

Octopus social tolerance experiment


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cephalopod, octopus, Octopus laqueus, behavior, social tolerance, modeling, maximum entropy model, culturing, elastomer

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MATERIALS TEXT

Artificial sea water (InstantOcean)
Live grass shrimp
Frozen shrimp
Live crabs
Frozen crabs
Critter keeper large (size ?)
Critter keeper small (size ?)

Plastic pool (size ?)
Clay pots large (size ?)
Clay pots small (size ?)
Aquarium (size ?)
Filtering system
Air pump
Air stones
Air lines
Black plastic board
Elastomer (Northwest Marine Technology # 10:1 VIE)
Mesh bag (dive bag, lingerie bag, or bag oranges are sold in)
Autoclave pan or other container to work with octopus in

Culturing

1

Octopus laqueus juveniles and adults can be housed in any stable recirculating or flow-through seawater aquarium at densities of up to 1 animal per 15 liters seawater. Medium or large clay pots can serve as dens, at least one per animal when not part of a housing experiment. Ideally, light cycles will match the local conditions of light and dark for where the animals were collected.

- 2 Monitor water quality (pH, nitrates, nitrites) using a commercial kit. PRIME can be added to the tank per kit instructions, particularly if water quality is declining rapidly. Half-volume water changes can be made daily to weekly depending on water quality conditions, particularly for temporary recirculating systems.
- 3 Animals can be fed live or frozen shrimp or crabs, with live crabs their preferred meal. To minimize contamination of the short term culturing tanks, animals can be fed daily in containers outside the aquarium. We typically transfer them to individual Critter Keepers that contain a small clay pot to serve as a den, feed them, and then transfer them back to the main tank. For long-term culturing in stable tanks, animals can be feed and then carcasses removed an hour or two later.

Elastomere tagging

- 4 Perform elastomere tagging at least 24 hours prior to a social tolerance experiment.
- 5 Eject at least 1 mL of color component into the mixing container. Measure the amount based on marks on the syringe. Be sure to recap the syringe tightly.
- 6 Eject 1/10 color component volume of curing agent into the mixing container. Be sure to recap the syringe tightly.
- 7 Mix thoroughly for a full minute using the popsicle stick that comes with the kit. The elastomer can be used for injections for around 1 hour, depending on temperature and local conditions. It can also be kept on ice to
- 8 Fill an autoclave pan or a low-sided container with artificial seawater to a minimum depth of 5 cm to serve as a injection tank. The low sides and large flat space make it easier to work with the animals for injection.
- 9 Place an octopus to be tagged into a mesh bag and transfer the mesh bag and octopus container with sea water previously used to tare a scale.

- 10 Record the weight and sex of the octopus. Males can be identified based on a pair of exceptionally large suckers on arms L2 and R2 and by curling of the heterocaudal fin when walking.
- 11 Transfer the octopus in its mesh bag to the injection tank.
- 12 Gently collapse the mesh bag so that the octopus is in a bottom corner of the bag.
- 13 Gently wedge the octopus against the corner or wall of the injection tank.
- 14 Inject a small amount of elastomer into the skin of the dorsal mantle going through the mesh bag and by holding the syringe at an angle that is near horizontal.
- 15 Check that the elastomere is secure in the skin.
- 16 Transfer the octopus in the mesh bag back to its tank and release the octopus.
- 17 Check on the octopus to ensure has fully recovered and exhibits typical behaviors later that day or the following day.

Social tolerance experiment

- 18 Depending on the specifics and size of the experimental tank, select an appropriate number of octopus of different sizes, and sexes to form an experimental cohort.
- 19 Depending on the specifics of the experiment, place an appropriate number of clay pots in the experimental tank.
- 20 Transfer animals to the appropriate tank. Time 0 of the experiment begins once all the tanks and animals are set up.
- 21 Select a set time to feed the octopus daily around or during the dark light cycle period, which is when *O. laqueus* is most active. This time should match culturing feeding times outside the experiment to minimize complicating variables in the experiments. Feed per instructions above. Try to minimize exposure to light during the transfers in and out of the feeding container when the transfer are done during the dark period (a few minutes total).

- 22 Monitor water conditions per instructions above daily.
- 23 Assess animals for signs of stress or aggressive interactions once to several times per day,. For instance, check for lesions in the skin or missing arm tips during feeding. If evidence of stress or aggressive interactions arise, terminate experiment in accordance with the animal care guidelines of the institute and as pre-determined for the experiment. Log all observations per identified animal, when possible.
- 24 Every 24 hours at a set time within a few hours after "sunrise" , score each tank and pot for octopus with each animal identified based on its elastomere markings. Repeat for several days or weeks, per the predetermined experimental design.
- 25 Weigh the animals at the start and end of the experiment.