Visual discrimination and memory of *Enteroctopus dofleini*, the Giant Pacific Octopus

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Spring 2013

Introduction:

Learning has been demonstrated in various invertebrates, but none have been studied as successfully and deeply as the octopus (Papini & Bitterman 1991). Octopuses have been the center of behavioral studies on marine organisms for the last 50 years (Hochner et al. 2006). Unlike other marine invertebrates, octopuses have strong cognitive and sensory capabilities as well as a complex brain and nervous system (Boal 1996; Williamson & Chrachri 2004; Hochner et al. 2006). They have a central nervous system consisting of about 500 million cells that is similar to a vertebrate brain (Hochner et al. 2006). Hochner et al. (2006) states that these sophisticated animals have shown sensitization, associative learning including tactile and visual discrimination, habituation, and spatial learning. They have a natural curiosity that is easily initiated and creates ideal conditions for experiments with emphasis on visual discrimination (Hochner et al. 2006). It has been shown in many studies that octopuses can learn and remember information for multiple days (Hochner et al. 2006; Papini & Bitterman 1991). It is commonly understood that the complexity of the octopus nervous system along with the unique folding of the octopus brain give this organism a strong memory and the propensity to learn (Hochner et al. 2006). It is likely that these are the reasons that species of octopuses have been used consistently in appetitive and operant conditioning (Crancher et al 1972; Papinim & Bitterman 1991). Operant conditioning is characterized by a specimen's spontaneous behavior resulting with reward or punishment, while appetitive (commonly referred to as classical) is more dependant on reflexes that are responding to a prior stimulus (Martin-Soelch et al. 2007).

Studies have shown that octopus have the ability to learn, retain spatial information, and solve visual discrimination problems especially in the presence of positive reinforcement (Young 1956; Boal 1996; Boal et al. 2000). In a study by Shashar and Cronin (1995) it was demonstrated that octopuses could be readily trained to pick targets with specific patterns purely based on if the pattern was present or not. Experiments conducted by Papini and Bitterman (1991) using 37 octopuses supported their hypothesis that octopuses are promising animals for comparative analysis of invertebrate learning. When octopuses forage in the wild they are able to return to and recognize their home den (Boal et. al 2000). This example of octopus memory studied by Boal et. al (2000) supports the claim that they are capable of remembering things they have previously encountered.

After observing the juvenile Giant Pacific Octopus at the Hatfield Marine Science Center aquarium we concluded that it would be a fascinating creature to work with. Based on this previous research as well as observations of an octopus's ability to open food filled jars, we questioned whether *Enteroctopus dofleini*, the Giant Pacific Octopus, could be taught to perform a task using operant conditioning. The objective of this experiment was to use positive reinforcement in the form of food to train an individual octopus to open a jar with a specific pattern on it. This will test the octopus's memory and its ability to visually discriminate between objects like in the study done by Shashar and Cronin (1995). We will be testing to see if the octopus behavior follows operant conditioning models as well as our hypothesis that the octopus will show a preference to the patterned jar that it is given positive reinforcement for opening. The null hypothesis of this experiment is that the octopus will show no learning or preference to the testing jar.

Methods:

This project was conducted in the aquarium at Hatfield Marine Science Center in Newport, Oregon. The tank housing the Giant Pacific Octopus was indoors and had a constant flow of seawater pumped from the bay. The experiment took place over a six-day period with the trials occurring during a one-hour window each day at eleven AM. The food used in each trial was determined by a senior aquarium staff member and was shrimp, squid, or fish. Opaque black and white patterned jars with screw top lids were approved by the octopus caretakers and used daily to hold the food for the experiment (Figure 1). We started the experiment by floating a water filled, food containing, striped jar in the octopus's tank and ran three trials over a two-day period. We recorded the amount of time it took the specimen to wrap its tentacles around the jar actively choosing it and resulting in a definite grab. We also recorded the time it took the octopus to fully unscrew the jar and come in contact with the food source.

After, we gave the octopus four patterned jars and ran four trials with the food consistently in the striped jar. We tested the octopus's memory and how well it had been conditioned by using three patterned jars that did not contain food and one patterned jar that did. The striped jar consistently contained food, and the other jars were scented. We chose to scent the jars that did not contain food in order to control for the octopus's sense of smell. We filled the jars with water before floating them in the tank and measured the amount of time it took for

the octopus to fully grab the striped jar as well as how long it took it to unscrew the lid in order to obtain the food. For each trial we changed the position of the patterned jars and order to see if the octopus recognizes the striped jar and returned to it consistently. When the octopus opened the striped jar it was allowed to eat the contents inside as a form of positive reinforcement for recognizing the specific patterned jar. After the octopus had a consistent time of finding the striped jar, it was given the same four jars but empty, and we ran one trial to see if it had been habituated to open the striped jar. We did this trial in order to see if the octopus was actually remembering the striped jar contained food or using its sense of smell to locate the food.

Finally, we gave the octopus four patterned jars, and put the food in the checkered jar that was randomly selected to test whether the octopus simply chose the jar because it recognized that jar had food in it or if it chose the jar because it was striped. We ran two trials with the food in the random jar and recorded the time it took for the octopus to find/open the jar that contained the food. Throughout the experiment we documented the number of times the octopus chose the striped jar first and how long it took, as well as the time it took it to obtain the food.

All data was recorded in a notebook and then transferred into an Excel data sheet. After obtaining all our data we ran a T-test as well as ANOVA statistical tests in Minitab to determine if our results were significant.

Results:

We compared the time it took the octopus to do a definite grab onto the striped jar when there was just the single striped jar containing food in the tank and when there were four patterned jars in the tank with the striped jar containing food (Table 1). After running a T-test to statistically compare the two groups, it was evident that the results were not significant (P-value= 0.192). Then we ran an ANOVA test with a Tukey comparison to find the significance of how long it took the octopus to make a definite grab on the striped jar between all three experimental conditions. The results from the ANOVA yielded significant results showing that there was a difference between the times it took for the octopus to grab the striped jar when presented with the single striped jar containing food, four patterned jars with the striped jar containing food, and the four jars with a random jar containing food (P-value< 0.0001, Figure 1, Table 1). The Tukey comparison showed that there was a significant difference between the times it took for the octopus to grab the single striped jar containing food in the tank and the other two experimental

trials ($P < 0.05$, Figure 1). It took significantly longer for the octopus to grab the striped jar when the food was in the checkered jar and all patterned jars were present. The least amount of time for a full grab of the striped jar occurred when the food was in the striped jar with all other patterned jars present in the tank (Figure 1). We also ran an ANOVA to determine if the data was significant in the amount of time it took the octopus to unscrew the lid and come into contact with the food in all three of the experimental conditions. The ANOVA results showed that there was no significant difference in times between the three groups (P-value= 0.909, Figure 2).

Discussion:

We concluded from this study that the octopus we worked with did not show preference for the striped jar, thus leading us to believe that it did not learn in this experiment. Our data showed that the amount of time it took the octopus to make a definite grab onto the striped jar containing food when there was one jar or four jars present was significant. We found that the octopus did not vary in the amount of time it took to come into contact with the food no matter what experimental trial we ran. The results were non-significant for the difference in time it took to grab a single striped jar containing food and four patterned jars. There was no difference in how quickly the octopus made a definite grab onto the striped jar based on the number of jars in the tank. This was an unexpected result because past studies showed that octopuses learn to target a specific pattern by visual discrimination (Hochner et al. 2006; Papini & Bitterman 1991). Shashar and Cronin (1995) demonstrated that octopuses could distinguish between targets on the basis of a particular pattern being present or not. Their study found that a correct choice of a pattern was based solely on the visual discrimination between targets (Shashar and Cronin 1995).

When comparing all three experimental trials and the amount of time it took for the octopus to grab the striped jar, it became evident that there could be another contributing factor other than visual recognition influencing the experiments. We hypothesize that a possible factor in the ability of the octopus to choose the right pattern could be scent. Even though all jars were scented, the jar containing the food may have had a stronger scent due to the actually food source being present. Grabbing the jar with the strongest scent would result in getting the food quicker. The octopus could have also learned from the previous trials that the striped jar would result in positive reinforcement, thus causing a difference in how long it took to grab the striped jar.

We tested to see if there was a difference in how long it took the octopus to unscrew the jar lid and come into contact with the food between the three different experimental trials. These data did not yield significant results because it took the same amount of time for the octopus to find food in the in each experimental trial. It did not matter when the food was put into a random jar; the octopus took about the same amount of time to unscrew the top. The octopus could have been using its sense of smell to find the jar that contained food and thus taken about the same amount of time to smell out the correct jar. These results could be due to the fact that the octopus we worked with had only been in captivity for a month and this experiment was its first time ever opening a screw top lid. The octopus struggled to unscrew the lid until the caretaker took the jar and showed it how the lid could be twisted on and off during the first trial. According to Shashar and Cronin (1995) octopuses are able to transfer learning experiences to other situations, such as observing something and then executing another similar task. Before being showed how the lid worked the octopus was just holding the jar in its tentacles with no visible signs of attempts to unscrew the lid.

When working with a sophisticated animal there are many variables that can play into an experiment (Hochner et al. 2006). One main factor that could have influenced our data was the mood of the animal on the different days we tested it. Octopuses are known to display different moods and personalities, much like a human adolescents (Mather and Anderson 1993). We noticed that some days the octopus seemed to be very lively and up to the task of opening the jars, while on other days it did not show much interest in them at all. Multiple times we observed color changes of the octopus during the trials and the aquarium staff associates those color changes with behavior and moods they have observed over the time period this octopus has been in their care. The octopus may have also been playing with the jars on certain days, which could contribute to why some days it took it a lot longer to open the jars during some trials. Octopuses are curious creatures that do play with objects when they are present in their tank (Mather and Anderson 1993, 1999). If the octopus was merely playing with the jars during trials it could easily influence our data.

There is also the factor of hunger levels to drive the octopus's time of interacting with the jars. On days with multiple trials it may not have been as quick to open a jar after the first trial because it no longer had hunger driving its time. One trial the octopus opened the jar, pulled out the food, and let to food sink to the bottom of the tank. It was clearly not interested in the food

that day leading us to believe that it probably was not hungry during that particular set of trials. Since these animals show very complex behaviors they often present a dilemma because their natural curiosity can lead them to behave “incorrectly” even after prolonged training about 20% of the time (Shashar and Cronin 1995). This could also be a contributing factor to why our data yielded results that did not fully support our hypothesis.

Human error is always a possibility in experiments and our study had areas that could be improved in order to minimize inaccuracy. We took turns observing the octopus and each of us could have interpreted the animal or our observations differently. There was also variability in how tight the lids of the jars were screwed on because different people sealed the jars for the trials. Since the octopus struggled to get the lid off when the jars were screwed on tightly, there was no exact point to which the jars were tightened.

Based on the data gathered from our experiments and statistical analyses our hypothesis of octopuses being able to learn and be conditioned to respond to a specific pattern was not fully supported. While the octopus we worked with did have some statistical significance in the amount of time it took it to grabbed the striped jar, there are too many variables possibly affecting the data to tell if it actually learned to visually discriminate the striped jar from the others or if a conditioned response was achieved. This study did however teach the octopus how to open screw top lids. Caution is advised when using these data and more testing with this animal would be beneficial to this study.

Figure 1. These are the four jars we used in the experiment. Each one had a different pattern that was drawn on with a non-toxic Sharpie. The patterns were referred to from left to right: big dots, stripes, checkers, and small dots. They were made of plastic and approved by the aquarium to be safe for the octopus to work with. The jars had screw top lids and floated in the water.

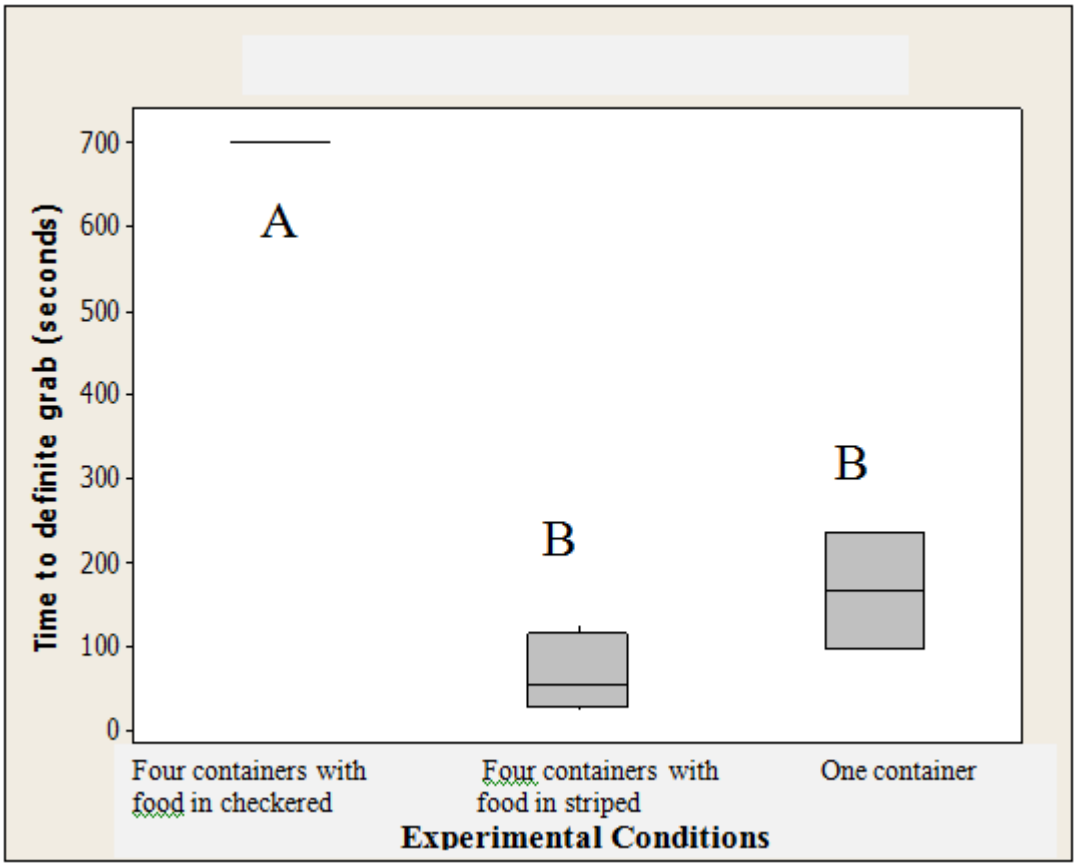

Figure 2. This boxplot shows the amount of time it took for the octopus to grab the striped jar in each experimental trial. The results are from an ANOVA test generated in Minitab. Four checkered containers with food in checkered is representing all four jars in the tank with the food placed in the checkered jar that was randomly selected. Four containers with food in striped represents four jars in the tank with the striped on containing food. One container is the data from when only the striped jar with food was in the tank. Groups A and B are from the Tukey comparison results. Four containers with food in checkered is significantly different from the similar B groups. All time was recorded in a notebook and then transferred into an excel sheet. As seen in the graph there is a significant difference in the amount of time it took the octopus to fully grab the striped jar the three experimental conditions.

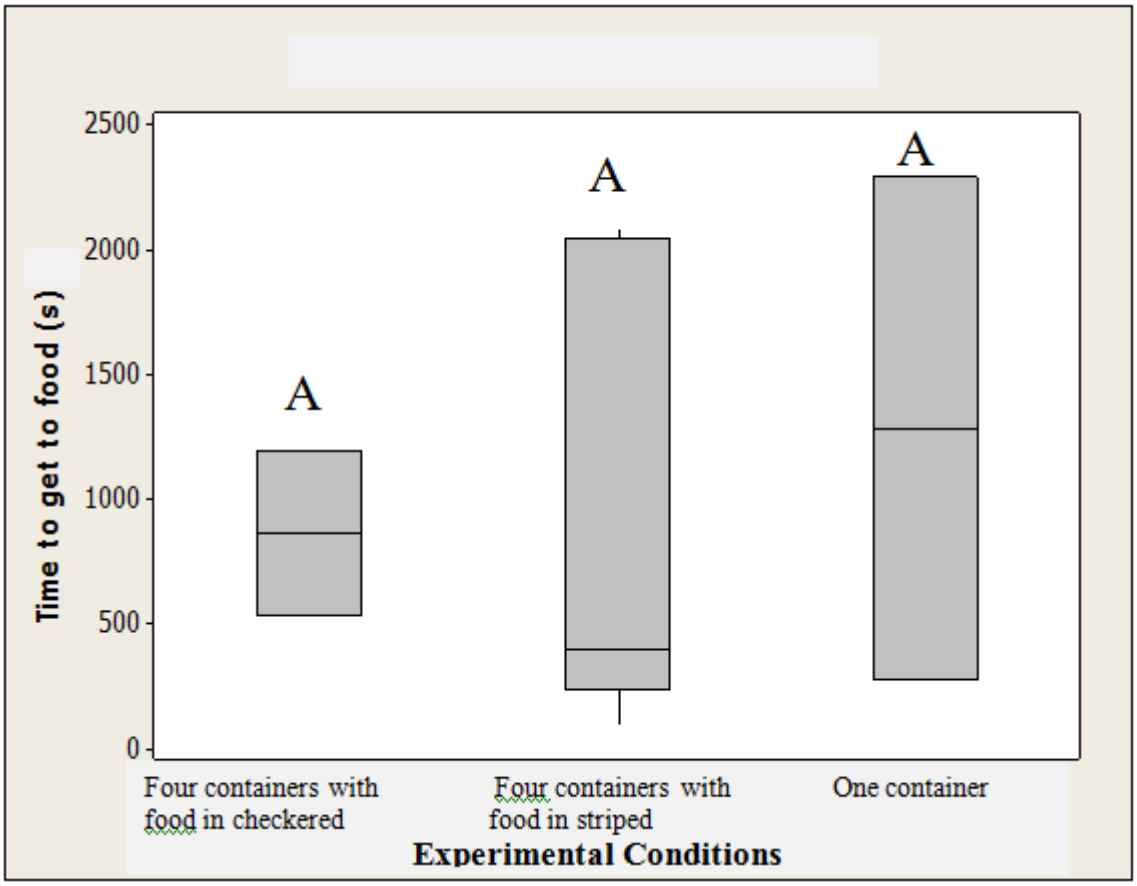

Figure 3. This boxplot represents the amount of time it took the octopus to come into contact with the food. In order to get the food the octopus had to unscrew the lid from the jar and time stopped as soon as the lid was off with a tentacle clearly in the jar. Data was analyzed and graphs were created using Minitab. Four checkered containers with food in checkered is representing all four jars in the tank with the food placed in the checkered jar that was randomly selected. Four containers with food in striped represents four jars in the tank with the striped on containing food. One container is the data from when only the striped jar with food was in the tank. Group A is the results from the Tukey comparison test. As seen in the graph there is no significant difference in the amount of time it took for the octopus to obtain the food.

Table 1. This table shows the data collected on each day along with notes about how the trials were conducted. Each day had multiple trials depending on the amount of time that was available for us to work in. The Day column contains what day the trials took place as well as what experimental condition was being tested. All testing occurred at the Hatfield Marine aquarium supervised by an octopus caretaker.

Day	Trial 1	Trial 2	Trial 3	Notes:
1	NA	NA	NA	The equipment we tried to use was deemed unsafe for the octopus so no data was collected.
2 Only the striped jar containing food in the tank	Time to grab striped jar: 1 minute and 6 seconds Time to open jar: 38 minutes and 10 seconds	NA	NA	Made new design for patterned jars and they were approved. First time specimen has ever opened a screw top jar. Only had time for one trail.
3 Only the striped jar containing food in the tank	Time to grab striped jar: 3.56 seconds Time to open jar: 4 minutes and 35 seconds	Time to grab striped jar: 56.3 seconds Time to open jar: 33 minutes and 50 seconds	NA	Showing progress on unscrewing the jar lid.
4 Four different patterned jars in the tank, striped jar containing food	Time to grab striped jar: 29 seconds Time to open jar: Did not open jars, they were pulled from the tank at 16 minutes and 29 seconds due to time constraints	Time to grab striped jar: 14 seconds Time to open jar: 18 minutes and 10 seconds	Time to grab striped jar: 7 seconds Time to open jar: 6 minutes and 45 seconds	Jars were pulled from the tank in trial 1 in order loosen the tops, since they were tighter than in previous trials.
5 Four different patterned jars in the tank, striped jar containing food	Time to grab striped jar: 13.6 seconds Time to open jar: 1 minute and 33 seconds	Time to grab striped jar: 5 minutes and 6 seconds Time to open jar: 6 minutes and 2 seconds	NA	Octopus opened checkered jar first, then small dots jar, and then striped jar, seemed to be playing with the jars.
6 Four different patterned jars in the tank, no jars containing food	Time to grab striped jar: 22 seconds Time to open jar: Striped jar never opened, jars pulled at 6 minutes and 52 seconds.	NA	NA	Octopus opened checkered jar but not interested in striped one.
6 Four different patterned jars in the tank, food in randomly selected jar (checkered)	Time to grab striped jar: never grabbed striped jar Time to open jar: never opened striped jar	Time to grab striped jar: 37 seconds Time to open jar: 12 minutes and 10 seconds	NA	Did not show preference for the striped jar, seemed to be playing with the jars.

Literature Cited:

Boal, J. G. 1996. A review of simultaneous visual discrimination as a method of training octopuses. Biological Reviews. **71**: 157–190.

Boal, J. G., A. W. Dunham, K. T. Williams, and Hanlon, R.T. 2000. Experimental evidence for spatial learning in octopuses (*Octopus bimaculoides*). Journal of Comparative Psychology **114**: 246–252.

Crancher, P., King, M. P., Bennet, A., and Montgomery, R.B. 1972. Conditioning of a free operant in *Octopus cyaneus* Gray. Journal of Experimental Analysis of Behavior **17**: 359–362.

Hochner, B., Shomrat, T., and Fiorito, G. 2006. Octopus: A Model for a Comparative Analysis of the Evolution of Learning and Memory Mechanisms. Biological Bulletin **210**: 308 - 317.

Martin-Soelch, C., Linthicum, J., and Ernst, M. 2007. Appetitive conditioning: neural bases and implications for psychopathology. Neuroscience Biobehavior Review **31**(3): 426 – 440

Mather, J. A. and Anderson R. C. 1999. Exploration, Play, and Habituation in Octopuses (*Octopus dofleini*). Journal of Comparative Psychology **113** (3): 333-338.

Mather, J. A. and Anderson R. C. 1993. Personalities of Octopuses (*Octopus rubescens*). Journal of Comparative Psychology **107**(3): 336-340.

Papinim, M. R. and Bittermamn, M. E. 1991. Appetitive conditioning in *Octopus cyanea*. Journal of Comparative Psychology **105**: 107-114.

Shashar, N. and Cronin, T. 1996. Polarization contrast vision in octopus. Journal of Experimental Biology **199** (4): 999.

Williamson, R. and Chrachri A. 2004. Cephalopod neural networks. Neurosignals **13**: 87.

Young, J. Z. 1956. Visual responses by Octopus to crabs and other figures before and after training. Journal of Experimental Biology **33**: 709–729.