LEARNING IN *OCTOPUS BOCKI*

CHARLOTTE JAMAR

Integrative Biology, University of California, Berkeley, California 94720 USA

Abstract. The goal of this paper is to elaborate on our understanding of cognitive abilities via *Octopus bocki*. In the realm of intelligence research, Octopuses are unique because they evolved their complex cognition independently from vertebrates. Thus, these animals, along with other cephalopods, provide us with models onto which we can compare and seek understanding of our own cognition. This study explores four objectives. First, the study aims to test learning, a key indicator of intelligence, by repeatedly giving octopuses a problem to solve. This study also investigates memory by testing the octopuses over the short term (one hour) as well as over the long term (one day). Third, this study compares the octopuses' performance on problems of different complexity. Lastly, the study compares the performance of juvenile octopuses versus adult octopuses. The experiment uses light to incentivize the octopuses to solve the problems, which consist of divider(s) the octopus has to get through. The results indicate that *O. bocki* is capable of learning and that there is significant variation in performance between problems of different levels of complexity and life history stages, but not between day or trials within days. The suggestion that *O. bocki* can learn has implications for the realm of species that have sentience.

Key words: Octopus bocki; learning; intelligence; Cephalopod; Moorea, French Polynesia

INTRODUCTION

Our brain provides us with the tools to understand the brain, yet the abilities of critical thinking, creativity, and collaboration it provides us bring us nowhere close to catching up to understanding it in its glorious complexity. In other words, our brains outsmart our minds. Additionally, the more we learn about the brain, the more we realize how much we do not understand about it (Gorman 2014).

As the human brain is the most complex brain, it can be helpful to use animal brains as models to better understand our own (Richter 2016, Hockner 2006, Zarella 2015). We can trace the evolution of the brain through phylogenetic analysis of animals that are alive today; from nerve nets in flatworms to chimpanzee brains similar in complexity to ours (Godfrey-Smith 2016). For some reason, human brains evolved to be exceptional among animals. However, by increasing our understanding of brains of other animals, we can piece together knowledge of our own brain.

Octopuses are in the cephalopod class, which also contains squid and cuttlefish. Six hundred million years ago, there existed a small marine invertebrate that was probably worm-like, had some sort of elementary nervous system, and possibly had eyes (Godfrey-Smith 2016). This early ancestor would differentiate into many lineages, one of which would lead to octopuses, and one of which would lead to all vertebrates. This latter lineage evolved the most "intelligent" animals – those with the largest brains, highest brain to body ratios, and most complex behavior. But intelligence also evolved in the cephalopods, in a clear case of convergent evolution (Packard 1972). Cephalopod intelligence is believed to have evolved due to predation and competition with vertebrates (Fiorito 2013). This independent evolution of complex neurological capabilities in cephalopods provides a unique system for comparison to the neurology of vertebrates, including humans (Richter 2016). Understanding these animals can help us understand ourselves.

Out of all of the invertebrates, octopuses have the most complex neurology (Richter 2016). Cuttlefish and squid have barely been studied because they are hard to observe in the wild and keep in the lab (Boycott 1965). *Octopus* is the choice genus for research, however there is still much not understood about its neurology and behavior (Richter 2016). Additionally, because invertebrates do not require a permit to research, Octopuses are great candidates for studying intelligence. There have been many studies on octopus problem solving, learning, object recognition, etc. These studies have mostly used the common octopus, *O. vulgaris* (Hockner 2006). The present study features *O. bocki*, a pygmy species found in the South Pacific and the

Philippines of which little is known. There has only been one published study on *O. bocki,* on its inking behavior as defense against predation (Caldwell 2005). However, there have been seven papers written in this class on *O. bocki*, one of which was on learning (Lebensohn 1999).

Thus, my general goal was to investigate intelligence of octopuses further by conducting experiments on learning in *O. bocki.* My study had four specific goals: (1) test problem-solving performance as a function of solution time over multiple trials (Fiorito et al. 1990); (2) compare the effect of long term (one day) and short term (one hour) temporal spacing between trials on memory and learning; (3) compare performance (as a function of solution time as well as success rate) on a problem with two levels of complexity; and (4) compare performance (as a function of solution time as well as success rate) between adult and juvenile octopuses.

METHODS

Biology of O. bocki

Octopus bocki is a pygmy species that has been recorded in the Philippines, Fiji, and French Polynesia, although *O. bocki* likely has a much broader range (Cheng 1996). The species is abundant in Moorea where it is the most common octopus (Caldwell, personal communication) and inhabits the crevasses of coral rubble with depth between 0.1-30 m (Encyclopedia of Life). *O. bocki*'s mantle reaches a length up to 3 cm. *O. bocki* can display a range of colors between white and reddish brown. *O. bocki* is sexually dimorphic; the males have enlarged suckers on their second and third tentacles (Cheng 1996). *O. bocki* is also thigmotactic, nocturnal, and very sensitive to light (Ramirez, Oakley 2015).

Collection Site

The octopuses were collected on the fringing reef adjacent to the UC Gump Research Station in Cook's Bay, Moorea, French Polynesia at 17.4905° S, 149.8264° W.

Octopus and food collection

Octopuses and their food (such as galathoid crabs, xanthids, stomatopods and alpheus) were collected from coral rubble. Coral rubble (~ 40x20x10 cm) was placed on cinder blocks in a plastic container (70x50x40 cm) on the shore next to the fringing reef. The coral rubble was rinsed with seawater so that the water level of the container reached approximately one centimeter below the coral. After 30-60 minutes, the coral and cinder blocks were taken out and any octopuses/ crabs/shrimp were collected from the container. The octopuses were transported back to the Gump Station and placed into their individual containers as described below and the crabs/ shrimp were also placed into their own container. The coral rubble was put back into the ocean. Occasionally, a hammer was used to break pieces of the coral rubble to look for crabs/ shrimp.

The animals were measured and octopuses whose mantle measured 5 cm or less were deemed juveniles, otherwise they were considered adults (Cheng 1996).

Octopus storage

Each octopus was kept in a plastic container (20x12x4cm) fitted with mesh held in place by rubber bands. The containers had two slots across the width of the container, 8 cm from the edge and three centimeters apart. To prevent the octopuses from getting bored, I filled the containers with sand and a variety of shells and PVC tubing that I switched out daily. The containers were each kept under running water and cleaned twice a week. The octopuses were fed a crab, whose carapace was no wider than the length of the octopus' mantle (Pearson 1994), every 48 hours after the trials were completed for the night. The container of live crabs/ shrimp was also kept under running water in the wet tables. At the end of the project, the octopuses were released into the wild.

Preliminary Trials

I decided to test learning by giving the octopuses a problem to solve with a reward as an incentive. However, finding the optimal experimental design required some trial and error. Originally, the problem consisted of gass vials with screw tops. I placed food (crab or shrimp) in the vial over night to determine whether the octopuses were able to open the vials. I tried this for several days but found no evidence that the octopuses could open the vials.

I decided to make the problem a maze and used the clear divider with a hole in it that I settled on using for the final experiment. I initially used an experiment tank, with a crab placed on the other side of the divider as an incentive to solve the problem. Because crabs were an unreliable incentive because controlling for hunger was difficult, I concluded that using light would be more effective.

The finalized experiment is described in the next section.

Experiment Setup

For this experiment, six octopuses were used and each were subject to the same experiment. Three of the octopuses had mantles 5cm long, which I refer to as juveniles, and three had mantles that were 1-1.2 cm long, which I refer to as adults. The octopuses had two weeks to acclimatize after capture before being tested. Experiments were conducted at night, between 1845 and 2345, since *O. bocki* are nocturnal (Lebensohn 1999). A headlamp red light was used when a test was not taking place. The octopuses were tested in their holding containers to reduce stress of transportation (Lebensohn 1999, Richter 2016). For each test, the octopuses were transported 1-2 meters in their containers from the wet table to a testing table in the wet lab.

The octopuses were tested on two types of problems: simple and complex. The simple problem consisted of one Plexiglas divider (12x4cm) and the complex consisted of two dividers 3 cm apart (the dividers split the length of the container). The dividers had a 1 cm circular hole on the bottom edge, 2 cm from the side of the container, that the individual needed to move through to reach its den on the other side (Figure 1). The experiment uses light as the incentive for the octopus to solve the problem.

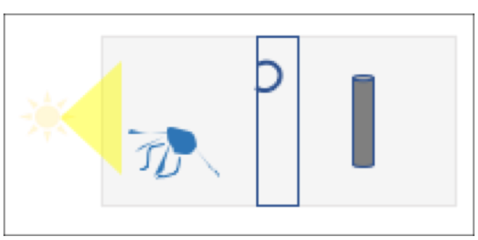

FIG. 1: Experimental setup for the simple problem. Includes octopus container, light source, octopus, divider with hole, and den.

Trial Organization

The first four days of trials were for the purpose of settling on an experimental design and thus varied. However, to avoid too much pseudo replication, these trials were included in the results analysis. As the octopuses were treated as independent test subjects, the first time they were given a problem (simple or complex) was considered trial one.

The trials were organized as such:

Day 1: To determine the feasibility of using light. All of the octopuses were tested on the simple and complex problems.
Day 4: Three trials were taken an hour apart. On the first trial, all of the octopuses were tested on a complex problem then a simple problem. On the second and third trial all of the octopuses were tested on two simple problems.
Day 5: One trial was given and the octopuses were tested on the complex problem if they had solved it previously, if not they were tested on the simple problem
Days 6 and 7: The octopuses were given three trials. All of the octopuses were tested on the simple problem and the octopuses that had solved the complex problem were also given that problem.
Days 8- 14 Three trials in which all of the octopuses were given the simple problem and then the complex problem (elaborated on below)

Each night the trials were spaced one hour apart and within each trial the problems were spaced 15 minutes apart. Within each trial, the octopuses were given a round of simple problems one at a time and then a round of complex problems. Each octopus was tested on a minimum of 25 simple problems and 20 complex problems.

Experiment

Once the octopus was moved to the testing table, the mesh and anything that could be used as a den (shells/PVC tubing/ algae) was removed. The container was rotated so that the octopus was on the side closest to the observer, the divider(s) were put in, and a piece of black PVC tubing (4x2cm) was placed on the side opposite the octopus and the observer to minimize distraction. When placing the dividers (the divider for the simple problem and the one closest to the octopus for the complex problem), the hole was put on the far side of the octopus (if the octopus was in the center of the opposing side the orientation would be chosen at random). For the complex problem, the orientation of the second divider opposed that of the first divider (so the holes were not stacked). The removal of potential dens and the placement of the dividers/ PVC tubing took less than 5 seconds.

Once complete, the observer simultaneously switched the red light to a bright white light, so that the light was directed on the octopus, and started a stopwatch. The problem was considered solved once the octopus passed through the divider(s) to reach the compartment with the PVC tubing. If after two minutes the octopus did not solve the problem and was not moving, the problem was considered failed. If the octopus had not solved the problem after two minutes but was still moving, the observer waited until the octopus either solved the problem or stopped moving to stop the stopwatch. The max time was 190 seconds. Once the octopus completed the problem, or had failed, the light was switched to red and stopwatch was stopped, the dividers and testing den were removed, the original dens and mesh were put back, and the octopus was put back into the wet table.

Statistical Analysis

I used a linear mixed- effects model to determine change in performance over trial number for each octopus on both the simple and complex problems, with 'Time" as the dependent variable and "Trial Number" as the independent variable and "Day" as a fixed effect. I used a linear regression to determine a relationship between mean time and success rate for each problem. A linear regression was used to test variation in performance on the simple versus complex problems for each octopus. In that test "Level" was the independent variable and "Time" as well as "Success rate" were the dependent variables.

A one-way ANOVA test was used to test variation between octopuses. A Tukey test was used to see where that variation lay.

Linear regressions were used to test effect of day on the solution times as well as the effect on the three trials within each day. For testing the effect of each trial within each day, trials one and two were made into a percent of the first trial (if the octopus failed, they were given a time of 190 s which was the maximum solution time).

Finally, a Welch two sample t-test was used to determine significant variation in performance between adults in terms of their solution times as well as their success rate.

Results

The average success rate of all of the octopuses across all of the problems was 65 ± 21%. Excluding Chuckles, all of the octopuses' performance did not vary significantly from each other (ANOVA, $F_{2.0}$ =4, p>0.05). On the simple problem, Chuckle's performance in terms of solution time was significantly lower than the other octopuses (Tukey's HSD, p <0.01). Chuckles' performance on the complex problem was only significantly different from two of the octopuses, Lindsey and Gus (Tukey's HSD, p<0.01). Compared to the two other juveniles Michael and Archie, Chuckles had a higher success rate (11% higher for simple and 35% higher for complex) but took longer complete the problems of both levels of complexity (43 s above for simple and 70 s for complex).

Performance was considered in terms of solution time as well as problem success rate. There was a nonsignificant yet notable correlation between mean time and success rate for the simple problem (Excluding Chuckles, correlation and linear regression, r =-0.7, $F_{0.77}$ = 4, R = -0.05, P>0.05, Figure 2 see appendix).

Solution time over all trials

For the simple problem, four of the octopuses (Archie, Chuckles, Constantin, and Lindsey) had a negative correlation between their time to complete the problem and the number of problems they solved (Figure 3). One, Michael, had a positive correlation, and one, Gus, had a near-zero rate of improvement. Lindsey was the only octopus with a significant correlation, with an improvement rate of -1 seconds/trial (LMER with day as fixed effect, F_4 = 25, SE= 0.06 p= 0.056,). For the complex problem, Archie and Michael only had two successes so a linear regression could not be performed. Two octopuses, Gus and Chuckles, had a negative correlation, Lindsey had a slightly positive correlation and Constantin had essentially no change over trial numbers (Figure 4). All correlations for the complex problem were nonsignificant.

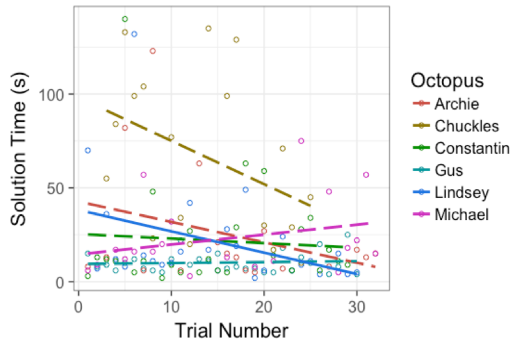

FIG. 3: Change in solution time over all trials for each octopus on the simple problem. Solid line indicates significant trend, dashed indicated nonsignificant trends.

Performance across days and within days

Neither the times per day nor the times per trials within each day varied significantly from each other (linear regression, p>0.05). Within each day, there were two main patterns identified (See appendix, Figure 5) for the simple problem. The octopuses showed more variation time for the second trial. The octopuses also tended to solve the second slower than the first but then solve the third problem faster than the first. However, Michael and Chuckles both increased their times over the three trials and Michael had more variation on the third day.

Performance by problem complexity

The octopuses performed significantly slower (+36 ± 19s) on the complex problem than the simple problem (linear regression, SE =5.8, F_{52} = 1, R^2=0.16, p<0.01). The average percent success on the simple problem was significantly higher by 28 ± 18% (linear regression, p <0.05, SE = 14.6, F_6 = 1, R_2 =0.3) on the complex problem than on the simple problem (see appendix, Figure 6).

Performance by life history stage

There was significant variation in performance between adult and juvenile octopuses in terms of solution time (Welch's t-test, t=-7.6, df = 297, p < 0.0) and percent success (Welch's t-test, t = 2.6, df = 10, p < 0.05,). Juvenile octopuses had an average success rate of 47 ± 1%, while adults had an average success rate of 81 ±-0.1% (Figure 12). For successful simple problems, juveniles had an average time of 37.54 s with a median of 19.00 s. The adults had an average time of 17.48 s and a median of 10.50 s (Figure 7).

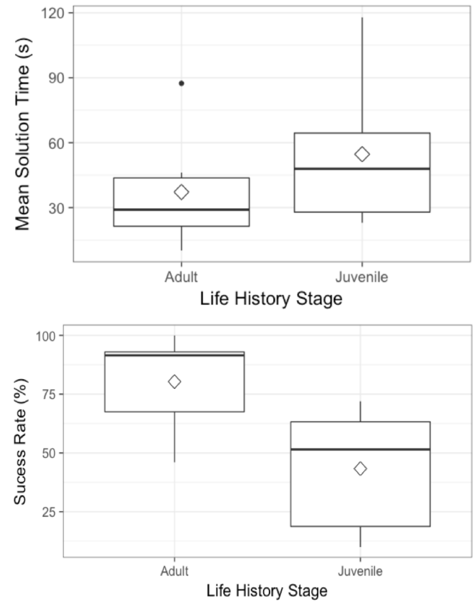

FIG 7: Performance by life history: mean solution times and success rate. The diamond indicates means and the horizontal bar indicates median.

DISCUSSION

The purpose of this project was to gain a better understanding of the problem solving and learning behavior of *Octopus bocki.* Although the study did not succeed in concluding that *O. bocki* is capable of learning, it provides evidence that suggests that they can. The study also highlights variation in behavior in response to differences in complexity of a problem as well as in life stages of the octopuses.

Learning

Although only one was statistically significant, four of the octopuses showed improvement in their times over all of the trials (I will focus on the simple problem as it had a higher rate of successes). This result indicates that *O. bocki* is likely able to learn, and therefore also has memory. Most studies on octopuses are done using the common octopus, *Octopus vulgaris*, thus comparably little research has been done on pygmy species, especially on *O. bocki* (Hochner 2006). To my knowledge, the only learning study on pygmy octopuses was done in this class (Lebensohn, 1999) and the results regarding *O. bocki*'s ability to learn were inconclusive. This may be the first study with supporting evidence that pygmy species can learn.

Pygmy species are less likely to have complex cognition compared to larger species because their brains are much smaller. Thus, these findings have valuable implications for expanding the known species that may have intelligence and sentience. Countries are beginning to require permits for conducting research on cephalopods as a result of evidence that cephalopods have sentience and can experience pain (Fiorito 2015). Self-awareness is also evident in octopuses' camouflage behavior. However, the mechanisms of the self-awareness, and maybe even conscious, are not known (Mather 2007).

Life history

There was significant variation in the performances between adult and juvenile octopuses. Adults had higher performance than juveniles in terms of success rate and solution time. Interestingly, the Lebensohn (1999) study, which studied learning in the context of object recognition, found lower performance in adults than juveniles. The variation in performance could be due to differences in neurological development between juvenile and adult species. Studies have found that *O. vulgaris*' brain grows through its lifetime (Sømme 2005), thus it is possible that the adult octopuses are more intelligent than the juvenile species. If the performance is different because of their different stages of neurological development, *O. bocki* could be a valuable species in developmental learning research. The variation in performance could also be attributed to behavioral differences unrelated to intelligence. If that is the case, the use of light in the experiment may have been the behavioral differentiating factor.

Future steps

If I could repeat this project or if I had more time, there are some things that I would do differently. Subjecting the octopuses to a variety of experiments before settling on the final design resulted in pseudo replication in my study. Instead, I would subject the octopuses to one experiment. Giving the octopuses two problems, simple and then complex, one after the other also caused pseudo replication. Instead, I would either subject them to just one level of problem or standardize the experiment for each level (for instance one week of just simple and then one week of just complex). I also would not use the "stick" incentive of light. If I had more time, I would use food as the reward, which would require spacing the trials out more. Additionally, I would give the octopuses a more complex problem, which could more definitively test learning. Finally, I would have conducted a time-series analysis on my data, which would consider each trial dependent

Moving forward, lesion studies should be used in combination with behavioral studies to expand our knowledge of octopus neurology. A pioneering researcher on octopuses, John Zachary Young, and his coworkers discovered that octopuses are capable of sensitization, habituation, and spatial and associative learning by combining lesion studies and behavioral experiments (Borelli 2008). Young, along with Boyle and Wells started mapped the octopus brain and began assigning functions to each lobe (Hochner 2006). Some connections in function have been made between the octopus brain and the vertebrate brain, but there is still much to be researched to understand their analogous systems (Borelli 2008.)

ACKNOWLEDGMENTS

For help on this project I would like to especially thank five people: Jonathon Stillman for guiding me through the development of the experiment, Suzanne Kelson, Audrey Haynes and Caleb Caswell-Levy for help with R, as well as Roy Caldwell who generously advised me with his expertise. Additionally, I thank Mishler for his suggestion that I use octopuses as my study species, Stephanie Carlson and Vince Resh for their feedback. Finally, I'd like to thank Mariama for her effort in helping me, as well as for the amount of cheer she contributes to the Gump Station.

I would also like to thank the people who made my time in Moorea a pleasure. I enjoyed spending time with each member of the class. Dean, I loved being your buddy because of our wonderful tête-à-tête's. Hannah, I cherished our runs, kayak rides, and conversations. I wish we could have spent your senior year together. Michael, thanks for always playing with me. I'm looking forward to finishing Bataille Corse.

Literature Cited

Alexandra Kuznetsova, Per Bruun Brockhoff and Rune Haubo Bojesen Christensen (2016). lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-33. https://CRAN.R-project.org/package=lmerTest

Ambrose HW III. 2007. *A Handbook of Biological Investigation*. Winston-Salem NC: Hunter Textbooks inc.

Amidio P, Fiorito G. 2013. *Invertebrate Learning and Memory: Chapter 23. Observational and Other Types of Learning in* Octopus. Volume 22 of Handbook of Behavioral Neuroscience. Elsevier Science. 293-302.

Borelli, L; Fiorito, G. 2008. *Behavioral Analysis of Learning and Memory in Cephalopods*. Behavioral Analysis of Learning and Memory in Cephalopods - Learning and Memory: A Comprehensive Reference - *1.31*.

Boycott BB. 1965. *Pyschobiology: The Biological Bases of Behavior.* San Francisco and London: W.H. Freeman and Company.

Boycott, B. B. 1954. *Learning in* Octopus vulgaris *and other cephalopods*. *Pubbl. Stn. Zool. Napoli* 25**:** 67–93.

Caldwell, RL. 2005. *An observation of inking behavior protecting adult octopus bocki from predation by green turtle (Chelonia mydas) hatchlings*. Pac Sci 59(1): 69-72.

Cheng, M. 1996. *The reproductive biology of two species of pygmy octopuses, Hapalochlaea lunulata and Octopus bocki*. Dissertation for UC Berkeley.

Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48. doi:10.18637/jss.v067.i01.

*Fiorito G, Planta vC, Scotto P. 1990. *Problem Solving Ability of* Octopus vulgaris *Lamarck (Mollusca, Cephalopoda).* Behavioral and Neural Biology: 53. 217-230.

Godfrey-Smith, P. 2016. *Other Minds: the Octopus, the Sea and the Deep Origins of Consciousness*. William Collins.

Gorman, J. "Learning How Little We Know About the Brain." *The New York Times*, The New York Times, 10 Nov. 2014, www.nytimes.com/2014/11/11/science/learning-how-little-we-know-about-the-brain.html.

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

H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2009

Johnson A. 1995. *Comparison of sensory cues in Octopus bocki by introduction of predator and prey organisms*. Not Published (UC Berkeley IB 158 Paper).

Lebensohn AM. 1999. *Learning capabilities of different size* Octopus bocki. Not Published (UC Berkeley IB 158 Paper).

Mather, A; Dickel, L. 2017. *Cephalopod Complex Cognition.* Current Opinion in Behavioral Sciences, Elsevier.

"Octopus - Octopus Bocki - Details." *Encyclopedia of Life*, eol.org/pages/588429/details#habitat

Packard, A. "Cephalopods and fish: the limits of convergence." *Biological Reviews*, Blackwell Publishing Ltd. 1972.

Pearson JA. 1994. *Food Preferences of Juvenile* Octopus bocki. Not Published (UC Berkeley IB 158 Paper).

Piéron, H. (1911). *Contribution a la psychologie du poulpe*. Bulletin de l'Institut Gondral Psychologique, 11, 111-119.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

*Richter JN, Hochner B, Kuba MJ. 2016. *Pull or Push? Octopuses Solve a Puzzle Problem*. PLoS ONE 11(3): e0152048. doi:10.1371/journal. pone.0152048

Sømme, Laurtiz S. 2005. *Sentience and Pain in Invertebrates: Report to Norwegian Scientific Committee for Food Safety*. Norwegian University of Life Sciences.

Wells MJ. 1978. *Octopus: physiology and behaviour of an advanced invertebrate*. New York: Flalsted Press. 63-75.

APPENDIX

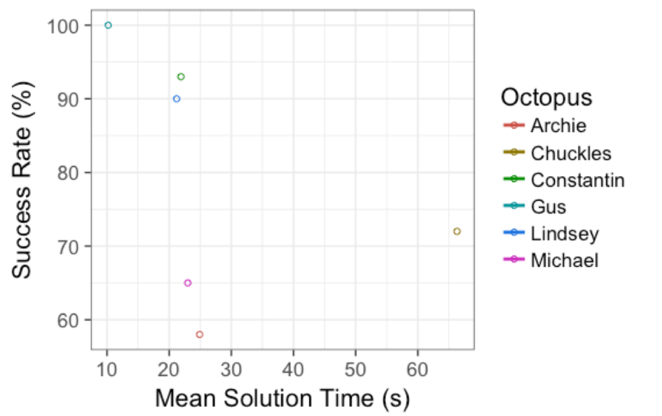

FIG 2: Relationship between the two measures of performance: success rate and mean solution time.

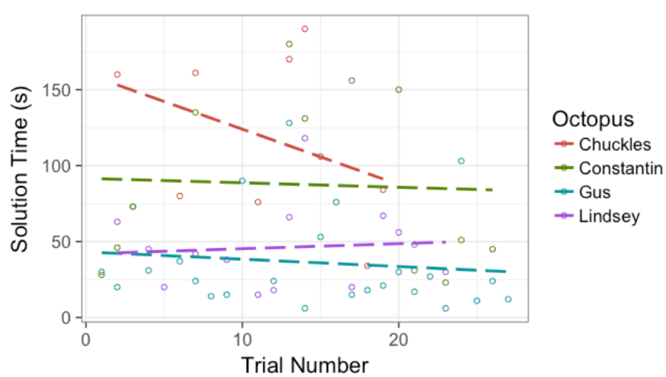

FIG. 4: Change in solution time over all trials for each octopus on the complex problem. All trends are nonsignificant, Archie and Michael were excluded because they each only succeeded twice on the complex problem

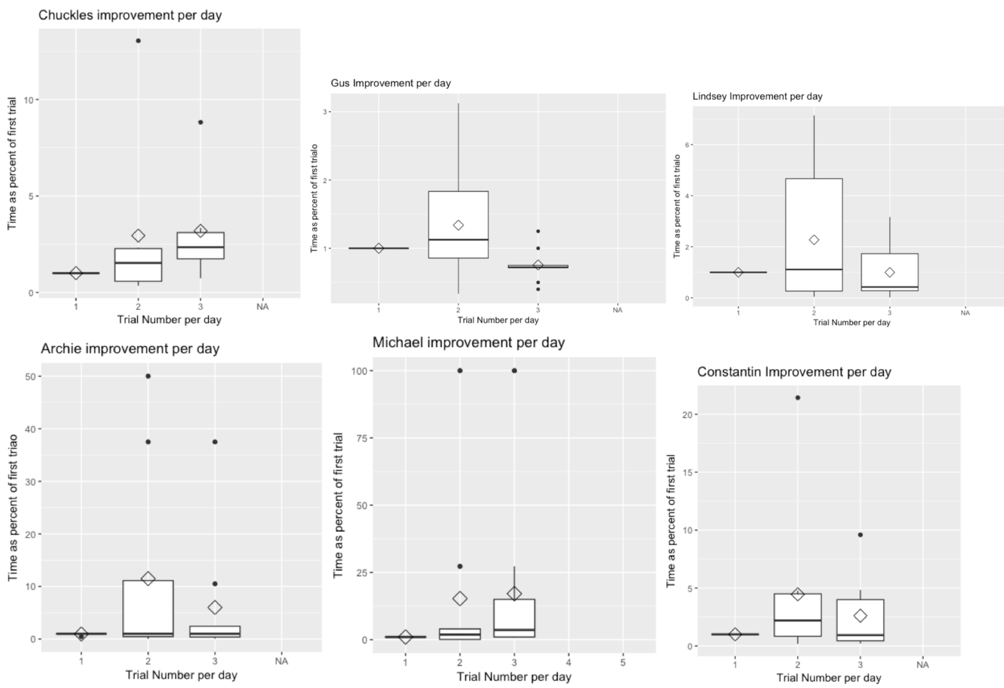

FIG 5: Performance on trials within each day for each octopus

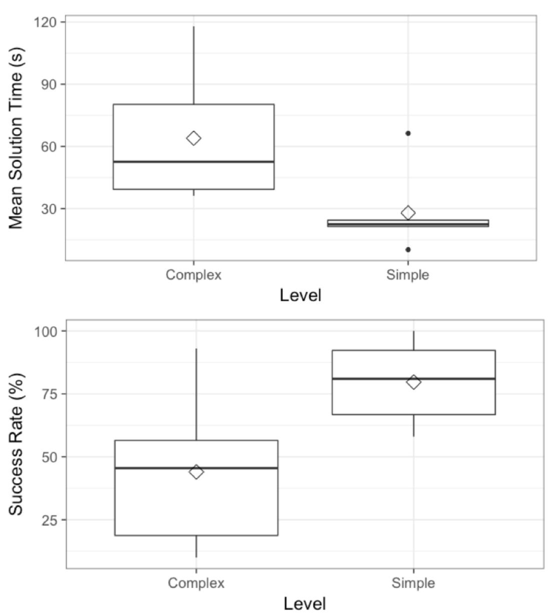

FIG. 6: Performance of all octopuses by level