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## Protocol for anesthesia and euthanasia in *Octopus maya*



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**We use this protocol and it's working**

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## Abstract

This protocol details the procedure of anesthesia and euthanasia in *Octopus maya*.

## Materials

### Materials and equipment

- Euthanasia container
- Seawater (prepared at 15°C and natural salinity levels)

### Feeding and stimulation materials

- Fresh crabs (*Callinectes* spp.) for feeding.
- Bags for holding dead crabs (*Callinectes* spp.) during gastric secretion stimulation. Ensure the bags are designed to allow contact but prevent ingestion.



## Feeding protocol for validation

20m

1

20m

### Note

Gastric juice for the assay is obtained after gastric secretion stimulation, while gastric juice post-digestion (chyme), obtained from animals fed with fresh crab (*Callinectes* spp.), is used as the standard.

Feed three octopuses ad libitum with fresh crab (*Callinectes* spp.) for  00:20:00 to stimulate gastric juice production.

## Fasting protocol for assay

12h 20m

### 2 Fasting protocol:

12h

Fast three octopuses for  12:00:00 to ensure complete digestion of prior meals.

### 3 Gastric secretion stimulation:

3.1 Place bags containing dead crabs (*Callinectes* spp.) into the tank with the octopuses.

3.2 Ensure the design of the bags allows the animals to contact the prey without ingesting them.

3.3 Leave the octopuses in contact with the bags for  00:20:00 to stimulate gastric secretion.

20m

## Anesthesia protocol

5m

4 Prepare seawater at a natural salinity level and chill it to  15 °C .

5 Place the octopus in the chilled seawater for  00:05:00 to induce hypothermia, which acts as a mild anesthetic.

5m



- 6 Monitor the octopus for signs of reduced activity and responsiveness, ensuring it is sufficiently anesthetized before proceeding.

## Euthanasia protocol

- 7 Gently remove the anesthetized octopus from the chilled seawater.
- 8 Using sterile dissection tools, perform euthanasia by cutting between the eyes into the brain. This method ensures immediate cessation of brain activity, adhering to ethical standards for cephalopod handling.

### Note

#### Notes and Precautions:

- Maintain the seawater at  15 °C throughout the anesthesia process to ensure effective hypothermia.
- Sterilize all dissection tools prior to use to prevent contamination of subsequent samples.
- Handle the octopus gently to minimize stress during the procedure.
- Follow all ethical guidelines and local regulations regarding cephalopod euthanasia.