**Animal Care and Husbandry at the University of Connecticut**

On arrival at UCONN, the water temperature of the bag is measured, and the squid are allowed to acclimate to the aquarium seawater temperature in the bags (open to air circulation) before release. Acclimation is conducted over a 2 h period with 200-300 ml water exchange every 20-30 min.

Almost all squid survive shipment, but a 10% loss within the first week is not unusual. The mortality may be attributed to stress of capture, transport, or failure to acclimate to captivity, or age of animal.

**Long-term holding**

Annual animal use

|  |  |  |
| --- | --- | --- |
| **Animal Type** | **Number (3 yr period)** | **Use** |
| Adult female | 270 | Breeding colony |
| Adult male | 270 | Breeding colony |
| Other wild-caught juveniles | 180 | Experimental |
| Hatchlings squid | 60,000 | Experimental |
| Raised squid | 1500 | Experimental |

The lab has room to house 15-30 adult females and 15-30 adult males at any given time. This ratio has proved to be successful in providing 2-6 clutches daily which, in turn, release about 150 hatchlings daily for experimental procedures. We can house up to 80 egg clutches at a time, but usually have about 20-30 at various stages of incubation. Roughly 500 hatchlings (annually) are used for long-term experiments lasting 4 days to 6 months. All other hatchlings are for used in experiments within the first 3-5 days of hatch.

Containment of exotic species

*E. scolopes* is a marine species, and is not native to Connecticut. As previously mentioned, they are found only within the Hawaiian Islands. However, as it is a tropical, salt-water species, introduction to any natural body of water locally would be fatal, due to temperature stress. We do not have any special precautions in place to reduce risk of introduction of this squid species locally. No special treatment is taken with effluent water.

Animal areas

The area in which the aquaria are kept is within the Nyholm laboratory in 2 aquarium rooms located in Torrey Life Sciences (TLS 82 and TLS 57). The PI, graduate students and/or trained undergraduates monitor the animals at least once each day.

The adult aquaria are on a 12h light/12 h dark cycle (lights on 0100 h; lights off 1300 h) This interval is equivalent to what the animal experiences in the wild, and provides ample time for lab maintenance and cleaning without disturbing these nocturnal animals. The air temperature is ambient for the lab space, about 24°C, which is also an environmentally natural temperature for the animals.

The egg-incubation aquarium is in a separate, light- and temperature- controlled aquarium with the same light/dark schedule and temperature.

Aquarium parameters

The main squid aquarium room in TLS 082 holds 2 rack systems (each system has 10 (15 gallon, 57 liter) tanks with a 50 gal (189 liter) reservoir for re-circulating seawater. A 300 gallon (1135 liter) reservoir tank is connected to each system. In addition, there is nursery tank (200 gallons; 757 liters) with its own recirculating system. Water temperature is ambient, about 24°C. Each system and nursery contains a sump with a protein skimmer, particle and carbon filters through which the seawater passes. The seawater also runs through a UV filter before returning to the animal-containment tanks. Artificial seawater is prepared to a desired 34 PPT salinity from Instant Ocean salts-mix and deionized water, and allowed to sit at least 24 h before use. Ammonia and nitrite levels are monitored and kept at zero (below detection). Nitrates are kept as low as possible, and are always <10 PPM. Each of the 2 adult and nursery aquaria is a closed system, so should an emergency arise, not all animals are lost. In fact, each system could be used as a separate quarantine system should the need arise.

Each tank is made of either darkened or opaque acrylic. The bottom of each tank is covered with approximately 2 cm of calcium-carbonate (marine) sand, and halved 4-in PVC pipes are placed in the tanks, within which the females lay eggs. The lids of each cube are transparent acrylic and allow light to penetrate, so that the squid experiences a natural light-dark cycle.

Each adult animal is housed in a separate tank and labeled with its own number. On occasion, 2-3 animals are kept per tank with no apparent increase in stress to the animals.

Should a power outage occur, the volume of water on the tables is sufficient to keep the squid immersed and oxygenated for several hours.

Feeding

Live freshwater glass shrimp (*Palaemonetes kadiakensis* or *P. paludosus*) are fed *ad libitum* to squid each afternoon, during the animals subjective “dark” cycle. Shrimp are enriched with a nutritional fish-flake food shortly before use. The glass shrimp are obtained through a licensed wholesale fish farm in Florida. In the lab, the shrimp are maintained in separate 50-gal aquaria.

When available, squid are fed saltwater white shrimp, *Penaeus vannamei*. *P. vannamei*. These can be collected from Long Island Sound during summer months.

Cleaning

On a daily basis, dead and dying feeder shrimp are removed from the tanks first thing in the morning. This prevents buildup of nitrogenous waste in the system and in the sand layer.

Each individual tank is cleaned between squid collections or when noticeably dirty. The squid are transferred to a separate holding tank for the duration of cleaning (< 20 minutes). Sand is removed and washed with very hot tap water, followed by a thorough rinsing with deionized water. Algae, leftover feed, and other detritus is removed from the sand layer through this process. The sides, bottom, and top of the tanks are also scrubbed.

Annually, the tanks are completely drained, the filters are changed, the UV light bulbs are changed, and deionized water is pumped through the table for a few days. The tanks are then refilled with seawater and allowed to run for at least a week before a new shipment of squid comes in or before new eggs are placed on the nursery.

Breeding of adults

The mantle lengths (ML) of all squid are measured in the first week of arrival after collection. A single female is matched with a similarly sized (or slightly smaller) male. During each breeding event (once every 2 weeks for each female), the male is moved into the female’s tank for 12 h overnight, and returned to its own tank the next morning for recovery. Each male has at least 2 days of recovery between mating events.

Record Keeping

Electronic animal records are maintained in the Nyholm lab for ease of input and access. Facility records, such as water parameters and purchasing receipts, are maintained in paper and electronic form. Temperature and salinity are recorded daily. Nitrate, nitrite and ammonia levels are checked 2x per week or more often if animals display signs of distress. Animal identifiers are designated by number and/or letter and monitored for the duration of the animal’s life. Animal death dates are recorded, and if the animal is euthanized, that is recorded as well. Mean longevity in captivity can thus be calculated.

Each egg clutch is assigned a unique identifier, which includes the cohort alphabet designation. For each clutch, the lay date, the approximate number of eggs, the female, and the PVC cave identifier are recorded.

From each clutch, the number of hatchlings is recorded. This information includes the number of hatchlings that hatched overnight (“earlies”) and the number of hatchlings that hatched at known times (“normals”). Any dead, sickly, or premature hatchlings are also noted. From these data, we can compute the number of hatchlings per clutch total, the number of hatchlings per time period, the number of hatchlings per female, and the clutch’s range of incubation time.

**Incubation and hatching**

Aquarium parameters

When a clutch is laid in an adult tank, it is recorded and moved (while immersed in a glass bowl filled with saltwater) to the incubation table. At no time is the clutch exposed to air.

The nursery can accommodate up to 20-30 containers that each can house of to 3 clutches each. The nursery has 200 gal of circulating seawater. The water temperature is kept at 23-24° C through the use of a chiller and heater attached to the system. Levels of ammonia, nitrite, and nitrate are in the water are maintained at zero (*i.e*., below detection), and salinity is kept at 34 PPT. The circulating water is exposed to UV illumination. Because constant aeration of the eggs is essential for their normal development, should a power outage occur, the egg table pump is attached to an outlet that receives power from the building’s emergency generator.

Hatching

Most eggs (70-80%) hatch within 2 h of dark, that is, between 1100 and 1400 h. Animals are collected regularly from the egg table using a 5 mL disposable plastic pipette to transfer them to a plastic beaker. During the day, animals are collected within 30 min of hatch and are placed into filter-sterilized (0.22 µM pore-sized filter membrane) Instant Ocean water (FSIO). Animals are washed 3 times in this filtered water to prevent any contamination from the egg table water. Overnight animals (the “earlies”), may remain on the table as long as 16 h, and are used for experiments that do not require either aposymbiosis (no colonization of the symbiotic light organ) or knowledge of the exact time of hatch.

**Anesthesia and euthanasia**

Recognition of stress or illness

Adult and raised squid are observed at least twice daily (first thing in the morning, and during the evening feeding). Signs of illness or distress include failure to bury in the sand during daytime hours, failure to eat, and failure to respond to gentle touch. Animals deemed to be ill are euthanized (see below). Lesions and scars may appear on females immediately after mating, but do not appear to affect the short- or long-term overall health of the animal, and usually disappear within a few days.

During cleaning, breeding and tank maintenance, it is sometimes necessary to remove the squid from its sand cover. This may evoke a temporary stress response (e.g., inking), but care is taken to minimize these occurrences, and to proceed as carefully and gently as possible. Because the viscous ink may clog gills, it is removed immediately from the water with a turkey baster.

Termination of experiments and euthanasia

Adult animals die either naturally, at the termination of an experiment, or are euthanized. Hatchlings and reared animals are euthanized at the termination of an experiment. Euthanasia is performed through over-anesthetization followed by a quick-freeze method. Anesthetization is performed as described above: exposure to either 2% ethanol or 0.12-0.15M MgCl2, in FSSW or FSIO.. Anaesthetized animals may be put into a dry, sealed container (a plastic bag or vial), and dropped into a bath of liquid nitrogen. Within a few seconds, the animal is completely frozen, and the tissue stored at -80˚ C. If organs need to be harvested after an experiment squid are first anesthetized as described above and the brain is quickly pithed and head severed with a scalpel or razor blade.

Carcass disposition and storage

After an experiment, or at the end of an adult’s life, the tissues (hemolymph, light organ, testes, eyes, *etc.*) are often harvested, and frozen for future use. Otherwise, after experiments and euthanasia, juvenile squid are placed in plastic bags and disposed of through Inserv animal waste via a collection site in Torrey Life Sciences;; similarly, after experiments and euthanasia, the entire carcass of adult/reared animals is put into labeled and sealed plastic bags, and stored in a dedicated freezer for future use or reference.