a squid jigging vessel at about six in the morning and transported to the laboratory where a black holding tank was made ready to receive them. The other black tank was set up for the simultaneous discrimination test (Figure 1).

In the holding tank, squid were trained to feed on sardine fillets (bait attached to a line) for eight days. Feeding was done regularly at night with the tank lighted by a 40W fluorescent lamp overhead. The behavior of the squid during feeding was closely observed in order to choose the squid that would be best suited for the test.

When the squid were regularly feeding on 2 g sardine fillet, two squid, that were good feeders and had fins with the least injury, were transferred to the adjacent test tank with the simultaneous discrimination apparatus installed as shown in Figure 2. The two squid tested were easily distinguishable because of a slight difference in size, and were marked as (A) for the better squid (♀, ML 21.5 cm) and (B) for the smaller one (♀, ML 20 cm).

In the test tank an overhead 20W fluorescent lamp covered with a translucent white plastic plate (1 mm thick) was provided in order for the observer to see the squid. A curtain of thick black cloth was hung above the side of this tank as shown in Figure 1 to prevent the penetration of bright light coming from the other tanks which were lighted with a bare 40W overhead fluorescent lamp. Another curtain made of black plastic sheet was hung in front of the simultaneous discrimination apparatus to minimize disturbance from movement of the observer.

Figure 3 shows the light projection set designated for this experiment. The light source was a halogen projector lamp (21V, 150W) commercially used for 8 mm cinema projector. Interference filters in the light path produced the desired color or hue. The intensity of each light was controlled by using neutral density filters (Wratten gelatin filter) at 0.5 log-unit steps. The light was then reflected off two front surface mirrors, down a black duct into the water, to a 10 cm square frosted glass which served as a screen. On the outside the glass was covered with a black adhesive plastic sheet with a 6 cm diameter hole cut out at the centre. This produced a circular 6 cm diameter screen appearing underwater. This circular screen serves as the "shape" which could be designated as a negative screen (−) or a positive screen (+) depending on the intensity or color of light displayed. An air bubble outlet was mounted at

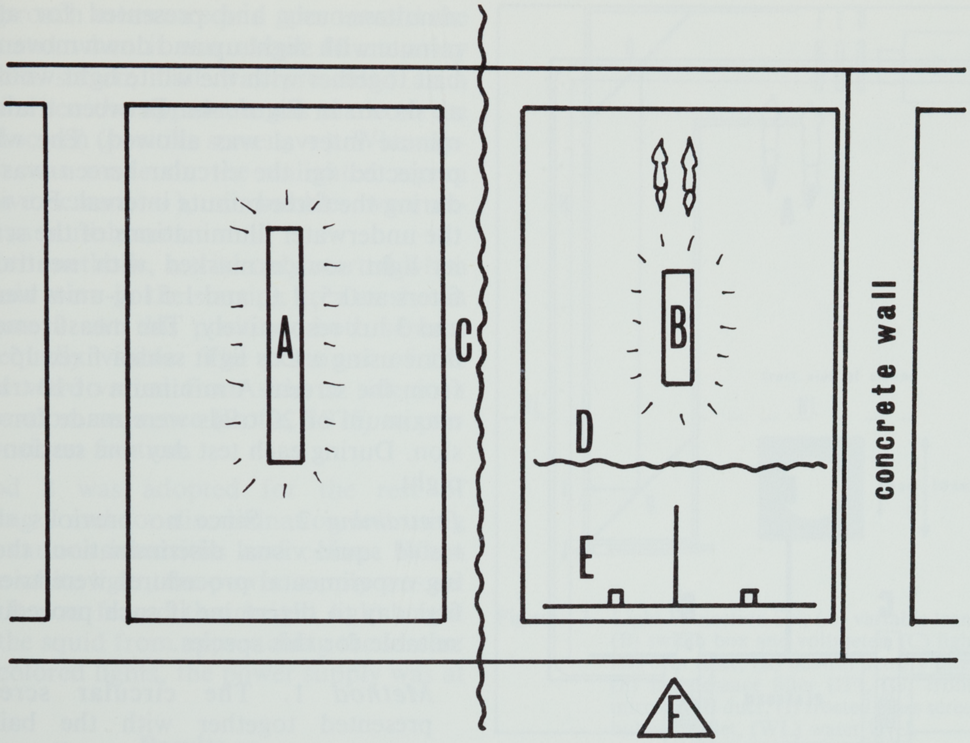

Figure 1. Floor plan showing experimental tanks used for simultaneous discrimination test (SDT); (A) 40w fluorescent lamp over holding tank, (B) 20w fluorescent lamp over experimental tank, (C) curtain, black cloth, (D) curtain, black plastic sheet, (E) SDT apparatus, (F) observer.

the lower corner of the duct with a plastic tubing connected to an ordinary aquarium air pump. The air bubbles when released serve as a form of punishment for an approach on the screen designated as negative screen.

The pair of light projection sets was connected to a switch box coupled with a voltmeter. The power source from an AC 100V outlet was led to a variable transformer before being connected to the switch box. This arrangement produced the same amount of electricity going to each light projection set.

Narrow band interference filters, green (523.5 nm) and blue (450 nm) were selected for this study in accordance with the spectral sensitivity curve for *T. pacificus* obtained by colorimetric method (equal energy spectrum) as reported by Orlov and Byzov (1962). The spectral sensitivity curve shows that at these wavelengths, the squid retina is equally sensitive, and so these colors should appear equally bright to the squid. Since the spectral sensitivity of the squid is high at the blue and green part of the spectrum, it should be easy for the squid to see these colors. The detailed characteristic of the interference filters used are shown in Table 1.

TABLE 1

Characteristics of interference filters based on data furnished by manufacturer (Vacuum Optics Corp., Japan).

	Green	Blue
Transmission maximum (nm)	523.5	450
Half-band width (nm)	13	20
Transmissivity maximum	45	41.3

The pair of light projection sets was mounted on a platform fixed at the upper edge of the deep wall of the test tank as shown in Figure 2. To this platform was also fixed a large piece of black painted marine plywood that fits down into the tank serving as a uniform background

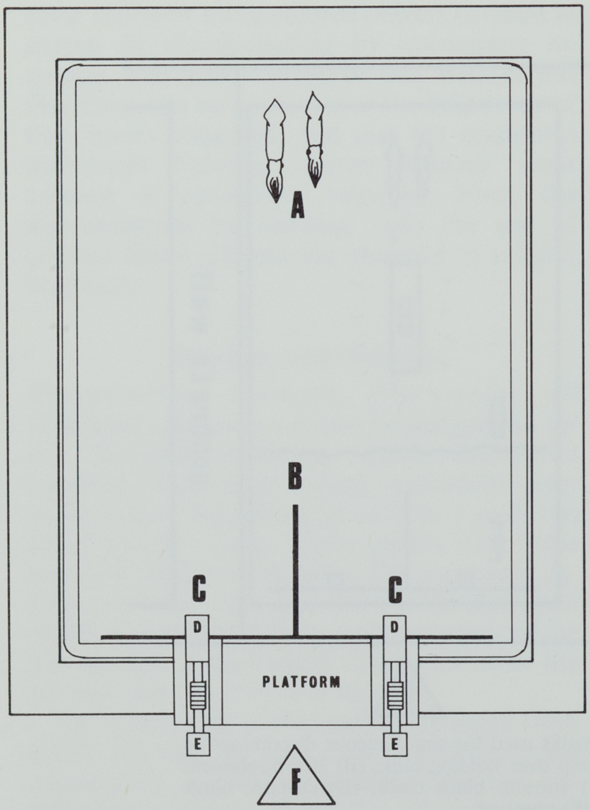

Figure 2. Overhead view of simultaneous discrimination test tank; (A) squid start position area, (B) partition, (C) feeding area, (D) screen, (E) halogen lamp, 21v, 150w, (F) observer.

of the circular screens underwater. At the centre of this background, a partition was fixed running lengthways down the tank. The length of the partition was 50 cm. This partition separated the left screen from the right screen so that when the squid was at one side the other side was not visible.

Experimental procedure

Pretraining 1. Upon transfer to the test tank the two squid were trained to feed on bait (sardine fillets) attached to monofilament nylon line (0.2 mm dia.) and hung in the feeding area (Figure 2, C). The feeding was done at night with white light projected on the circular screen. Bait was lowered in each feeding area simultaneously and presented for about one minute with slight up and down movement. The bait together with the white light would appear as shown in Figure 4a. Between trials a three-minute interval was allowed. The white light projected on the circular screen was kept on during the three-minute interval. For reference, the underwater illuminations of the screen with its light source masked with neutral density filters at 0.5, 1.0, and 1.5 log-units were 80, 20, and 3 lux respectively. The measurements were done using a Cds light sensor fixed 15 cm away from the screen. A minimum of 10 trials and a maximum of 20 trials were made for each session. During each test day one session is run at night.

Pretraining 2. Since no previous study has tested squid visual discrimination, the following experimental procedures were tried on the first day to determine if such procedures were suitable for this species.

Method 1. The circular screen was presented together with the bait as in Pretraining 1. The squid was allowed to seize the bait at the circular screen with white light projected (positive screen, +) at the same intensity as in Pretraining 1.
The other screen was also presented but without light (negative screen, (−) as shown in Figure 4b. For this screen, the squid was not allowed to seize the bait. Instead punishment was administered in a form of air bubbles.
The screens were exposed throughout the session and a trial begun by introducing bait into the feeding area of each screen as in Pretraining 1. A minimum of 10 trials were made for each session.

Method 2. Each trial started with the presentation of the screen by slowly pulling up the black plastic plate (11 × 11 cm) hung in front of the screen. Both screens were presented simultaneously and were exposed for one minute as shown in Figure 4b. After each trial, the black plastic plates were lowered to cover the screen. A three-minute interval was allowed between trials. A minimum of 10 trials were made for each session.

An approach means a squid goes toward the screen and when it was about 10 cm away from the screen, bait was presented as a reward when the approach was made to the lighted screen (positive screen, +). When approach was made on the unlighted screen (negative screen, −), air bubbles were released as punishment.

For both methods, a trial only started when the squid were in the starting area as shown in Figure 2. At this position, both screens were equally visible. The positions of the screens were varied from side to side in a semi-random sequence (Roffe, 1975) to prevent position fixation.

Method 2 was adopted for the rest of Pretraining 2 and for discrimination test using light of various intensities and colors. When using the white light, the power supply was set at 10V. It was feared that bright light might prevent the squid from approaching the screen. For the colored lights, the power supply was at 15V.

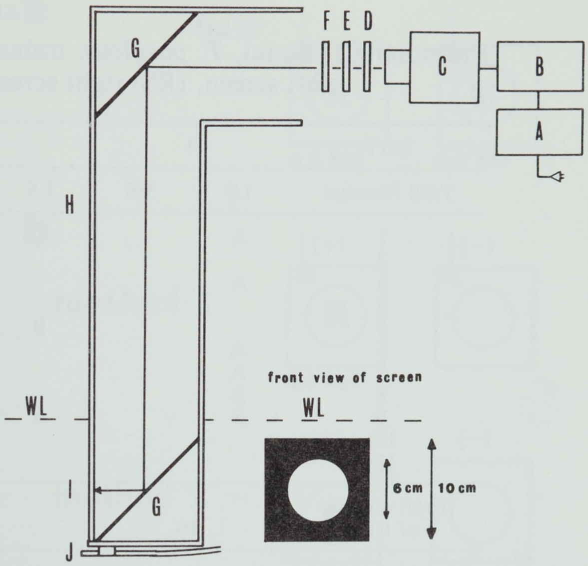

Figure 3. Light Projection Set; (A) variable transformer, (B) switch box and voltmeter, (C) light source, (D) heat filter, (E) neutral density filter (NDF), (F) interference filter (IF), (G) front surface mirror, (H) duct, (I) frosted glass screen, (J) air bubble outlet, (WL) water level.

Results

Simultaneous discrimination test. (SDT)

Pretraining 1. Training was started on the third day after transfer of squid into the test tank. The daily performance of each squid is shown in Table 2. Training was continued until a squid took ten pieces of bait during one session. This was necessary because the following discrimination test required a minimum of 10 trials per session, and for each trial bait was presented as a reward for an approach to the positive stimulus. On the 4th day, squid B took ten pieces of bait for one session while squid A made no approaches to the lighted screen.

A high percentage of successful trials can be attained from a squid that is able to consume ten pieces of bait or more per session. In the holding tank, squid stopped responding to bait presented after consuming enough amount of food. A squid, satisfied with say three pieces of bait, will most likely not respond to more trials.

The low percentage of successful trials will result in slow learning on the part of the squid. During this training, squid A did not perform well, but it was hoped that this squid would catch up during the following experiments.

Pretraining 2. Day one of training was devoted to the testing of Methods 1 and 2. Method 1 was not suitable because simultaneous presentation of bait produced problems of equal stimulation. Since baits attached to lines were presented or introduced manually, it was difficult to equalize their movement. Furthermore, the baits appeared to be stronger cues than the lights since the latter were exposed throughout the session, and trials were started with the introduction of the bait and were ended with its retraction.

At first, the observer was reluctant to use Method 2 because it was not known whether the squid could be attracted to the screen when presented by itself. To the surprise of the observer, the squid made clear approaches towards the positive screen presented with the light intensity the same as that of Pretraining 1. No approach was made towards the negative screen which was not lighted. This method was then applied for the rest of Pretraining 2 and

TABLE 2

Pretraining 1; Squid, *T. pacificus*, trained to feed at designated feeding area, (LS) left screen, (RS) right screen, (A) squid A, (B) squid B.

DAY	1		2		3		4	
Trial Number	LS	RS	LS	RS	LS	RS	LS	RS
1	A			A			B	
2				A				B
3	A			B	No		B	
4				B	response			B
5			B		at		B	
6	A			B	all			B
7	A			A			B	
8	B			B			B	
9	B		A				B	
10								B
11				A				
Total number of trial	10		11		11		10	
Attack level %								
Squid A	40		45		0		0	
Squid B	20		45		0		100	

$$\text{Attack level \%} = \frac{\text{Total response per squid}}{\text{Total number of trials}} \times 100$$

Note: Response means an attack on the bait presented at the feeding station.

for the succeeding simultaneous discrimination tests.

On training days, two and three, both squid easily discriminated between the positive and negative screens making perfect scores as shown in Table 3. Squid A with 12 attacks (60%) for day two performed better than squid B with 2 attacks (10%). The low level of attacks by squid B was not considered in the analysis of percentage of correct attacks. However, on day three, squid B recovered by attacking 70% of the time, all of which were correct. For day two, only the positive screen was presented (Figure 4c). Here the situation was simple, and as expected squid A made no mistake making clear approaches toward the positive screen. On day three, both screens (Figure 4d) were presented simultaneously and both squid made no mistake.

SDT using white lights with different intensities. Differences in intensity also serve as cues in stimulus discrimination in addition to wavelength difference. The next set of experiments was designed to determine the intensity level (expressed in terms of neutral density filters) at which squid stop differentiating. Figure 5 illustrates the combination of screens used.

Experiment SDT 1-1 (Figure 5a). This is an extension of Pretraining 2, Day 3 using the same pair of screens. The performance of each squid for two days is presented in Table 4. Again, the two squid made no mistake except for once by squid A on day two which is negligible.

Experiment SDT 1-2 (Figure 5b). Both screens were lighted with the light source of the negative screen masked by a 1.5 NDF. The light of the positive screen masked by O.5 NDF was the same as in the previous experiment. The results of the one day test are presented in Table 5. Squid B made a perfect score while squid A had 75% correct response which is still statistically significant.

In this experiment the intensity difference between the screens presented was about 26 times and here the squid made clear discrimination within a one day test. A typical approach

TABLE 3

Pretraining 2; test on the learning ability of the squid, *T. pacificus*; (LS) left screen, (RS) right screen.

DAY	2		3	
Trial Number	LS	RS	LS	RS
1	–	+	–	+
2	+	–	+B	–
3	+	–	+BA	–
4	–	+A	–	+AB
5	+	–	+B	–
6	–	+A	–	+AB
7	+A	–	+	–
8	+A	–	+	–
9	–	+A	–	+AB
10	+	–	+AB	–
11	–	+A		
12	–	+A		
13	+AB	–		
14	–	+B		
15	+A	–		
16	–	+		
17	–	+A		
18	+A	–		
19	–	+A		
20	+	–		
Total responses				
Squid A	5(+)	7(+)	2(+)	3(+)
Squid B	1(+)	1(+)	4(+)	3(+)
Total number of trials	20		10	
Attack level %				
Squid A	60		50	
Squid B	10		70	
Correct responses %				
Squid A	100		100	
Squid B	–		100	

$$\text{Correct response \%} = \frac{\text{Total response to positive screen}}{\text{Total response}} \times 100$$

Note: Day one (1) of training was devoted to the testing of Methods 1 and 2 (see results).

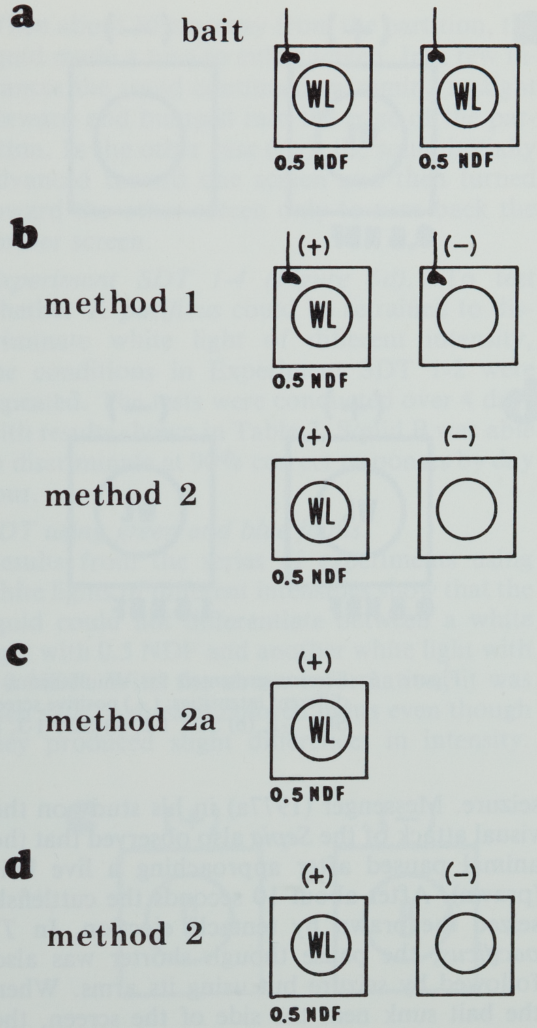

Figure 4. Screens presented for Pretraining Test; (WL) white light, (+) positive screen, (–) negative screen, (NDF) neutral density filter; (a) for Pretraining 1, day 1-4, (b) for Pretraining 2, day 1, (c) for Pretraining 2, day 2, and (d) for Pretraining 2, day 3.

where discrimination was clear is shown in Figure 7 (p. 222). In these cases, the squid made an almost straight approach towards the positive screen from the starting position. When the squid was about 10 to 20 cm away from the screen, it paused. At this position, the negative screen at the other side of the partition was no longer visible. Any reinforcement given at this position would be related only to the screen approached.

The pause in front of the screen allowed the observer to introduce the bait. It is interesting to note that later in the experiment, when the reward was delayed, the squid would continue moving toward the screen its arms open as if for

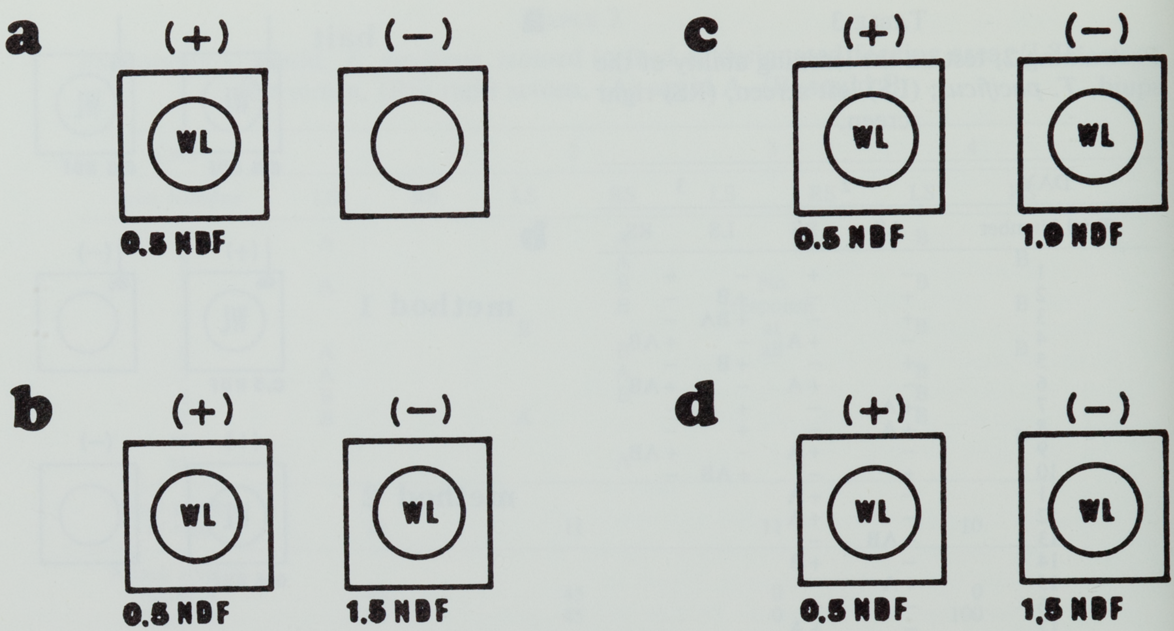

Figure 5. Screens presented for simultaneous discrimination test (SDT) using white light (WL) of different intensities; (+) positive screen, (−) negative screen, (NDF) neutral density filter; (a) SDT 1-1, (b) SDT 1-2, (c) SDT 1-3, and (d) SDT 1-4.

seizure. Messenger (1977a) in his study on the visual attack of the *Sepia* also observed that the animal paused after approaching a live bait (prawn). After about 10 seconds the cuttlefish seized the prawn by tentacle ejection. In *T. pacificus*, the pause though shorter was also followed by seizure but using its arms. When the bait sunk near the side of the screen, the squid would yaw head first towards the bait and seized it.

Experiment SDT 1-3 (Figure 5c). In this test, the light intensity difference was reduced with the light source of the negative screen masked by a 1.0 NDF. The positive screen was four times brighter than the negative screen. The results of this two-day experiment, tabulated in Table 6, indicated that the two squid were not able to discriminate. During day one of experimentation, squid A performed at chance level of 55% correct responses while squid B performed at 72% which is above chance level. On day two, squid B now performed at chance level of 40% while squid A had 10% correct responses.

TABLE 4

Simultaneous discrimination test (SDT) 1-1; discrimination between a lighted screen (+) and an unlighted screen (−); (LS) left screen, (RS) right screen.

DAY	1		2	
Trial Number	LS	RS	LS	RS
1	+	−	+A	−
2	+	−	−	+A
3	−	+A	+	−A
4	+AB	−	−	+BA
5	−	+	−	+BA
6	−	+A	+B	−
7	+B	−	−	+AB
8	−	+B	+B	−
9	+	−	−	+BA
10	−	+B	+B	−
11	−	+B		
12	+B	−		
13	−	+B		
14	+	−		
15	−	+		
16	−	+		
17	+	−		
18	+	−		
19	+	−		
20	−	+		

TABLE 4 continued

Total responses				
Squid A	1(+)	2(+)	1(+)	5(+), 1(−)
Squid B	3(+)	4(+)	3(+)	4(+)
Total number of trials		13		10
Attack level %				
Squid A		23		70
Squid B		50		70
Correct responses %				
Squid A		100		85
Squid B		100		100

In this experiment, the nature of approaches were different from approaches where discrimination was possible. Figure 7 also shows examples of two approaches in which the squid was not able to discriminate. In one case (-o-), the squid went from the starting position straight towards the partition. During this type of approach, both screens are probably visible to the squid, producing equal stimulation. When about 30 cm away from the partition, the squid made a turn to either screen. In a few instances the squid continued swimming straight forward and bumped into the edge of the partition. In the other case (-x-), the squid initially advanced toward one screen and then turned toward the other screen only to turn back the former screen.

Experiment SDT 1-4 (Figure 5d). To test whether *T. pacificus* could be retrained to discriminate white light of different intensity, the conditions in Experiment SDT 1-2 were repeated. The tests were conducted over 4 days with results shown in Table 7. Squid B was able to discriminate at 90% correct responses by day four.

SDT using green and blue lights.
Results from the series of experiments using white lights of different intensities show that the squid could not differentiate between a white light with 0.5 NDF and another white light with 1.0 NDF. With the above information, it was then safe to present colored lights even though they produced slight differences in intensity.

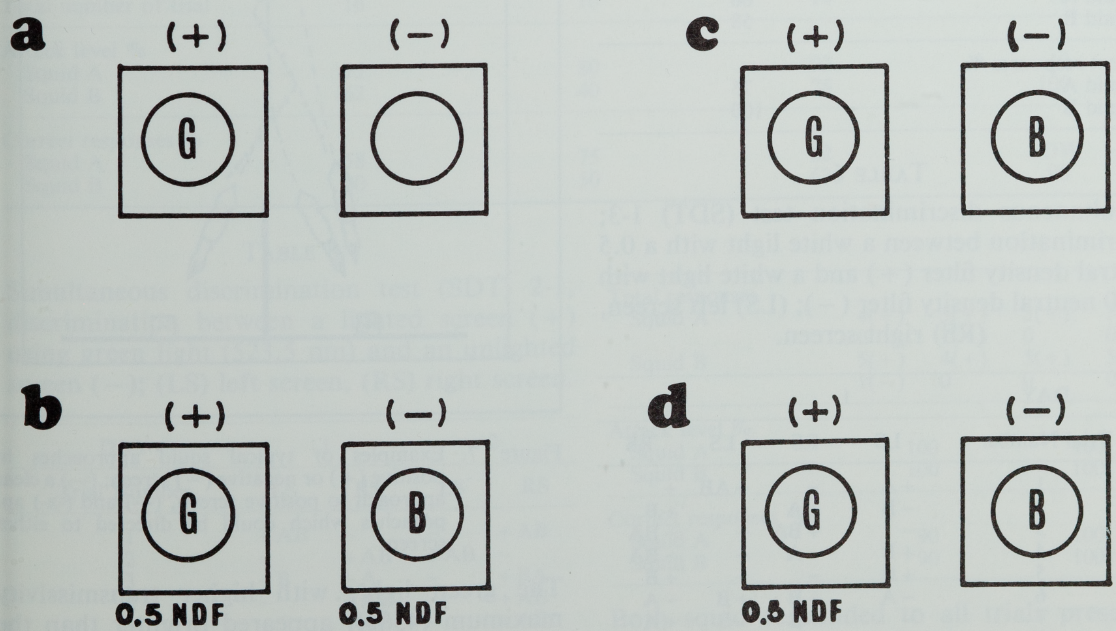

Figure 6. Screens presented for simultaneous discrimination test (SDT) using green and blue lights; (+) positive screen, (−) negative screen, (NDF) neutral density filter; (a) SDT 2-1, (b) SDT 2-2, (c) SDT 2-3, and (d) SDT 2-4.

TABLE 5

Simultaneous discrimination test (SDT) 1-2; discrimination between a white light with a 0.5 neutral density filter (+) and a white light with a 1.5 neutral density filter (−); (LS) left screen; (RS) right screen.

DAY	1	
Trial Number	LS	RS
1	−	+AB
2	+	−A
3	−	+BA
4	−	+B
5	+	−
6	+	−
7	+B	−
8	−	+A
9	−	+AB
10	+	−A
11	+AB	−
12	−	+AB
Total responses		
Squid A	1(+)	5(+), 2(−)
Squid B	2(+)	5(+)
Total No. of trials	12	
Attack level %		
Squid A	66	
Squid B	58	
Correct responses %		
Squid A	75	
Squid B	100	

TABLE 6

Simultaneous discrimination test (SDT) 1-3; discrimination between a white light with a 0.5 neutral density filter (+) and a white light with a 1.0 neutral density filter (−); (LS) left screen, (RS) right screen.

DAY	1		2	
Trial Number	LS	RS	LS	RS
1	+A	−	−AB	+
2	−B	+A	−	+B
3	−	+BA	−	−BA
4	+B	−A	+	−BA
5	+B	−	−	+B
6	−A	+B	+B	−A
7	−	+B	−B	+A
8	−	+BA	+	−AB
9	+A	−B	+	−AB
10	+	−BA	−	+B
11	−A	+B		
12	+B	−		

TABLE 6 continued

Total responses				
Squid A	2(+)	3(+)		1(+)
	2(−)	2(−)	1(−)	5(−)
Squid B	3(+)	5(+)	1(+)	3(+)
	1(−)	2(−)	2(−)	4(−)
Total number of trials	12		10	
Attack level %				
Squid A	75		70	
Squid B	91		100	
Correct responses %				
Squid A	55		10	
Squid B	72		40	

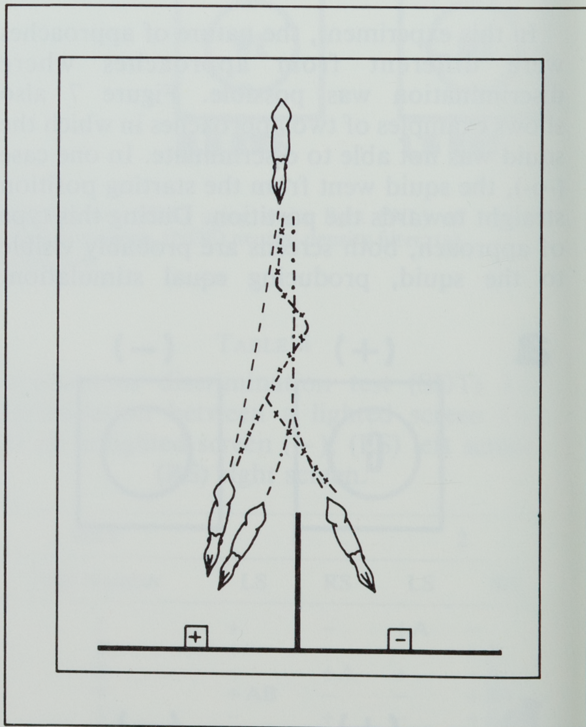

Figure 7. Examples of typical squid approaches to positive (+) or negative (−) screen; (---) a clear approach to positive screen, (-·-) and (-x-) approaches which could be directed to either screen.

The green light, with higher transmissivity maximum (45%), appeared brighter than the blue light (41.3%).

Experiment SDT 2-1 (Figure 6a). In order to establish a positive image the green light was presented together with an unlighted screen.

TABLE 7

Simultaneous discrimination test (SDT) 1-4; rediscrimination between a white light with a 0.5 neutral density filter (+) and white light with a 1.5 neutral density filter (−); (LS) left screen, (RS) right screen.

DAY	1		2		3		4	
Trial Number	LS	RS	LS	RS	LS	RS	LS	RS
1	−A	+B	+	−A	−	+A	+AB	−
2	−	+A	−	+A	+	−A	+B	−
3	−	+A	−B	+A	+B	−A	−	+B
4	+B	−	−	+AB	−	+AB	−	+B
5	+BA	−	+B	−	−	+A	−	+B
6	−	+A	+	−B	−B	+A	+B	−
7	+	−B	−	+A	+AB	−	+B	−
8	−A	+B	+	−A	+B	−	−B	+
9	+	−AB	−	+A	−B	+A	+B	−
10	+	−A	+A	−	+B	−A	+B	−
11	+	−A			+B	−A		
12	−	+AB			−B	+A		
13	−	+AB			+A	−B		
14	−	+B			+B	−A		
15	+B	−						
16	+A	−						
Total responses								
Squid A	2(+) 2(−)	5(+) 3(−)	1(+)	5(+) 2(−)	2(+)	6(+) 5(−)	1(+)	0 0
Squid B	3(+)	5(+) 2(−)	1(+) 1(−)	1(+) 1(−)	6(+) 3(−)	1(+) 1(−)	6(+) 1(−)	3(+)
Total number of trial	16		10		14		10	
Attack level %								
Squid A	75		80		93		10	
Squid B	62		40		78		100	
Correct responses %								
Squid A	58		75		62		LOW	
Squid B	80		50		63		90	

TABLE 8

Simultaneous discrimination test (SDT) 2-1; discrimination between a lighted screen (+) using green light (523.5 nm) and an unlighted screen (−); (LS) left screen, (RS) right screen.

DAY	1		2	
Trial Number	LS	RS	LS	RS
1	+AB	−	−	+AB
2	−	+AB	+AB	−
3	+B	−A	−	+BA
4	−	+AB	−	+AB
5	−	+AB	−	+AB
6	−B	+A	+AB	−
7	+AB	−	+AB	−
8	+AB	−	+AB	−
9	+AB	−	−	+AB
10	−	+AB	+AB	−
Total responses				
Squid A	4(+) 0	5(+) 1(−)	5(+) 0	5(+) 0
Squid B	5(+) 1(−)	4(+) 0	5(+) 0	5(+) 0
Attack level %				
Squid A	100		100	
Squid B	100		100	
Correct responses %				
Squid A	90		100	
Squid B	90		100	

Both squid responded to all trials presented during the two-day test as tabulated in Table 8. The performance of both squid were the same making 90% correct responses on day one 100% on day two.

TABLE 9

Simultaneous discrimination test (SDT) 2-2; discrimination between positive screen (+) with green light (523.5 nm) and negative screen (−) with blue light (450 nm) both masked with 0.5 neutral density filters; (LS) left screen, (RS) right screen.

DAY	1	
Trial Number	LS	RS
1	−B	+A
2	+B	−A
3	−B	+A
4	+B	−A
5	−B	+A
6	−B	+A
7	−B	+A
8	+B	−A
9	+B	−A
10	+AB	−
Total responses		
Squid A	1(+)	5(+)
	0	4(−)
Squid B	5(+)	0
	5(−)	0
Attack level %		
Squid A	100	
Squid B	100	
Correct responses %		
Squid A	60	
Squid B	50	

TABLE 10

Simultaneous discrimination test (SDT) 2-3; discrimination between positive screen (+) with green light (523.5 nm) and negative screen (−) with blue light (450 nm); (LS) left screen, (RS) right screen.

DAY	1		2	
Trial Number	LS	RS	LS	RS
1	+B	−A	−B	+A
2	−AB	+	−A	+B
3	−	+AB	−	+AB
4	−A	+B	+B	−
5	+A	−B	+A	−B
6	+B	−A	+	−AB
7	+	−A	−A	+B
8	−	+AB	+	−AB
9	+	−AB	−A	+B
10	−AB	+	+A	−B
Total responses				
Squid A	1(+)	2(+)	2(+)	2(+)
	3(−)	4(−)	3(−)	2(−)
Squid B	2(+)	3(+)	1(+)	4(+)
	2(−)	2(−)	1(−)	4(−)
Attack level %				
Squid A	100		90	
Squid B	90		100	
Correct responses %				
Squid A	30		44	
Squid B	55		50	

Experiment SDT 2-2 (Figure 6b). To test color discrimination a green positive screen was matched against a blue negative screen with both light sources masked by 0.5 NDF. One day test showed both squid performing at chance level for this situation as tabulated in Table 9.

Experiment SDT 2-3 (Figure 6c). To produce a brighter screen for this experiment, the 0.5 NDF were removed for both screens. The two day tests demonstrated that again, the two squid performed at chance level as tabulated in Table 10.

Experiment SDT 2-4 (Figure 6d). In this experiment, the light source of the green screen was masked with a 0.5 NDF, while the blue shape had none. This final series of test was conducted over four days, and the results tabulated in Table 11 clearly indicated that the squid were not able to discriminate between the two colors.

Discussion

Studies on discrimination learning in octopus are always done with the test animal in isolation. This was not followed since the squid, *T. pacificus*, in isolation as observed in holding tanks were not feeding well and were seen repeatedly hitting the walls with their fins. In the experiments, the response of one squid to screens presented did not affect the response of the other. As early as day 2 and 3 in Pretraining 2, it could be seen that an approach of one squid on a screen was not always followed by the other. There were individual approaches as well as approaches in pair. Two baits were always made ready before each trial so that each squid can be given its share when both

TABLE 11

Simultaneous discrimination test (SDT) 2-4; discrimination between positive screen (+) with green light (523.5 nm) masked with a 0.5 neutral density filter, and negative screen (−) with blue light (450 nm); (LS) left screen, (RS) right screen.

DAY	1		2		3		4	
Trial Number	LS	RS	LS	RS	LS	RS	LS	RS
1	−B	+A	−A	+B	+	−A	+A	−
2	−A	+B	+B	−A	+	−	+	−AB
3	+	−AB	+	−AB	+A	−	−B	+A
4	+A	−B	+	−A	−	+A	+B	−A
5	+A	−B	−A	+B	+A	−	−	+B
6	−AB	+	+A	−B	−A	+	+B	−
7	+AB	−	−A	+B	+	−	−B	+
8	−A	+	+A	−	−	+A	−B	+
9	+AB	−	−AB	+	−A	+	−B	+
10	−	+AB	−A	+	−	+A	+B	−
Total responses								
Squid A	4(+)	2(+)	2(+)	3(−)	3(+)	3(+)	1(+)	1(+)
	3(−)	1(−)	5(−)	0	2(−)	1(−)	0	2(+)
Squid B	2(+)	2(+)	1(+)	3(+)	0	0	3(+)	1(+)
	2(−)	3(−)	1(−)	2(−)	0	0	4(−)	1(−)
Attack level %								
Squid A	100		100		90		40	
Squid B	90		70		0		90	
Correct responses %								
Squid A	60		20		66		50	
Squid B	44		57		0		44	

squid made a correct approach to a positive screen.

The learning ability of the squid was established when it made discriminations between screens presented with different intensities (SDT 1-1 and 1-2). The same rapid learning ability is also displayed by the octopus (Messenger & Sanders, 1972) where after only four sessions, all octopuses in the one-cue experiment made brightness discriminations at a level significantly above chance. This learning ability of the squid was further demonstrated when it discriminated screens previously presented (SDT 1-4) after going through a two-day test (SDT 1-3) where it was not to discriminate. Similar behavior was also observed for octopus where after a non-discrimination stage using violet/grey vertical rectangles, the animal was able to discriminate reintroduced black/white vertical rectangles (Messenger, 1977b).

In Experiment SDT 2-1 where an unlighted screen was presented together with a screen with green light projected, there were two contrasting cues, one was the big difference in intensity and the other was color. Using the two cues, even an animal without color vision can still distinguish one screen from the other using the intensity difference as the cue which is similar to the situation in Experiment SDT 1-1. The high percentage of correct attacks even for the first session suggest that the squid was using intensity difference and not color as the primary cue.

Assuming that the squid was using color as the cue, it would be difficult to attain a high percentage of attacks at the very start of experimentation since the squid would be viewing the colored screen as a new image. In the pretraining sessions, it took four days before a squid was able to attain a 100% attack level. During this period, the squid was getting itself accustomed to the white light projected on the screen and associating it with the bait presented simultaneously. Therefore, it appears that a new screen, presented without the presence of bait, requires a period for learning the consequences of an approach to this screen. But, without color vision, this colored screen would only appear as a lighted screen, brighter than the unlighted screen which would be a condition similar to Experiment SDT 1-1. Thus the 100% attack level and 90% correct responses were attained by the squid at the first session and then perfected in the session that followed. The nature of approaches here was the same with those of SDT 1-1 and other experiments where discrimination was possible.

For color discrimination test using blue and green lights projected on screens, at first both colored lights were masked by 0.5 NDF with the intention of presenting them at low intensity. The results were not significant with both squid making aproaches to either screen in all trials. To increase the light intensity, the neutral density filters were removed. Again, the squid responding to almost all trials showed familiarity to both screens presented. Even at higher intensities, the performances were not significant. Recall that at these conditions, the green screen appeared slightly brighter than the blue screen. But this difference in intensity was not enough for the squid to discriminate between the screens presented. In the final experiment (SDT 2-4) with the green screen masked by 0.5 NDF to lessen the intensity difference, the results for four sessions were the same as those of the previous experiments.

Fehring (1972) in hue discrimination experiments used a similar technique on loggerhead turtles which were suspected to have color vision. He reported fast discrimination with most specimens making perfect scores after only two days. Since *T. pacificus* rapidly learned to discriminate in intensity discrimination tests (SDT 1-1 and 1-2), then if the squid possessed color vision, they should have learned at about the same rate during the color discrimination tests (SDT 2-2, 2-3, and 2-4).

The findings in the present study agree with the work of Orlov and Byzov (1962). With regard to color vision, *T. pacificus* and *O. vulgaris*, studied by Messenger *et al.* (1973) and Messenger (1977b, 1979), are the same. With regard to squid fishing, the use of colored underwater lights does not seem practical in view of the findings in this study. Since both colored and white lights are treated similarly by the squid, it is not necessary to expend the extra energy to produce colored lights.

Acknowledgement

I wish to thank the Director and Staff of the Hokkaido Hakodate Fisheries Experimental for their facilities. I also thank Dr S. Igarashii and Mr T. Mikami for their assistance and encouragement during the course of this study, and Drs C. Roper and R. Hanlon for their careful reading and criticism of the manuscript. This study was supported by the Japanese Ministry of Education and Culture.

References

Fehring, W. K., 1972. Hue discrimination in hatching loggerhead turtles (*Caretta caretta caretta*). Anim. Behav. 20: 632-636.

Flores, E. E. C., Igarashi, S., Mikami, T., & Kobayashi, K., 1976. Studies on squid behavior in relation to fishing I. On the handling of squid, *Todarodes pacificus* Steenstrup, for behavioral study. *Bull. Fac. Fish. Hokkaido Univ.* 27: 145-151.

Flores, E. E. C., Igarashi, S., & Mikami, T., 1977. Studies on squid behavior in relation to fishing II. On the survival of squid, *Todarodes pacificus* Steenstrup, in experimental aquarium. *Bull. Fac. Fish. Hokkaido Univ.* 28: 137-147.

Flores, E. E. C., Igarashi, S., & Mikami, T., 1978. Studies on squid behavior in relation to fishing III. On the optomotor response of squid, *Todarodes pacificus* Steenstrup, to various colors. *Bull. Fac. Fish. Hokkaido Univ.* 29: 131-140.

Messenger, J. B. & Sanders, G. D., 1971. The inability of *Octopus vulgaris* to discriminate monocularly between oblique rectangles. *Intern. J. Neuroscience* 1: 171-173.

Messenger, J. B. & Sanders, G. D., 1972. Visual reference and two-cue discrimination learning in *Octopus*. *Anim. Behav.* 20: 580-585.

Messenger, J. B., Wilson, A. P., & Hedge, A., 1973. Some evidence for colour-blindness in *Octopus*. *J. exp. Biol.* 59: 77-94.

Messenger, J. B., 1977a. Pre-capture and learning in the cuttlefish, *Sepia*, p. 347-376. In Nixon, M. and Messenger, J. B. (eds.). *The Biology of Cephalopods*. 615 pp. Academic Press, London.

Messenger, J. B., 1977b. Evidence that *Octopus* is colour blind. *J. exp. Biol.* 70: 49-55.

Messenger, J. B., 1979. The eyes and skin of *Octopus*: compensating for sensory deficiencies. *Endeavour, New Series*. 3: 92-98.

Orlov, O. Yu & Bysov, A. L., 1962. Soviet study on the eyesight of squids. Translated from *Priroda Mosk* 51: 115-118.

Roffe, Y., 1975. Spectral perception in *Octopus*: A behavioral study. *Vision Res.* 15:353-356.

Sutherland, N. S., 1957. Visual discrimination of orientation and shape by the octopus. *Nature Lond.* 179: 11-13.

Sutherland, N. S. & Muntz, W. R. A., 1959. Simultaneous discrimination training and preferred direction of motion in visual discrimination of shape in *Octopus vulgaris* Lamarck. *Pubbl. Staz. zool. Napoli* 31: 109-126.

Sutherland, N. S., 1960. Visual discrimination of shape by *Octopus*: squares and rectangles. *J. Comp. Physiol. Psychol.* 53: 95-103.

Sutherland, N. S., 1960a. Theories of shape discrimination in *Octopus. Nature Lond.* 186: 840-844.

Sutherland, N. S., 1963. Visual acuity and discrimination of stripes widths in *Octopus vulgaris* Lamarck. *Pubbl. Staz. zool. Napoli* 33: 92-109.