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## **The Use of Dwarf Octopus (*Octopus joubini*) as a Model for Hands-On Research Experience in Comparative Psychology**

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The Laboratory of Comparative Psychology and Behavioral Biology at Oklahoma State University has been developing comparative psychology teaching exercises for over 30 years. In this paper, we provide suggestions for using the dwarf octopus (*Octopus joubini*) to teach students about the importance of observation, and the relationship of observation in the creation of ethograms and experimental design. Throughout this paper, students learn how to properly make an observation of a novel animal, create an ethogram, and conduct an experiment. We present a sample observation activity and three additional experiments, during which students will observe and record behaviors and environmental conditions. Students learn saltwater tank upkeep, time management, creation and coding of operational definitions, attention to changes in animal behavior, and the experimental process. These observation studies allow students to observe and record behavioral data to understand comparative psychology and experimental design.

*Keywords:* comparative psychology, ethograms, inquiry-based learning, octopus

### **比較心理学の実践的研究体験のモデルとしてのヒメダコの利用**

オクラホマ州立大学の比較心理学・行動生物学研究室は、30年以上にわたって比較心理学の授業演習を発展させてきた。この論文では、ヒメダコ (*Octopus joubini*) を使って、観察の重要性、エソグラムや実験計画の作成における観察との関係性について生徒に教えるための提案を行う。この論文を通して、生徒は新奇な動物を適切に観察し、エソグラムを作成し、実験を行う方法を学ぶ。我々は、生徒が行動や環境条件を観察し記録する、観察活動の例と3つの追加実験を紹介する。学生は、海水水槽の維持管理、時間管理、操作定義の作成とコーディング、動物の行動変化への注意、実験プロセスを学ぶ。これらの観察研究は、学生に行動データを観察・記録させることで、比較心理学や実験計画を理解させるものである。

キーワード：比較心理学，エソグラム，探求型学習，タコ

### **El Uso del Pulpo Enano (*Octopus Joubini*) Como Modelo para la Investigación Práctica en Psicología Comparada**

El Laboratorio de Psicología Comparada y Biología del Comportamiento de la Universidad de Oklahoma State ha estado desarrollando ejercicios de enseñanza de psicología comparada durante más de 30 años. En este artículo, ofrecemos sugerencias para utilizar el pulpo enano (*Octopus joubini*) para enseñar a los estudiantes sobre la importancia de la observación y la relación de la observación en la creación de etogramas y el diseño experimental. A lo largo de este trabajo, los estudiantes aprenden cómo realizar correctamente una observación de un animal notable, crear un etograma y realizar un experimento. Presentamos una actividad de observación de muestra y tres experimentos adicionales, durante los cuales los estudiantes observarán y registrarán comportamientos y condiciones ambientales. Los estudiantes aprenden el mantenimiento del tanque de agua salada, la gestión del tiempo, la creación y codificación de definiciones operativas, la atención a los cambios en el comportamiento animal y el proceso experimental. Estos estudios de observación permiten a los estudiantes observar y registrar datos de comportamiento para comprender la psicología comparativa y el diseño experimental.

*Palabras clave:* psicología comparada, etogramas, aprendizaje basado en la investigación, pulpo

For over 30 years the Laboratory of Comparative Psychology and Behavioral Biology at Oklahoma State University has been developing a range of classroom-tested, inquiry-based exercises in comparative psychology. These exercises range from live animal demonstrations of conditioning phenomena using invertebrates such as bees and flatworms to the use of action figures to demonstrate how to make comparisons. These papers have appeared in various venues such as handbooks related to the teaching of psychology and various publications.

The *International Journal of Comparative Psychology* published a special issue devoted to the teaching of comparative psychology (Abramson, 2020a). This issue contains over 50 classroom activities. A summary of the activities that our laboratory has developed is available in Abramson (2020b) and Abramson et al. (2020). The focus of the present contribution is to relate our experiences using the dwarf octopus (*Octopus joubini*) to teach students about the importance of observation. To the best of our knowledge, we believe that the present contribution is the first time an octopus has been used in a psychology classroom.

The dwarf octopus has many qualities that make it ideal for behavioral observations. The octopus is a unique animal that can be used in classroom or laboratory studies because of their natural environment, intelligence, and complex behaviors. *O. joubini* have translucent skin that can change colors, ranging from pale beige to striking red. A video of their camouflage abilities can be found at <https://youtu.be/DFxKXUOI3FQ>. This species is native to warm waters off the coast of Florida and the Caribbean Islands. The social behavior of this species is limited to mating and the rest of their lives are spent in isolation. They are nocturnal; therefore, they feed and hunt only at night. Their diet consists of crabs (*Brachyura*), snails (*Gastropoda*), shrimp (*Caridea*), and other small prey.

*O. joubini*, given their unique characteristics, are exciting animals for students to observe. This paper outlines how students can learn to observe animals properly and create ethograms to track their behavior using a unique animal. To begin, students must first observe, create operational definitions, and then create ethograms to track that animal's behavior. Operational definitions are concrete definitions of behavior that can be quantitatively measured in empirical research (Boring, 1945). Precise definitions are paramount to a researcher's ability to make explicit conclusions, replicate findings, and disseminate data accurately (Jasny et al., 2011; Lurquin & Miyake, 2017). Students will not only learn how to properly observe an animal, create an ethogram, and code behavior, but also will learn how to purchase the animal, maintain the animal, and conduct various experiments.

## Method

Pygmy octopuses (*Octopus joubini*) can be purchased from Salty Bottom Reef Company in New Port Richey, Florida (<https://www.saltybottomreefcompany.com/dwarfoctopus>). These octopuses are seasonal and are only available from October to March with a life span of six to eight months. In 2023, octopuses cost \$55.99 USD per pygmy octopus and are approximately five inches (0.127 meters) long.

Pygmy octopuses should be housed separately in a tank that is at least 10-gallons (0.038 cubic meters). Two months before the octopuses arrive the tank must be filtered. We used a Fluval U3 underwater filter (<https://www.petco.com/shop/en/petcostore/product/fluval-u3-underwater-filter>), but any saltwater filter that can filter the size of your tank will work. We also recommend live sand and live rock to the tank to help control the tanks water quality.

The Institutional Animal Care and Use Committee (IACUC), University Animal Care Committees (UACCs), or Animal Welfare and Ethical Review Bodies (AWERBs) may need to be notified depending on where the research is held. Before ordering the octopuses, check the university to see if there are ethical regulations that need to be followed for octopuses. Review boards can be university-dependent. For instance, invertebrates are not included in most IACUCs, however, fish are. Therefore, it is important to check to see if octopuses are included at the university where the octopuses will be held. Ethical review boards are used to protect animals and make sure proper care and management are administered. If an ethical review board is needed at your university for octopuses, submit the form before the octopuses arrive, to ensure that you can keep them on your campus and know what additional care rules you must follow.

Once the appropriate review board is notified and approved and the octopuses are ordered, they take approximately two weeks to arrive. They will arrive in a box, which contains individual double-sealed bags. It is vital that the instructors read the instructions that come with them to successfully transition the octopuses into their new tanks. For instance, instructions involve acclimating the octopuses by having them float in the bag while in the tank so that the octopuses can acclimate to the tank temperature. After all instructions are followed, slowly open the bag up and let the octopuses climb out into the tank. Make sure to only house one octopus per tank. Octopuses are solitary animals. Further, make sure to check on your octopuses regularly to ensure they are adapting well to their new environments. For example, check to make sure the octopuses are eating and moving around their tank a few days after releasing them. At first, they will look for a hiding place, but after 24 hr should move around the tank at night and begin eating. Octopus color can also be a sign of a healthy octopus. If your octopus is changing colors or pink, this is a good sign. If the octopus is beginning to look pale, this could be due to environmental problems (e.g., water or temperature) or an unhealthy octopus.

Octopuses should consume small prey like hermit crabs, snails, or small shrimp once a day. Frozen prey can also be used; however, they need to be thawed out before being fed to the octopus to ensure there is not a sudden drop in tank temperature. The temperature of the tank should be a stable temperature between 75-79°F (24-26°C). Frozen animals will be consumed however, live animals are more exciting for the octopuses and will help enrich their environment.

Once the octopuses are settled, water changes must occur every two weeks. A gallon of distilled water (3.8 L) should be mixed with half a cup (118 ml) of sea salt in a pitcher with 5 ml of conditioner (<https://www.petco.com/shop/en/petcostore/product/imagitarium-water-conditioner-4oz-2728425>). Mix the sea salt, conditioner, and water 24 hr before the water change, to allow for the salt to be dissolved before it is poured into the tank. After 24 hr, confirm that the salt is completely dissolved. When confirmed, siphon out one gallon of water and debris from each tank into an empty pitcher, afterward discard the old tank water.

Before adding the prepped new gallon of water to the tank, check the specific gravity and pH of each tank and of each of the prepped gallons. A discrepancy in specific gravity or pH could be detrimental to the octopus. To check specific gravity, place one drop of tank water using a pipette onto the end of a refractometer. We used the Imagitarium Aquatic Refractometer (<https://www.petco.com/shop/en/petcostore/product/imagitarium-aquatic-refractometer>), which can be purchased at Petco, however, any refractometer will be satisfactory. Then close the lid and wait 30 s for the device to calibrate. Next, look through the eyepiece at the light to check the reading. The specific gravity should range from 1.025 to 1.035. Gravity is shown by the line between the white and blue halves on the refractometer. To clean the device, wipe off excess tank water from the end prism and repeat the process for each tank.

To check the pH, take a test strip from a pH test kit and swish it around in tank water for 10 s. We recommend API 5-IN-1 TEST STRIPS (<https://www.petco.com/shop/en/petcostore/product/api-5-in-1-test-strips-freshwater-and-saltwater-aquarium-test-strips>) from Petco, however, any pH test kit will be sufficient. Next, hold the test strip next to the references on the pH case. The pH should be neutral (8.0-8.4). Once a gallon of water is removed and the specific gravity and pH are recorded, begin mixing in your new gallon of water. Add the new water in slowly to limit the amount of sand that will get caught in your filter and disrupt your octopus. Some sand will mix into the water, but after a day or two, the new saltwater should settle, and the tank water should be clear. Make sure to complete this process every two weeks for every tank.

## Results

### Sample Student Activity

Based on our students' experiences with the activities we recommend the following steps. Octopuses are nocturnal, which makes the evening (i.e., 6:00 p.m. to 12:00 a.m. CST) an ideal time to monitor the animals. To make this a class exercise, the instructor will need to set up a camera at night to monitor the octopuses' movements. In class, students can watch the videos to learn what behaviors are present and how to code them. For the first couple of weeks, students will want to take notes on each animal's behavior to identify the natural behaviors. Instructors will want to make sure they have feeding time behaviors being recorded and natural behaviors present in the recordings for class. This will allow the students to see behavior changes before, during, and after feeding. Instructors, research assistants, or class volunteers can be in charge of feeding the octopuses and setting up the camera.

Once students have observed the animal’s behavior for a few weeks, they will create an ethogram in class. With the help of an instructor, students will create operant definitions for each behavior they have elected to include in their ethogram. Ethograms should include variables such as date, time, behavior, feeding measures, general notes, and water conditions (i.e., pH levels). Additional experiments for classrooms and help on how to create an ethogram can be found in Abramson et al. (2020) and Abramson (2020b). A sample of the behaviors coded by student (N = 5) observations are provided in Table 1.

**Table 1**

*Example of Octopus Operational Definitions and Counts*

Variable	Operant Definitions	Counts
Jetting	Head extends followed by rapid movement in a specific direction	24
Crawling	Subject slowly moves on base of tank	49
Clinging	Subject is attached to tank walls or corners	93
Scaling	Subject slowly moves on glass	81
Hiding	Subject is not visible to observer	90
Seizing	Grabbing or reaching for food	29
Camouflage	Changing the color of skin to match external surroundings	41
Freezing	Stopping all movement in response to external stimuli	13
Swimming	Subject moving through water at a constant pace	8
Retreat	Movement toward area not visible to observer	34

## ***Results of Sample Student Activity***

Our students recorded a total of 462 observations over five months. This observational activity taught students how to operationally define and code behavior. They learned how to properly observe behavior, create operational definitions, and code behavioral data. The next step is to experiment. Below is a list of experiments that will add to each student's behavioral training.

### **Possible Experiments**

Experimentation is the next step in understanding the scientific method and animal behavior. At this point, students have learned how to properly observe an animal's behavior through operational definitions, the creation of an ethogram, and consistent coding practices. Now students must learn how to conduct a study and implement behavioral manipulations. Below is a list of three experiments that can be completed with the pygmy octopuses with the help of an instructor. First, read the literature provided on each study and understand the purpose. Then identify the methods and begin the study. Pay attention to the animal's behavior and make sure the methods are consistent between subjects. Once completed, instructors can help students analyze the data and interpret the outcomes.

In each of these experiments, instructors, research assistants, or class volunteers can conduct the experiments. A video recording must be conducted during the duration of each experiment. The class will then analyze the videos in class and discuss the behavior changes and how the experimental measures changed the octopus's behavior.

#### ***Jar Opening Experiment***

Fiorito and colleagues (1990) conducted a problem-solving study. The purpose of the study was to determine if octopuses were intelligent enough to open a jar and to determine if they would improve over time. To conduct this study, present each octopus with a clear jar containing a crab, sealed with a plastic plug. Then observe the amount of time it takes for each octopus to seize the crab after the jar is placed in the tank, along with the behaviors displayed leading up to retrieving the crab. Complete this process once a night until the octopus reaches the criterion. Make sure to record each trial so that it can be analyzed in class the next day. A trial is every day the experiment is conducted. Fiorito et al. (1990) observed a sequence of five behaviors in 88% of the trials, including attack, exploration, opening, insertion, and seizing. These are examples of variables to include in your ethogram with the addition of time and day.

Based on Fiorito and colleague's (1990) findings, students should expect the octopuses to attempt to attack the prey through the jar. It may take multiple trials or even shaping for the subject to seize the prey. Once the prey is seized, students should expect to find a decrease in solving time after a successful trial, displaying learning.

#### ***Cap Pushing Experiment***

Abramson and colleagues (2016) conducted a study on learning and memory in honey bees (*Apis mellifera*) using the Cap Pushing Response (CPR) in which honey bees are trained to push a cap to reveal a hidden food source. The purpose of this study was to use the CPR to explore operant conditioning through shaping. To conduct a version of this experiment with octopuses, present the octopuses with a cap or jar that covers a hermit crab. Observe how many trials it takes for the octopuses to master pushing or lifting the cap to get the crabs. To avoid observational learning between octopuses, put solid barriers between the sides of the tanks.

If the octopus is not able to push the cap or jar to reach the hermit crab, try using a clear cover or slightly leaving the jar uncovered and slowly covering up the hermit crab - similar to what was done in Wincheski and colleagues (2023). Three different methods to shape honey bees to perform the CPR are outlined in Wincheski et al. (2023). If octopuses are not able to perform the behavior on their own, a modified version of one of the shaping procedures with the honey bees should be implemented.

Based on the results of previous research (Abramson et al., 2016; Fiorito et al., 1990), students should expect the octopuses to display a similar learning curve to the honey bees in the CPR study. It will be interesting to see if the octopuses can complete the CPR without shaping or help from the researcher.

### ***Partition Experiment***

Triplett (1901) conducted an experiment testing the learning of perch (*Perca*). The purpose was to determine if perch would associate punishment with a particular food item. In this case, a minnow (*Phoxinus Phoxinus*). When the minnow was placed on one side of a partition, the perch would immediately try to consume it but after repeatedly bumping into the partition, would eventually stop. The interesting part of this experiment was that when the partition was lifted the perch did not go after the minnow. However, when a new minnow was placed into the tank, the perch immediately attacked it.

This study can be done with dwarf octopuses and the results can be compared to the behavioral observations of Triplett's 1901 experiment. Like Triplett, instructors should implement a partition in each octopus's tank separating them from their live prey. Make sure to switch their diet to another food source before the experiment. Triplett (1901) observed that the perch showed interest in the experimenter after switching their diet, which influenced the study. The octopuses wouldn't seize the food in the presence of the researcher. Students and instructors can eliminate this possible effect by recording the octopuses after adding the crabs instead of observing them directly. After observing the interactions between the octopuses and the crabs, instructors should remove the partition and observe the interactions after the partition is removed. Each video can then be evaluated in class by the students and create discussion about experimental design, behavioral changes, and data analyzation.

Based on Triplett's (1901) experiments, students should expect the octopuses to attempt to attack and eat the hermit crabs through the partition. Over time, the octopuses should habituate to the partition and no longer attack the hermit crabs, even after the removal of the partition.

## **Discussion**

Students made behavioral observations, created operational definitions, observed and coded behaviors based on these definitions, and learned how to properly care for dwarf octopuses. They learned these crucial skills while working with a species that they normally would not have interacted with during day-to-day life. The students observed and helped care for three dwarf octopuses in a laboratory setting. They acquired husbandry skills such as maintaining proper environmental conditions (e.g., tank-specific gravity and temperature) and animal care (e.g., feeding proper amounts of Gulf shrimp, hermit crabs, and snails). The students exhibited well-developed responsibility, accountability, and teamwork skills as duties and responsibilities were split amongst them. They also learned about comparative research methods described in Abramson (2023).

Students can take the information they learned about observation, operational definitions, and how to organize and code data and expand their training by introducing manipulation and experimental design when conducting these experiments. Additionally, students will expand on their foundational knowledge and apply the skills they acquired. We believe that students need to gain a strong foundation in observation before adding in experimental design. These future experiments will enhance their experimental training along with highlighting the importance of observation, experimental design, and operational definitions. This activity is a perfect way to get students excited about comparative psychology, while also teaching them important foundational research skills.

It should also be noted that this exercise is appropriate for courses in experimental psychology and comparative psychology, but also in courses such as animal behavior. The exercise is also useful as a source of independent student projects.

## Conclusion

### Benefits of Students

Students learned not only how to code behavioral data, but also how to define behaviors and the importance of operational definitions. Octopuses give students a behavioral repertoire that is novel to most students and forces them to observe the octopuses to understand why each behavior is occurring without prior knowledge. Students are provided with the opportunity to research a live animal using observations, create a list of their behaviors, and record data to understand the animal's needs. This exercise excites students and introduces them to research.

### Benefits of Instructor

The instructor should monitor students and help direct them, keep students focused, and point out behaviors that the students may not notice. The instructor can see what operational definitions the students make and provide them with feedback to raise awareness of unnoticed behaviors and strengthen the definitions. Instructors can explain how important pre- and post-behaviors are in understanding target behaviors and how external (e.g., environment) and internal (e.g., hunger) influences can affect behavior. The exploration of environmental enrichment apparatuses with students and animal husbandry can also be beneficial to students and instructors. This experiment is beneficial to the instructor's classrooms because it gives students real-life examples of research and presents the instructors with opportunities to teach students and answer questions as they arise. Instructors will see that with the addition of this exercise, students will be more involved in their class and take a real interest in research methods and design.

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