J.1.10 Cephalopod Care and Use Policy
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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.
  - 3.2 The Cephalopod Advisory Board includes the resident Advisory Group and other resident and outside experts appointed by the MBL to provide guidance to help ensure that MBL policies and procedures are as effective and useful as possible. A key goal of the Board, which meets biannually, is to help ensure that humane considerations are informed by up-to-date, appropriate research. The board includes:
    - 3.2.1. experts in nociception, cephalopod behavior, cephalopod husbandry, and animal research standards and policies.

- **4. Background**

- 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,

        - 5.2.2.2. Wild-caught non-native species that are maintained or reared in captivity through part of their life cycle, and
        - 5.2.2.3. Non-native species that are cultured through their full lifecycles at MBL facilities.
    - 5.2.3. Wild-caught squid
      Local squid (*Doryteuthis pealeii*) are collected by the MBL between the months of May and December. During all aspects of collection and handling, the welfare of squid is a priority. MBL employees must follow best practices in all aspects of capture, transfer, and housing of wild specimens. Different methods may be employed depending on the number of specimens required and the time needed to transport animals back to the laboratory. See Appendix III for details.
    - 5.2.4. Importation of non-native cephalopod species.
        - 5.2.5.1 The MBL takes rigorous measures to ensure that non-native animals and their potential pathogens do not escape into the local environments. Pathogens associated with imported animals also pose a serious risk to the MBL's cultivation efforts. Therefore, the importation of any cephalopod species to the MBL must be performed in consultation with MRC staff and coordinated at least one month in advance to ensure proper containment and care. It is imperative that species are imported lawfully, according to US regulations and the foreign government at the shipment's origin. While at the MBL, all imported cephalopods must be contained within the MRC facility. Finally, as with the importation of any exotic species to the MBL, the following guidelines must be followed. Cephalopods arriving at the MBL that do not adhere to these guidelines will either be shipped back to the origin or confiscated and euthanized.
    - 5.2.5. Cultured Cephalopods
      Caring for cephalopods is difficult due to their rapid growth rates, high protein-based metabolisms, sensitive physiologies, and species-specific husbandry requirements. The MBL has an experienced staff to lead husbandry efforts for the multi-generational culture of several cephalopod species. Animals and seawater systems are closely monitored to provide optimal care and mitigate stress and disease in our animals.
- 5.3. Training
    - 5.3.1. All members of the MBL staff handling cephalopods are highly trained and maintain rigorous humane standards based on multiple scientific reports (Sykes et al. 2012; Iglesias et al. 2014; Vidal 2014). Only staff/volunteers/interns who have completed training are allowed to provide care for the cultured cephalopods at MBL. During training and orientation, staff, interns, and volunteers learn husbandry fundamentals including, but not limited to: routine maintenance, animal feeds, embryo

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 are measured multiple times per day. Complete nutrition is provided throughout each life stage, approximating natural diets. Stress contributors (i.e. vibrations, bright lights, loud noises, visual disturbances, etc.) are kept to a minimum. Embryos are closely monitored through incubation and hatchlings are carefully transferred to rearing systems. All animals are provided with environmental enrichment where appropriate to promote animal welfare and optimal reproduction.
- 5.5. Water Quality
  - 5.5.1. Optimal seawater is supplied to all cephalopod systems based on the ideal standards for water chemistry requirements as reviewed by Vidal et al. (2014). Water quality parameters (temperature, salinity, pH) are measured daily and a detailed water quality analysis (NH_4, NO_2, NO_3, PO_4, etc.) are performed weekly.
- 5.6. Monitoring Systems
  - 5.6.1. Each cephalopod culture system is monitored 24hrs/day, 7 days a week utilizing the advanced monitoring technology. Critical parameters (temperature, pH, light, etc.) can be checked and/or manipulated from a mobile device or laptop remotely any time of day. If equipment malfunctions, alarms alert our team's mobile devices so that immediate action is possible. Redundant monitoring systems are equipped on most systems. Infra-red cameras are used for nocturnal observation of some species. Nocturnal observation allows for increased familiarity with each species so we can create and optimize species-specific husbandry care protocols.

- **6. Policy for Investigators**
  - 6.1. All researchers at the MBL, whether they are resident scientists, visiting scientists, training course participants or other potential users, must abide by the following policies while using cephalopods on the MBL campus. Those who do not will lose access to animals and possibly face other sanctions. Researchers intending to use cephalopods within our facilities must read these guidelines and procedures carefully and commit to the humane treatment of cephalopods while

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.
- 6.3. Care
  - 6.3.1. The responsibility of care of cephalopods must be detailed in the approved IACUC protocol prior to initiating research.
- 6.4. Interactions with the press and social media
  - 6.4.1. Any contacts with the press regarding the MBL cephalopod program or cephalopod research conducted at the MBL must be discussed in advance with the Office of Communications. The posting of any videos or still images of research or educational activities using MBL cephalopods at the MBL on any social media platform must conform to MBL social media.
- 6.5. Anesthesia
  - 6.5.1. Use of anesthesia at MBL generally follows the Guidelines for the Care and Welfare of Cephalopods in Research (Fiorito, et. al., 2015). Pain, defined as both a sensory response to noxious stimuli and a subsequent emotional response resulting in altered behavior, has not been definitively demonstrated in cephalopods. There are data, however, suggesting that cephalopods sense and respond to noxious stimuli (Crook, et al., 2013, Aluplay, et al., 2014, Crook, et al., 2014). Until more definitive research becomes available to allow a more informed policy, the MBL requires anesthesia for any procedure that may result in a noxious stimulus (including physical methods of euthanasia) unless its omission is scientifically justified and approved by the MBL IACUC. The preferred methods of anesthesia are based on recent research in cephalopod nociception and anesthesia (ibid.; Andrews, et al., 2013, Butler-Struben, et al., 2018,). See Appendix IV
- 6.6. Euthanasia

        - 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 cared for. Eggs and/or embryos require clean seawater and proper oxygenation to develop and should be checked daily. Dead or diseased embryos should be removed promptly to ensure that the remaining embryos are not compromised. After eyes become pigmented, embryos and hatchlings should be anesthetized prior to euthanizing via fixation, rapid freezing, or decapitation and cranial sectioning.
    - 6.8. Disposal of dead animals and tissues
        - 6.8.1. Non-viable or euthanized non-native cephalopods including embryos, eggs, or tissues are to be disposed of according to the MBL non-indigenous animal policy. *Doryteuthis pealeii*squid adults, eggs, or dead embryos that have not been treated with any substances may be disposed of in collection buckets in the tank room of the Marine Resources Center.

The undersigned agrees to abide by the policies set out in Section 5 of this document.

Investigators Print Name:

Investigators Signature:

Appendix I – Cephalopod Use Form

PI/Course Director		Primary Care Person	
Phone #		Phone #	
Email:		Email:	
MBL Building/Room		MBL Building/Room	
MBL Account #			
		Alternative Care Person	
		Phone #	
		Email:	

Species	
Size Age Number	
Source (Vendor or MBL)	
Research Goals	

Housing and Water Quality

Number of Tanks		Temperature	
Volume(s) of Tanks		pH	
Housing instructions			

Husbandry

Feeding Protocol	
Responsible for feeding	Investigator ☐ MRC ☐
Tank Maintenance	
Responsible for maintenance	Investigator ☐ MRC ☐
Security Measures	
Responsible for security	Investigator ☐ MRC ☐
Habitat Design	
Responsible for design	Investigator ☐ MRC ☐
Other Requirements	

Appendix II– Personnel Contact Information

Personnel	Telephone	Email
Manager of Cephalopod Operations	508-289-7700	ceph@mbl.edu
Director, Marine Research Services	508-289-7477	mrc@mbl.edu
MBL Veterinarian	508-289-7522	vet@mbl.edu

Appendix III – Procedures for wild-caught squid

- **1. Collection by Trawler**
    - 1.1. Under normal circumstances, large numbers of squid are required by the MBL community. To accommodate this demand, the collection vessel Gemma uses a modified otter trawl; a large nylon-mesh net that is towed along or near the bottom. Although this method is effective in catching squid in numbers, it can cause physical damage and stress to the animals. To reduce stress and damage, MBL collecting vessels employ the following strategies and practices:
        - 1.1.1. A smaller and softer fabric liner is added to the interior cod-end of the net to reduce the squid's contact with the more abrasive nylon twine.
        - 1.1.2. Metal hoops are added to the cod-end to prevent the net's collapse, which can compress the squid.
        - 1.1.3. The cod-end is divided into two sections, one above the other in order to segregate soft-bodied squid from more robust fish and crustacean by-catch.
        - 1.1.4. Short tows reduce the length of time squid remain in the net and exposed to the risks identified above. Tows are typically 15-20 minutes each.
        - 1.1.5. Minimize the numbers of tows to collect only sufficient squid for immediate requirements.
        - 1.1.6. Immediate transfer of the catch from the net to a large volume sorting tank full of fresh, circulating seawater. The vertical distance between the net and the surface of the tank is kept to a minimum before opening the net and releasing the catch.
        - 1.1.7. Squids are separated from the bycatch as quickly as possible for transfer to a dedicated squid holding tank. Staff ensure any transfers minimize the time the animals are exposed to the air.
- **2. Collection by Jig**
    - 2.1. Squid jigs are the preferred method for collecting squid because it causes significantly less physical damage and stress compared to trawling. Jigs are used

when the number of squid required is amenable to the technique and especially when the squid are intended for long-term study. Squid jigs are small artificial baits armed with rings of small, sharp, barb-less tines that are fished using sport fishing rods, from boats or piers. When performed carefully and followed by best practices in holding and transfer (see below), squids can be collected in near-perfect condition.

Squid are caught by their arms or tentacles when they strike the bait, being held by the tines. Jigged squid can be transferred to a holding tank without any abrasive body contact; the squid is simply lifted out of the water via the fishing line, the jig body is grasped and inverted over the surface of the tank to release the animal. The squid will quickly fall off the barbless tines into the water and the animal itself is never touched. The disadvantage of jigging is low catch per unit effort compared with trawling.

- **3. Temporary containment of wild-caught squid**
    - 3.1. Wild-caught squid are transferred to containment systems aboard ship for transport to MBL using procedures and techniques that prioritize their health and welfare. Freshly collected *Doryteuthis* are active animals that require clean oxygenated seawater. They have a single-celled microvillus epidermis that renders them susceptible to skin abrasions and subsequent infections. Stressed squids are prone to inking and their condition will rapidly deteriorate when exposed to inked water for even short periods. Regardless of the method of capture, temporary holding of squids requires active monitoring and interventions. Our practices include:
    - 3.2. Utilizing as large a volume of water as is practical aboard the vessel.
    - 3.3. Active circulation and exchange of water. Raw clean seawater should be continuously pumped through the holding tank to flush out ink and metabolites.
    - 3.4. Lining primary containers with an inner ring of a soft, flexible liner to minimize abrasive collisions.
    - 3.5. Temperature should be kept as close as possible to the ambient conditions at the collection site. At the height of the summer, surface temperatures in Eel Pond can exceed 27C on a day when squid may have been collected in water 10C cooler. At these times the intake pump on the collecting vessel should be turned off prior to entering Eel Pond, and the squid removed from the vessel as quickly as possible. Bags of ice or seawater block ice may be used in the summer to help moderate the temperature.
- **4. Transfer of wild-caught squids to the MRC from vessel or truck**
    - 4.1. Squid must be transferred from the collecting vessel to the MBL holding facility (MRC) utilizing techniques that minimize stress and the risk of damage to the specimens. Our practices include:

        - 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, should be fed. They will feed on live feeder fish. *Fundulus* (mostly *heteroclitus* but also *F. majalis*) are suitable. Silversides (*Menidia*), sand lance (*Ammodytes*), and grass shrimp (*Palaemonetes)* are also suitable prey when available. Squids are voracious predators and require regular feedings (2X/day).
- **7. Substrate**
    - 7.1. In nature, *Doryteuthis pealeii* often sit on the bottom in a camouflaged body pattern. Covering the bottom of the tank with 1-2 inches of sand or fine gravel provides substrate that better mimics natural conditions, and this can lead to reduced stress. This addition is recommended when squid are being housed for more than 48 hrs.

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

The doses above will provide anesthesia for short procedures.

1. If the animal is taken out of the water for more than 5 minutes, then fresh aerated seawater should be directed gently over the gills for continued blood oxygenation.
2. Out of the water, the animal must be placed on a non-absorbent and smooth surface to protect the skin (plastic cling wrap works well).
3. For procedures lasting more than 10 minutes, half-strength anesthesia should flow over the gills to maintain the animal under anesthesia for the duration of the procedure.

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.

Appendix V – Euthanasia Procedures

We recommend an overdose of $MgCl_2$ 1:1 dilution of 7.5% $MgCl_2$ in home tank seawater or 5% EtOH in seawater. For the ethanol treatment, start at 2% ethanol until the animal is sedated (slowed respiratory rate, pale color, loss of sucker intensity, failure to right itself when turned on its back, lack of response to mantle pinch) and then slowly add ethanol in 1% increments every 3-5 minutes until 5% is reached. **Animals should be left in the water for a minimum of 10 minutes following the cessation of respiration and then followed with cervical transection and/or brain pithing.**

In cases where the anesthetic may interfere with the objectives of the research, and for experiments using adult *Doryteuthis pealeii*, a swift cervical transection between the head and mantle followed by a transection of the head between the eyes should be performed using a sharp blade or scalpel. Alternatively, for procedures that require preservation of the brain, the squid may be placed on ice as long as the skin of the animal is protected from direct contact with the ice such as wrapped in a thin cloth or paper towel. For all other methods of euthanasia, approval from the MBL veterinarian is required.

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