J.1.10 Cephalopod Care and Use Policy
Initiated by: Cephalopod Advisory Group
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## 1. Policy Statement

Cephalopods are not regulated by law in the United States however, the Marine Biological Laboratory's Institutional Animal Care and Use Committee (IACUC) oversees the use of cephalopods and a cephalopod-specific IACUC approved protocol is required prior to use of cephalopods in any research conducted at the MBL. Furthermore, the MBL has established the following policies to ensure the humane treatment and highest husbandry standards for maintaining these animals. The welfare of the animals and the integrity of the research for which they are used is of the utmost importance.

## 2. Definitions

- 2.1 The term cephalopod as used in this document includes all eggs, embryos, hatchlings, juveniles, and adult members of the class Cephalopoda (squid, octopus, cuttlefish, nautilus).

## 3. Personnel and Oversight Organization (See Appendix II)

- 3.1 The Cephalopod Advisory Group consists of a set of resident MBL faculty and staff who serve as the authority for the review and approval of the husbandry practices outlined in this document. MBL policies and procedures are based on empirical data and are being continually refined. The group consists of:
    - 3.1.1. Resident researchers and staff who work on cephalopods;
    - 3.1.2. The Manager of Cephalopod Operations serves as the contact for issues related to cephalopod culture;
    - 3.1.3. The MBL Veterinarian oversees the health and welfare of all animals used in research at the MBL;
    - 3.1.4. The Director of Marine Research Services is accountable for all aspects of animal collection, water treatment, and marine life support infrastructure.
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- 4.1. The research community at the Marine Biological Laboratory (MBL) has long used cephalopods as models for scientific inquiry and recently has established efforts to refine capture, transport, maintenance and husbandry methods as well as generating genetically tractable cephalopod models. The MBL has established rigorous policies and procedures for the humane treatment of cephalopods in experimentation, teaching, and husbandry given the behavioral complexity of these animals. These items are divided into two sections.

    4.1.1. MBL institutional policies pertaining to the collection, housing, and care of cephalopods.

    4.1.2. Responsibilities of investigators using cephalopods for research at MBL.

5. Institutional Policy
    - 5.1. The humane treatment of cephalopods during experimentation

        5.1.1. The MBL strives to base its policies for the humane care of cephalopods on sound scientific data and principles. Compared to vertebrates, cephalopods have not been well studied, particularly in terms of their nociceptive capabilities (that is, responses to noxious stimuli). All investigators, including researchers and instructors, using cephalopods at the MBL must adhere to the guidelines and procedures for experimentation as developed by the MBL. These guidelines include, but are not limited to, procedures for anesthesia prior to experimentation and/or euthanasia. It is stressed that different cephalopods often exhibit diverse physiology and behaviors, and as a result our procedures should be read carefully because they are often species-specific. By establishing these policies, we aim to ensure that all cephalopods used at the MBL are treated with the highest humane standards to ensure their ethical treatment. This section outlines the policies and standard operating procedures (SOPs) for housing cephalopods and using them for research at the MBL.
    - 5.2. Capture, Housing, and Husbandry of Cephalopods

        5.2.1 Cephalopods used in MBL research and education consist mostly of non-native species and a single local species of squid. Cephalopods that are captured from the field locally and abroad are transported to the MBL using methods that result in high survivorship. Stocking densities, water quality, and stress levels are monitored when possible to allow for safe transport. Once at the MBL, cephalopods are provided with enclosures to support their spatial, water quality, and photoperiod requirements. Optimal nutrition and animal care are provided.

        5.2.2. Cephalopods originate from one of three sources:

            5.2.2.1. Wild-caught local species,

harvesting techniques, animal transport methods, quarantine procedures, seawater system design, stress recognition, sterilization methods, etc. Training incorporates a detailed husbandry protocol checklist as a means to standardize each team member's retention of skills/responsibilities within the lab.

- 5.4. Animal Care

  5.4.1. The MBL has established stringent guidelines that allow animals to successfully reproduce and live their natural lifespan under laboratory conditions. Life support systems are designed for each individual life-stage, for every species in our collection. Water flow, aeration, temperature, water levels, animal health, and other system parameters

at the MBL. All researchers and course directors and assistants working with cephalopods at MBL shall abide by the following procedures:

6.1.1. All cephalopod use at the MBL must be performed under an approved IACUC protocol.

- 6.2. Visiting Investigator Animal Requests

  6.2.1. Cephalopods cultured from eggs at the MBL are not available on-demand without prior notification. The acquisition of these cephalopods requires the following procedures from the investigator.

  6.2.1.1. The investigator must review this policy document and agree to operate under the conditions outlined in this section (Policy for Investigators).

  6.2.1.2. An approved IACUC protocol must be in place prior to obtaining any cephalopods.

  6.2.1.3. Coordination with the manager of cephalopod operations must be initiated by the investigator.

6.6.1. MBL requires the use of IACUC approved procedures for euthanasia for all cephalopods that are used for research in our facility prior to tissue harvest, or at the end of the research project. See Appendix V for approved euthanasia procedures.

- 6.7. Care, Handling, and Euthanasia of Cephalopod Embryos

  6.7.1. Cephalopod eggs are deposited externally and not cared for by the parents except for octopus species. Eggs and/or embryos can be maintained throughout their development until hatching if properly

when the number of squid required is amenable to the technique and especially when the squid are intended for long-term study.

4.1.1. Large fish totes on wheeled carts are used to transfer live squid smoothly and quickly from the fishing vessel to the Marine Resources Center.

4.1.2. Pneumatic tires or an air-filled bladder, such as an inflated tire inner tube, placed beneath the water-filled tub holding the squid are employed to minimize vibration during transport that may stress the squid.

4.1.3. Keeping the container covered to prevent squid from escaping and damaging themselves on the dock and to reduce stressed induced by bright ambient light.

4.1.4. Releasing the squid into the tanks by immersing the container and letting the squid swim out gently, under their own power. This is much less stressful than pouring the container out above the surface.

5. Handling of wild-caught squid in the MRC

- 5.1. Wild-collected squid are typically housed in one of two 1800-gallon fiberglass tanks with a continuous feed of seawater. Ambient seawater is suitable for May and early June and again from late September until the end of the season. Chilled seawater (15C) is used during the summer.
- 5.2. Captive squid will often court and spawn in the tanks, producing small to mid-size mops of egg capsules. Competition among males for females can result in additional skin damage and promote faster death as they jostle, bite and impact the sides of tanks. They may also ignore food. When animals are required to be held for periods longer than 48 hrs, they should be segregated by sex and size class into different tanks. Alternative methods for long-term maintenance and growth of mid-sized squids have been published; these require a good deal of time and investigators interested in this should consult MRC personnel who have performed these experiments (Hanlon, Hixon & Hulet, 1983; Hatfield et al., 2001).

6. Feeding

- 6.1. Feeding trawl-caught squids is impractical because they tend to be used quickly once in the lab. Squid collected for longer-term (> 48 hrs) use, however,

## Appendix IV –Anesthesia Procedures

### Suggested anesthetics

7.5% $MgCl_2$ (75g dissolved in 1L DI water) mixed with home tank sea water in a ratio of 1:4 dilution for light sedation; 1:2 for complete anesthesia.

Or

2-4 % ethanol (95% lab grade ethanol) in home tank sea water for complete anesthesia. Start at 2% and increase in 1% increments to effect (see details below).

### Anesthesia protocol

1. The animal should be immersed in an appropriately sized container filled with the seawater/anesthesia solution described above until the animal stops responding to a physical stimulus (a gentle pinch with a blunt pair of forceps on the mantle).
2. If after 5 minutes the animal is not adequately anesthetized, then increase the dose by 1% for ethanol or change the ratio for $MgCl_2$ (from a ratio of 1:4 to 1:3, for example).
3. When using the MgCl solution, the animal should be left in the solution for 10 minutes beyond the time when they stop reacting to the gentle stimuli to ensure complete loss of sensory input.
4. Other common signs of anesthesia include: slowed respiratory rate, pale color, loss of sucker intensity, failure to right itself when turned on its back (note: this final sign will not work in animals with large cuttlebones such as cuttlefish Sepia officinalis).
5. At this point the animal (treated with either procedure) can be removed from the anesthetic bath and the procedure performed as rapidly as possible.
6. The time it takes to reach a state of non-responsiveness will vary by species.

### Maintenance

Recovery

1. After the procedure, the animal should be placed in a tank with fresh, aerated seawater in isolation from other individuals and monitored until full recovery (ventilating regularly, ability to right itself).
2. In the event that the animal is not ventilating, manual assisted ventilation should be started. Gentle compressions of the mantle with the observer's hand at a rate of about 1 per second and gently directing fresh sea water into the mantle cavity with either a small tube connected to a water pump or a syringe.

Butler-Struben, H. M., Brophy, S. M., Johnson, N. A., & Crook, R. J. (2018). In vivo recording of neural and behavioral correlates of anesthesia induction, reversal, and euthanasia in cephalopod molluscs. Frontiers in Physiology, 9(109). doi:10.3389/fphys.2018.00109

Crook, R. J., Dickson, K., Hanlon, R. T., & Walters, E. T. (2014). Nociceptive sensitization reduces predation risk. Current Biology, 24(10), 1121-1125.

Crook, R. J., Hanlon, R. T., & Walters, E. T. (2013). Squid have nociceptors that display widespread long-term sensitization and spontaneous activity after bodily injury. Journal of