

Laboratory Rearing and Maintenance of

Loligo pealei larvae and juveniles

by

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Preface

The work described herein was conducted as part of the Aquavet Program,
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during the summer 1981.

of

Introduction

The Atlantic Coast squid, Loligo pealei, is extensively used at the Marine Biological Laboratories in neurological studies of its giant axon. Unfortunately, Loligo pealei, is available only during the summer months of the year and this severely limits the study of this animal. A practical alternative to this problem would be to use aquaculture techniques to raise Loligo pealei in the lab and provide researchers with a year round supply of squid.

Artificial rearing of Loligo pealei, however, has proved to be a most difficult assignment. Many people have attempted to raise Loligo pealei with little or no success. The most successful attempt thus far has been made by a group working out of the Marine Biomedical Institute of the University of Texas. This group (Hanlon, Yang, Hixon, and Hulet) had marginal success and were able to raise hatchling Loligo pealei in three separate trials for a maximum of 16, 29, and 40 days.

In this paper, I will describe my attempts at rearing Loligo pealei hatchlings using a wide variety of rearing containers and food concentration. In addition, I will document my attempts at maintaining wild caught juvenile Loligo pealei in the lab.

Materials and Methods

Collection and Hatching of Squid Eggs

Loligo pealei eggs were collected from the MBL supply department. The supply department obtained squid eggs in one of two ways. Occasionally, eggs were brought up in their bottom trawls. More often, however, captured adult squid laid fresh egg masses when they were held overnight in a large circular tank.

Loligo eggs obtained from MBL supply were suspended in aquaria or buckets with monofilament line and allowed to mature in running seawater. Periodically a single egg case would be separated from the main egg mass and looked at under a dissection scope. When the embryonic squids neared stage 30, the outer egg sac was removed and the squid allowed to hatch out. The removal of the outer egg case was not essential, but it did speed the hatching process. When the eggs were incubated at the normal seawater temperature of 21-22°C, it took between 1.5 and 2 weeks for the squid to begin hatching out. If, however, the squid eggs were incubated at a temperature between 27-30°C, the squid began hatching out within one week.

When a particular experiment was initiated, the hatching tank would be completely cleared of squid hatchlings. The eggs would then be allowed to hatch out for a set number of hours until the needed number of squid were hatched. Timing the hatching process enabled the squid hatchlings to be aged precisely. Hatchling squid were dipped out of the hatching tank with a small beaker as needed.

Copepod Collection

Copepods were used as the basic food source for hatchling and small juvenile Loligo pealei. The majority of copepods were collected in one of two ways. The first method was used to collect small copepods (200-300 μ) to feed to freshly hatched squid and consisted of simply hanging a small mesh zoo- or phytoplankton net off one of the local piers. The current would run through the net and the copepods would be filtered out. The net would then be periodically checked during the day and emptied of copepods. The second method used to collect copepods was a boat plankton tow. The boat tow was conducted mainly in Great Harbor and a large mesh zooplankton net was used to collect copepods larger than 500 μ for use with the smaller juvenile squid. A third option that was not used was to collect copepods from the seaweed at low tide. Large numbers of copepods could be collected from the seaweed, but it also involved tediously separating the sediments from the copepods.

After the zooplankton was collected, either off the pier or from a boat tow, it would then be filtered through some netting with a mesh equal to about 2 mm. This initial screening would clear out the larger debris (seaweed, ctenophores, etc.). The plankton would next be filtered through a 500 μ sieve and then through a 200 μ sieve. The fraction larger than 500 μ would be fed to the smaller juvenile squid while the fraction between 200 and 500 μ was fed to the squid hatchlings.

The fraction between 200 and 500 μ was more than 90% copepods and was ideal for use with hatchling Loligo. The four main species caught between 200 and 500 μ were Microcalanus pusillus, Eurytemora sp., Acartia tonsa, and Labidocera sp. The dominant species between 200 and 500 μ was Acartia. The main advantage to using the fraction less than 500 μ was that almost all the crab zoea were excluded. Crab zoea were not favored by the hatchlings as food. The fraction greater than 500 μ was also comprised mainly of copepods, but it also included crab zoea, crab megalops (Cancer), and larval shrimp (Caridea). The dominant copepod larger than 500 μ was Labidocera.

After the copepods were graded into different sizes, the fraction between 200 and 500 μ was diluted to 500 or 1000 mls and allowed to settle. The dead copepods on the bottom were then siphoned off. Quantification was performed by pipetting out one ml of suspended copepods into a counting cell and counting the live copepods. Three counts would be made and then averaged. The copepods larger than 500 μ were not quantified.

Feeding Hatchling Loligo pealei

Probably the most accurate method of quantifying the proper number of copepods needed for the squid hatchlings reared in glass beakers was to use a glass pipette. The dead copepods were first allowed to settle to the bottom of the container and then the live copepods suspended in the water column would be pipetted out. The pipette was then held up to the light and the copepods counted.

For the larger rearing systems (buckets and 60 gal tank), the glass pipette method was too tedious. For these systems, the concentration of the captured copepods was first estimated using the counting cell as previously mentioned and then the proper volume of suspended copepods was added to each system. To minimize errors in estimation of the copepod concentration, the dead copepods were removed prior to counting and the container holding the copepods was well mixed before the 1 ml sample was removed for the counting cell. In addition, the copepod holding container was also mixed before adding the solution to each rearing container. This mixing overcame the problem of the copepods orienting to the light and stratifying themselves in the water column.

Whenever there were extra copepods, they would be placed in a holding tank with running seawater. Copepods could be kept alive in this fashion for over a week. Maintaining extra copepods on hand alleviated somewhat the day to day variation in plankton tow catches, and assured a constant supply of live copepods.

Fresh, live copepods had to be supplied to each rearing system daily, but not the same amount of copepods was needed each day. The amount of copepods needed varied because even though some would be eaten and some would die, a large portion of the copepods would survive and remain trapped in the rearing container by the fine mesh screens of the water baffles. In order to keep the concentration of copepods in each rearing system constant, each tank would have to be subsampled first before any new copepods could be added. Ideally, it would have been best to subsample the entire water column (top, middle, and bottom), but this would have been very stressful to the squid and so only a single surface subsample was taken on each system. Between 100-500 mls were removed for each subsample depending on the copepod concentration of the rearing tank at that time. The copepods in the subsample would be counted and an estimation made of the tank copepod concentration. Then, depending what that concentration was, more copepods would be added to bring the concentration up to the desired level.

Feeding Behavior of *Loligo pealei* hatchlings

When viewed under a dissection scope, *Loligo pealei* hatchlings were found to capture and feed on copepods. An attempt was made to quantify the feeding behavior of *Loligo* hatchlings and to see if they display a size preference when feeding on copepods. The procedure included grading live copepods into three sizes using plankton sieves (200-300 μ , 300-400 μ , and 400-500 μ), offering these different sized copepods to 10 squid hatchlings held in an observation dish, and counting the number of catches made in 30 min.

Only one copepod was caught in 30 min in each of the different size categories. Although it seems that there was no size preference between copepods 200-500 μ , there was not enough data to make any firm conclusions.

Beaker Systems

The first attempts at raising Loligo pealei hatchlings was made using glass beakers of various sizes. Three different size beakers were used: 100, 500, and 1000 ml (actually 600 ml beakers were used, but they were only filled to the 500 ml level). Ten beakers of each size were used and one squid was placed in each beaker.

The daily procedure included checking the water temperature, counting the number of hatchlings that died overnight, transferring the hatchlings to a beaker of fresh seawater, adding new copepods, and covering the beakers with a dust cover. The most traumatic part of the procedure was the daily transfer of squid to beakers of new water. Initially, this was done using a piece of standard aquarium air tubing. Later, a larger siphon was made by cutting a plastic 10 ml pipette in half and attaching a piece of $\frac{1}{2}$ -inch diameter plastic tubing to the cut end. Both siphons were made of clear plastic and so enabled you to check whether or not a hatchling was actually siphoned into the hose. The larger siphon seemed to be less traumatic to the squid.

Cetyl alcohol was sometimes used to try and modify the survival time of the hatchling squid. Cetyl alcohol or hexadecanol lowers the surface tension of the water and has been used in other aquaculture systems to prevent larvae from getting trapped in the air-water interface. Many hatchlings were seen to get trapped on the surface of the water and die, but there was no improvement in survival time when cetyl alcohol was used.

In addition to using glass beakers, an attempt was also made to rear Loligo hatchlings in "PVC beakers". Ten PVC beakers were constructed out of a length of PVC pipe (diameter = 4 inches) which was cut into sections about 5 inches high. Each beaker held about 750 mls and was washed, acid cleaned, and cured in seawater prior to use. Both PVC and glass beakers were exposed to fluorescent lighting with a photoperiod of 10.5 hours on and 13.5 hours off (on at 8:00 a.m. and off at 6:30 p.m.).

Bucket Systems

Three different rearing systems using plastic buckets were used. The first consisted of a plain white, 18.5 liter bucket with black plastic sheeting taped around the outside of it. The second bucket was dark brown in color and held approximately 12 liters. The third bucket was a shallow (4 inches) black plastic pan that held only 7 liters. All three buckets were flow through systems and were equipped with a stem pipe drain in the center and all three had water baffle-screens around each drain opening to prevent both squid and copepods from being washed out of the system.

The stem pipe drains were constructed out of grey PVC pipe and were positioned so that the water flowing in would drain out towards the center of the bucket at the surface of the water. This was done by simply drilling a hole out of the bottom of the bucket and installing a length of PVC pipe. The water

level was controlled by simply altering the height of the PVC pipe. Water baffle-screens were constructed out of 100 ml plastic beakers. Large rectangular sections were cut out of the sides of the beakers and these holes were then covered over with plankton screening set in place with silicone sealer. Later some of the baffles were colored black with an indelible ink marker. The coloring of the screens was done to prevent the hatchlings from being attracted to light reflected off the white screens. This problem was also alleviated by placing a black bottle cover above the baffle. The bottle cover would cast a shadow over the baffle and eliminate the attraction problem as long as it was kept in place. Problems arose when the covers had to be removed in order to clean the baffle screens.

The use of covers over the baffles worked because the light source was directly overhead. The lighting used for the bucket systems was separate from the fluorescent lighting of the main study area. This was done by enclosing the buckets in an aluminum box that was painted black. In addition, the entire area was enclosed with black plastic sheeting. The light source consisted of two dissection scope lights which were fixed directly over two buckets (only two buckets were run at any one time). The reostat controls for the two lights were outside the black box and the lighting could be controlled without opening the box. Different light settings were used in attempts to modify behavior and extend the life span of the squid hatchlings.

The daily procedure started with the turning on of the lights and the gradual increasing of the light intensity up to the desired level. The light intensity was checked each day with a light meter (photographic). Next, the temperature of the water would be checked and the water flow measured. The filter screens would then be washed, the dead hatchlings removed, and an estimate made of the remaining live squid. Later, a subsample would be taken and the proper number of copepods added to the tank. Finally, at the end of the day the water flow would again be checked and the lights turned off.

Night Lighting for Juvenile Squid

Juvenile Loligo pealei were caught by night lighting in Great Harbor. Night lighting was usually done on calm nights when there was little or no wind chop from a small outboard motorboat. The boat would be tied up to a free buoy and a light hung over the side to attract squid. The smaller juvenile squid would usually appear in ones and twos and would come in quite close to the light. The larger juveniles, on the other hand, tended to school and would stay out at the marings of the light. Whenever juvenile Loligo would come in close enough, they were caught with dip nets. The squid would then be placed in plastic buckets and transported to the rearing tanks.

Collection of Food for Juvenile Squid

Both fish and shrimp were fed to the larger juvenile Loligo pealei. The two species of fish that were used were Menidia menidia (silversides), and Fundulus heteroclitus (killifish). The species of shrimp that was used was

Palaemonetes vulgaris (glass shrimp). All three species were caught near shore with dip nets. Killifish could be caught at any time during the day, while silversides were most easily netted during the evenings when they would come in close to shore. Glass shrimp were found in great abundance all along the coast hiding in the seaweed. Excess shrimp and fish were kept without feeding in flow-through tanks and could survive for up to a week without significant death loss.

Feeding Wild Caught Juvenile Loligo pealei

Feeding trails using fish (live and dead) were run with the juvenile squid in 60-gal tank #1 and feeding trails using shrimp (live and dead) were run with the squid in 60-gal tank #2. Trials using live fish or shrimp were performed by adding a set number of live fish or shrimp to the tank and then counting the number remaining the following day. If all the fish or shrimp were consumed, the amount of live food would be increased and if only a small percentage of the live food was consumed, the number added to the tank would be decreased. The preference of juvenile squid for silversides or killifish was also tested. This was done by placing an equal number of silversides and killifish in 60-gal tank #1 and checking the next day which species were consumed more.

Feeding trials using dead fish and shrimp were also run. Freshly killed fish or shrimp were dropped one by one into a tank and juvenile squid observed to see if they would grab the food. The squid were fed at set intervals (2, 3, or 4 hours) during the day and the dead food that fell to the bottom was checked at each feeding interval to see if the squid were willing to pick up dead food off the bottom.

Sixty gallon Fiberglass Tanks

Sixty gallon fiberglass tanks were used to rear both hatchling and wild caught juvenile Loligo pealei. These 60-gal tanks were circular (diameter = 22 inches) and constructed out of 1/8-inch thick fiberglass sheeting. When used, the tanks were actually only filled to the 50-gal level which is equal to approximately 189 liters. The tanks were equipped with a center stem pipe drain and had black plastic sheeting taped to the outside. The tops of the 60-gal tanks were protected with plastic sheeting covers. These covers kept debris from falling into the tanks and also prevented people passing by from startling the squid. The photoperiod for the 60-gal tanks was the same as that used for the beakers, 10.5 hours on and 13.5 hours off.

The 60-gal tanks used for the larger (3.5 cm) juveniles was basically unmodified. Salt water was piped into the top of the tank at a flow rate of 2 l/min and drained out the center PVC drain. The center drain was initially covered with some large mesh screening, but later was left completely open. There was little chance for the larger juvenile Loligo to be washed down the drain since they spent little time near the surface and were sufficiently strong enough to avoid the current going into the drain. The water flow was checked every second day while the bottoms of the tanks were siphoned daily.

The 60-gal tanks used for the smaller (6 mm) juvenile Loligo were only slightly modified. The water flow was also 2 l/min, but was fashioned as an elbow attached to the side of the tank and positioned so that the inflow came in just under the surface of the water. This created a circular flow of water which was necessary to keep the small juveniles off the sides of the tank. The drain for the small juvenile tank was also in the center, but was surrounded by a large baffle-screen made out of a 1000 ml plastic beaker and some plankton screening. This screen prevented both squid and zooplankton from being lost from the system and had to be brushed periodically to remove sediments and zooplankton. The bottom of the small juvenile tank was not siphoned regularly because the cleaning process was severely traumatic to the small squid and caused many of them to crash into the walls of the tank. Instead of siphoning the bottom, a large number of glass shrimp were released into the tank. These shrimp acted as both a food source for the growing squid and as scavengers to clean the bottom of the tank.

One 60-gal tank was also used in rearing experiments with hatchling Loligo pealei. This 60-gal tank was modified in the following fashion. First, a large diameter PVC pipe was placed over the center stem pipe drain. This larger PVC pipe had windows cut into the bottom of it and these windows were covered with plankton screening. This set up allowed the water to drain out the bottom of the tank and had the advantages of pulling collected debris off the bottom while avoiding attracting hatchlings on the surface to its white screens. The filter screens were brushed daily and the bottom of the tank was siphoned every other day. The water inflow was modified in two ways. At first, the water was channeled into a PVC pipe which had a row of holes drilled into it. This created a row of water jets which sprayed across the surface of the water. These jets were intended to break up the surface tension of the water and at the same time oxygenate the water. Unfortunately, the water jets also had the effect of smashing the hatchlings up against the sides of the tank. The second way in which the water was pumped in was through a simple elbow as was used with the small juvenile tank. The elbow was attached to the side of the tank and the water came in underneath the surface of the water creating a circular water pattern.

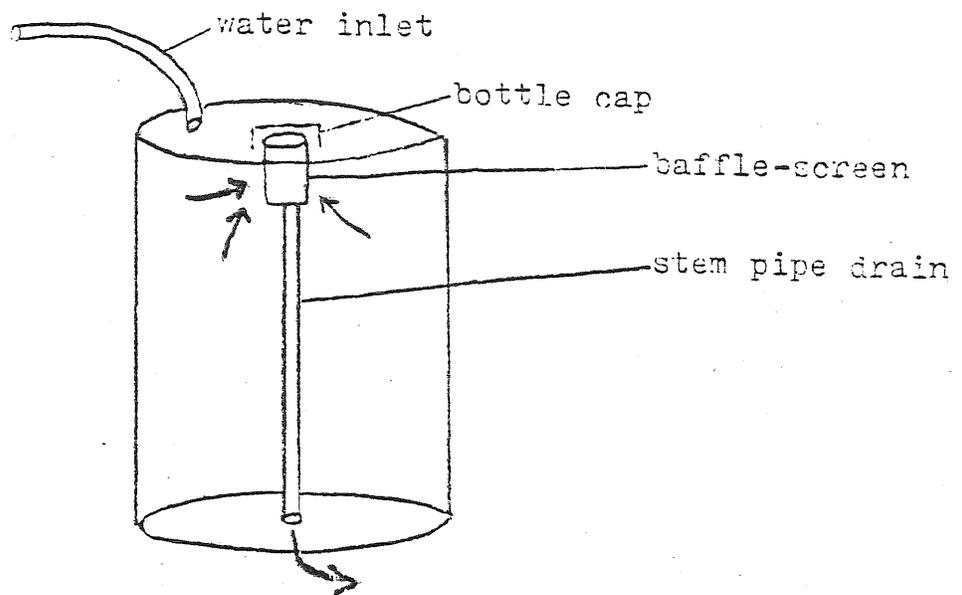


Figure 1. Bucket System.

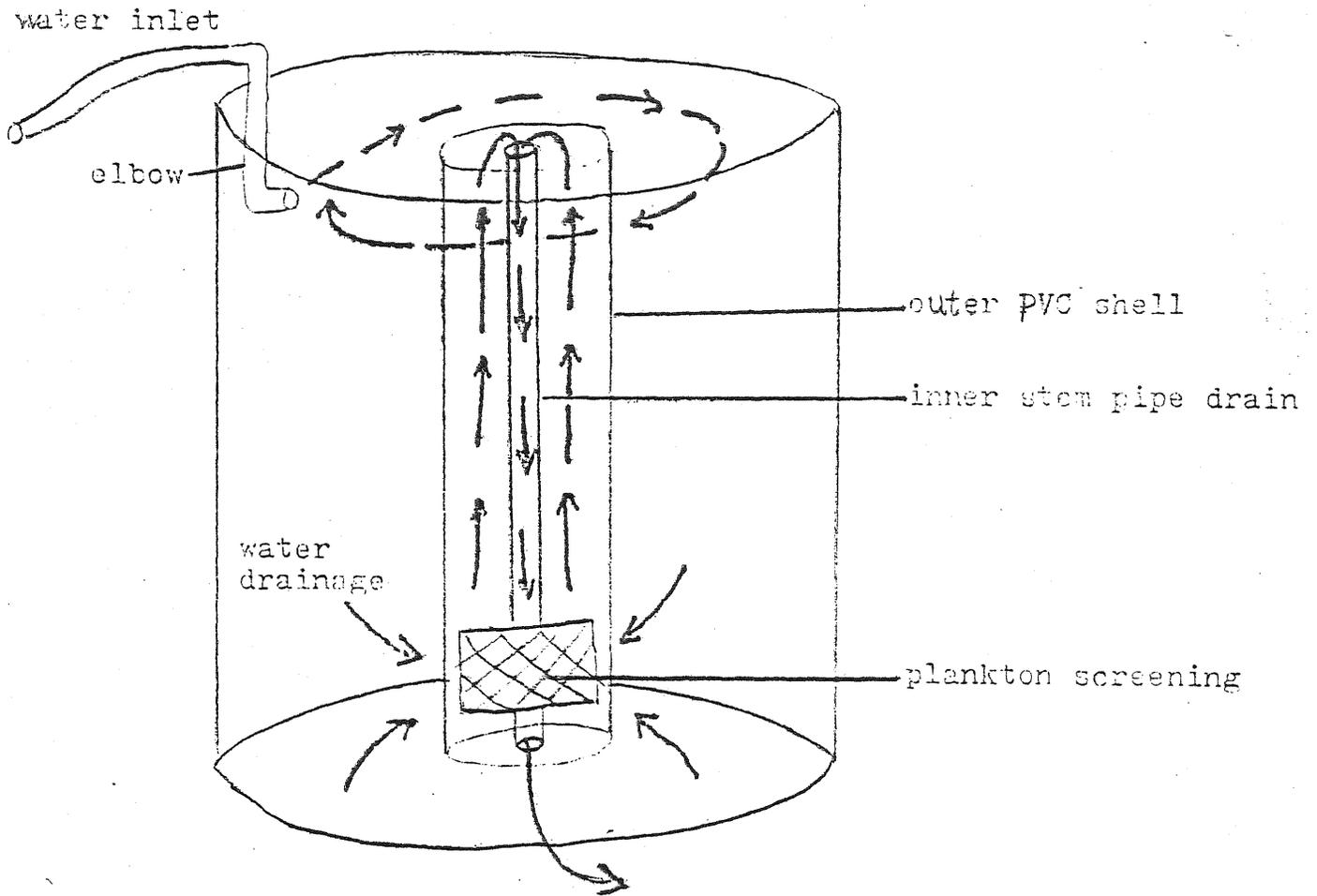


Figure 2. Sixty-gallon tank - set up for hatchlings.

Results:

Beaker Systems

Experiment #1

1. Conditions - 0.25 copepods/ml
 - daily water change
 - cetyl alcohol
2. 6/28 - 6/30 all dead

Experiment #2

1. Conditions - 0.25 copepods/ml (altered collection site)
 - daily water change
 - cetyl alcohol
2. 7/1 - 7/4 all dead

Experiment #3

1. Conditions - 0.1 copepods/ml
 - daily water change
 - no cetyl alcohol
2. 7/5 - 7/8 all dead

Experiment #4

1. Conditions
 - a. beakers 1-5 (100 ml) - no food, no water change, no cetyl alcohol
 - b. beakers 6-10 (100 ml) - no food, no water change, cetyl alcohol
 - c. beakers 11-15 (500 ml) - no food, water change, cetyl alcohol
 - d. beakers 16-20 (500 ml) - 0.1 cop./ml, water change, cetyl alcohol
 - e. beakers 21-25 (1000 ml) - .05 cop./ml, water change, cetyl alcohol
 - f. beakers 26-30 (1000 ml) - 0.1 cop./ml, water change, cetyl alcohol
2. 7/7 - 7/10 all dead

Experiment #5 (only 500 - 1000 ml beakers run)

1. Conditions

- a. beakers 11-15 (500 ml) - no food, no cetyl alcohol, water change
- b. beakers 16-20 (500 ml) - .05 cop./ml, water change, no cetyl alcohol
- c. beakers 21-25 (1000 ml) - .025 cop./ml, water change, no cetyl alcohol
- d. beakers 26-30 (1000 ml) - .05 cop./ml, water change, no cetyl alcohol

2. 7/10 - 7/12 all dead

PVC beakers

1. Conditions - .025 cop./ml, water change every two days, no cetyl alcohol

2. 7/14 - 7/16 all dead

Bucket Systems

1. Bucket #1

a. Experiment #1

- 1) Conditions - 50 squid
- 0.25 copepods/ml
- flow = 100 ml/min

2) 6/27 - 6/30 all dead

b. Experiment #2

- 1) Conditions - 50 squid
- 0.25 copepods/ml
- flow = 100 ml/min

2) 6/30 - 7/3 all dead

c. Experiment #3

- 1) Conditions - 50 squid
- 0.1 copepods/ml
- flow = 200 ml/min

2) 7/5 - 7/8 all dead

d. Experiment #4

- 1) Conditions - 50 squid
- no food
- flow = 200 ml/min

2) 7/7 - 7/10 all dead

e. Experiment #5

- 1) Conditions - 50 squid
- 0.025 copepods/ml
- flow = 200 ml/min

2) 7/10 - 7/14 all dead

2. Bucket #2

a. Experiment #1

- 1) Conditions - 50 squid
- 0.25 copepods/ml
- flow = 100 ml/min

2) 6/27 - 6/30 all dead

b. Experiment #2

- 1) Conditions - 50 squid
- 0.25 copepods/ml
- flow = 100 ml/min

2) 6/30 - 7/3 all dead

c. Experiment #3

- 1) Conditions - 50 squid
- 0.1 copepods/ml
- flow = 200 ml/min

2) 7/5 - 7/8 all dead

d. Experiment #4

- 1) Conditions - 50 squid
- 0.05 copepods/ml
- flow = 400 ml/min

2) 7/8 - 7/11 all dead

e. Experiment #5

- 1) Conditions - 50 squid
- 0.025 copepods/ml
- flow = 400 ml/min

2) 7/11 - 7/14 all dead

f. Experiment #6

- 1) Conditions - 50 squid
- 0.025 copepods/ml
- flow = 400 ml/min
- 2) 7/14 - 7/16 all dead

g. Experiment #7

- 1) Conditions - 25 squid
- 0.05 copepods/ml
- flow = 300 ml/min
- 2) 7/16 - 7/19 all dead

h. Experiment #8

- 1) Conditions - 24 squid
- 0.25 copepods/ml
- flow = 1000 ml/min
- 2) 7/26 - 7/28 all dead

3. Bucket #3

a. Experiment #1

- 1) Conditions - 25 squid
- 0.025 copepods/ml
- flow = 300 ml/min
- no light
- 2) 7/14 - 7/16 all dead

b. Experiment #2

- 1) Conditions - 20 squid
- 0.05 copepods/ml
- flow = 300 ml/min
- 2) 7/16 - 7/18 all dead

c. Experiment #3

- 1) Conditions - 14 squid
- 0.25 copepods/ml
- flow = 1000 ml/min
- 2) 7/26 - 7/28 all dead

Sixty-gallon Tank - Hatchlings

1. Experiment #1
 - a. Conditions - 378 hatchlings (2 hatchlings/1)
 - 0.25 copepods/ml
 - flow = 2 l/min (water jets)
 - (189 liter tank with 2 l/min = 120 l/hr = 180 l/1.5 hr
 - one full turnover every 1.5 hours)
 - b. 7/15 - 7/28 all dead
2. Experiment #2
 - a. Conditions - 378 hatchlings
 - 0.20 copepods/ml
 - flow = 2 l/min (elbow - no jets)
 - b. 7/28 - 8/1 all dead
3. Experiment #3
 - a. Conditions - 189 hatchlings (1 hatchling/1)
 - 0.20 copepods/ml
 - flow = 1 l/min
 - b. 8/1 - 8/4 all dead
4. Experiment #4
 - a. Conditions - 378 hatchlings
 - 0.20 copepods/ml
 - flow = 3 l/min
 - b. 8/4 - 8/7 all dead (silt in water, clogged filters)
5. Experiment #5
 - a. Conditions - 378 hatchlings
 - 0.20 copepods/ml
 - flow = 3 l/min
 - b. 8/7 - 8/11 all dead (copepod shortage)
6. Experiment #6
 - a. Conditions - 378 hatchlings
 - 0.25 copepods/ml
 - flow = 2.5 l/min
 - b. 8/10 - 8/12 all dead (phytoplankton clogged the filters and the drain overflowed)

7. Experiment #7

- a. Conditions - 378 hatchlings
 - 0.25 copepods/ml (supplemented with brine shrimp)
 - flow = 2.5 l/min

- b. 8/12 - 8/15 all dead (insufficient food ?)

Large Wild Caught Loligo pealei

Night lighting on 7/31/81

45 squid caught

13 squid died during capture - average weight = 1.7 grams
- average mantle length = 3.5 cm

32 squid survived - 21 placed in 60 gal tank #1
- 11 placed in 60 gal tank #2

Silversides

20 randomly sampled silversides - average weight = 0.12 grams
- average length = 2.38 cm

Glass shrimp

Ranged in size from 1-3 cm

Consumption of live silversides

<u>Date</u>	<u>Consumption (fish/day)</u>
8/1	4.4
8/2	4.0
8/4	4.8
8/5	5.8
8/6	7.2
8/7	7.1
8/8	5.5
8/9	5.5
8/10	4.8
8/11	4.9
8/12	4.8
8/13	<u>4.6</u>

Total = 63.4 = 5.3 silversides/squid/day

Preference of Loligo pealei for silversides

Tank #1 - 50 silversides and 50 killifish
36/50 or 72% of the silversides were consumed
10/50 or 20% of the killifish were consumed

Consumption of Dead Silversides

<u>Date</u>	<u>Consumption</u>
8/14	9:30 am - 17/28 silversides grabbed 1:30 pm - <u>8/24</u> silversides grabbed 25 fish/14 squid = 1.8 fish/squid
8/15	9:30 am - 12/13 silversides grabbed 10:30 am - 1/4 silversides grabbed 3:30 pm - 12/12 silversides grabbed 5:30 pm - <u>8/13</u> silversides grabbed 33 fish/13 squid = 2.5 fish/squid
8/16	9:00 am - 9/13 silversides grabbed 11:00 am - 1/8 silversides grabbed 1:00 pm - no feeding 4:00 pm - <u>7/11</u> silversides grabbed. 17 fish/11 squid = 1.5 fish/squid
8/17	9:00 am - 11/12 silversides grabbed 12:00 pm - 5/12 silversides grabbed 3:00 pm - 8/12 silversides grabbed 6:00 pm - <u>4/12</u> silversides grabbed 28 fish/11 squid = 2.5 fish/squid
8/18	8:00 am - 9/12 silversides grabbed 12:00 pm - 9/12 silversides grabbed 4:00 pm - 4/12 silversides grabbed 8:00 pm - <u>10/12</u> silversides grabbed 32 fish/11 squid = 3.0 fish/squid/12 hours (6.0 fish/squid/24 hours)

Large Wild Caught Juvenile Loligo pealei

Consumption of Live Shrimp

177 shrimp consumed in 24 hours
177 shrimp/7 squid/24 hours = 25.3 shrimp/squid/day

Consumption of Dead Shrimp

8:00 am - 4/8 dead shrimp grabbed
12:00 pm - 5/8 dead shrimp grabbed
4:00 pm - 7/8 dead shrimp grabbed
8:00 pm - 5/8 dead shrimp grabbed

- at 4:00 pm all the uneaten shrimp that were lying on the bottom were gone and presumed eaten.
- total consumption - 29 shrimp/7 squid/12 hours = 4 shrimp/squid/12 hours
- 4 shrimp/squid/12 hours = 8 shrimp/squid/day

Small Wild Caught Juvenile Loligo pealei

Night Lighting on 8/7/81

Over 100 small juvenile Loligo pealei caught
84 survived the capture process and were placed in 60 gal tank #3
- by day 2 only 11 survivors
- 20 dead juveniles measured - average mantle length = 6.0 mm

<u>Date</u>	<u># squid surviving</u>
Day 0 (8/7)	84
Day 2	11
Day 11	9 (1 new squid added)
Day 12	8
Day 13	9 (1 new squid added)
Day 14	9

Sizes of the surviving 9 squid on day 14 (8/21/81)

- 1) 7 mm
- 2) 15 mm
- 3) 15 mm
- 4) 15 mm
- 5) 15 mm
- 6) 15 mm
- 7) 15 mm (caught 8/20 - day 13)
- 8) 24 mm (caught 8/14 - day 7)
- 9) 24 mm

Feeding

The small juvenile squid caught 8/7 were fed mainly zooplankton and small glass shrimp. The zooplankton was the fraction larger than 500 μ and consisted of copepods, larval shrimp, crab zoea and crab megalops. The copepods and the

larval shrimp were readily captured and eaten, but the crab zoea and megalops were largely ignored. Small glass shrimp were occasionally captured by these small juvenile Loligo.

Growth

Average initial size = 6.0 mm

Average size after 14 days = 15.1 mm (average of the 7 squid surviving the full 14 days)

9.1 mm of growth/14 days = 0.65 mm growth/day

Discussion

Beaker Systems

A total of five trials using 30 beakers (glass) were run. Variations were made in the food (copepod) concentration, the frequency of water changes, and the use of cetyl alcohol. Copepod concentrations of 0.025, 0.05, 0.10, and 0.25/ml were used. Trials were run with and without the use of cetyl alcohol and the water in the beakers was changed either daily or every second day. Unfortunately, at no time was the life span of the experimental hatchling Loligo pealei extended beyond that of the control group. The control group, kept in 100 ml beakers without food, cetyl alcohol, or change of water, was able to survive for a maximum of three days. In all the trials using beakers as rearing containers, the hatchling squid died within three days of hatching out. The one trial using home made PVC beakers also did not extend the survival time of the hatchling squid beyond three days. The precise reason for the failure of the beaker systems is not known, but there was a noticeable rise in the pH (from 7.6 to 8.0 in the 100 ml beakers and from 7.6 to 7.8 in the 500 and 1000 ml beakers) of the beaker water left standing overnight with one squid hatchling in each beaker and a copepod concentration of 0.05/ml. In addition, the daily transfer of hatchling squid to beakers of new water could have been traumatic enough to be lethal.

Bucket Systems

Bucket #1 was a plain white, 18.5 liter plastic bucket. One control was run using 50 squid hatchlings, a water flow rate of 200 ml/min, and no food. The unfed control hatchlings survived for three days. Four other trials were run using copepod concentrations of 0.25, 0.10, and 0.025 copepods/ml and water flow rates of either 100 or 200 ml/min. No improvement over the three-day control time was made. The main drawback to bucket #1 was that it was white. The white sides of the bucket readily attracted the squid hatchlings which spent much of their time bumping up against it. This probably resulted in epithelial damage to the squid and their early deaths. Attempts were made to modify bucket #1 using an opaque two-inch lip around the rim of the bucket. This was designed to cast a shadow over the white walls of the bucket, but failed because incoming light was reflected off the bottom and walls of the bucket and cancelled out the shadow effect created by the two-inch lip. The squid hatchlings placed in this modified bucket did not stay away from the sides anymore than they did in the unmodified bucket.

Bucket #2 was a dark brown bucket that held 12 liters. Copepod concentrations of 0.025, 0.05, 0.10, and 0.25 copepods/ml were used and water flow rates of 100, 200, 300, 400, and 1000 ml/min were tried. The dark brown walls of bucket #2 appeared to solve the problem of the squid hatchlings bumping into the sides of the bucket. In dim light, the hatchlings would more or less evenly distribute themselves throughout the water column. Problems occurred only when the light intensity was turned up and the hatchlings were attracted to the white screens of the baffles. This was alleviated by coloring the screens black with a permanent ink marker. Although the problem of the squid hatchlings damaging themselves against the sides of the tank were alleviated, the maximum survival time for squid hatchlings raised in bucket #2 during eight trials was still only three days. The reasons for the lack of increased survival time is not known. The oxygen tension of the buckets remained virtually unchanged throughout the eight trials, the pH increased only slightly (7.6 to 7.7) in three days time, and there was no detectable buildup of nitrates or nitrites in the water (ammonia could not be run with the fresh water Hach kit used).

Bucket #3 was a flat black pan that held only seven liters. Like bucket #2, the squid hatchlings seemed to evenly distribute in the water column. Unfortunately, also like bucket #2, squid hatchlings survived only three days at the most. Food concentrations of 0.025, 0.05, and 0.25 copepods/ml and water flow rates of 300 and 1000 ml/min were used.

Sixty-gal Tank - Hatchlings

Seven trials were run using a 60-gal fiberglass tank trying to raise Loligo pealei hatchlings. And although no improvement was made over the three-day survival time of the control group, this system probably holds the most promise in achieving some success in rearing Loligo hatchlings. It was found that the ideal inflow pipe should be arranged as an elbow and that the water should come in just under the surface at a rate of between 2.5 and 3 l/min. This arrangement created a circular flow pattern and kept the hatchlings off the walls of the tank. The majority of hatchlings could be seen to orient themselves against the flow of water and were positioned approximately half way between the center drain and the walls of the tank.

There were several reasons why no success beyond three days was achieved even when the proper water flow pattern was created. The first time the optimum water flow pattern was set up, silt was accidentally flushed into the tank and all the hatchlings crashed. The second time, phytoplankton clogged the drain filters and most of the hatchlings were washed out of the tank. And the third time, not enough copepods could be collected and the tank had to be supplemented with brine shrimp. I strongly suspect that if these three problems had not intervened, at least some of the Loligo pealei hatchlings reared in the 60-gal tank would have survived for longer than three days.

Sixty-gal Tank - Large Juveniles

Thirty-two large juvenile Loligo pealei were used for feeding studies with live and killed food. The average size of these juvenile squid (determined from 13 squid that had died during capture) was 1.7 grams in weight and 3.5 cm in mantle length (as measured from the tip of the mantle to the eye). It was determined that the juvenile squid definitely preferred live silversides over live killifish. When equal numbers of silversides and killifish (of approximately the same size) were placed in the same tank with a group of juvenile squid, 72% of the silversides were eaten while only 20% of the killifish were consumed. The most likely reason for this favoring of silversides over killifish was that the killifish seemed to be stronger and more elusive than the silversides. In addition, the killifish tended to huddle in a group near the bottom, while the silversides were more evenly spread out in the water column. Live silversides were fed only to the squid in tank #1 and over 12 days, the average consumption was 5.3 silversides/squid/day.

The larger juveniles were also fed freshly killed silversides. When hungry, the squid would accept dead food fairly readily. A variety of forms of dead fish were tried. It was found that the squid would accept whole dead fish, fish cut in half, and even fillets off the sides of the larger silversides. Feeding intervals of two, three, and four hours were tried. The feeding interval of four hours proved optimal. With a four-hour interval almost all the squid in the tank would be ready to feed again. The juvenile squid were fed at 8:00 a.m., 12:00 p.m., 4:00 p.m., and 8:00 p.m., for a total of four feedings. The average consumption for those four feedings was 3.0 fish/squid/12 hours. If the 12-hour feeding is extrapolated out to 24 hours, the consumption comes out to be 6.0 fish/squid/day - which comes close to the consumption of live silversides which was 5.3 silversides/squid/day. The only problem is that in order to achieve the 6.0 fish/squid/day consumption rate, the dead fish would have to be fed at four-hour intervals throughout the day (and night) because the squid would not pick up dead fish off the bottom. The live silversides, on the other hand, only had to be given once a day.

Glass shrimp were also fed to the larger juvenile Loligo pealei. The average daily consumption of live glass shrimp was 25.3 shrimp/squid. Dead glass shrimp were also accepted as food and more importantly dead shrimp lying on the bottom were also eaten. No squid was actually seen picking up a dead shrimp from the bottom, but the leftover dead shrimp would always be missing from the bottom when the next feeding time came up. It is presumed that because the natural habitat of shrimp is the bottom, the squid were much more willing to accept dead shrimp lying on the bottom. This proves to be a significant advantage over the feeding of dead fish which were not readily picked up from the bottom.

When the feeding trials had ended after three weeks, 10 of 21 squid in tank #1 had survived and 7 of 11 squid had survived in tank #2. Deaths in both tanks could be attributed to bacterial infection after the juvenile squid sustained epithelial damage. The 60-gal tanks may simply not have been large enough. Whenever the squid were startled, they would dart quickly from one

side of the tank to the other. The 60-gal tanks were only 22 inches in diameter and nervous squid often smashed into the sides of the tank, especially before they were accustomed to the daily siphoning procedure. Damaged areas of the squid epithelium would get bacterially infected and this probably led to some deaths. Some epithelial damage could also have been carried over from the capture process. Captured juveniles were transported to the rearing tanks in fairly small (18.5 l) rigid plastic buckets and the squid did a lot of wall bumping in these buckets.

Besides bacterial infection, other deaths could also be attributed to starvation and cannibalization. Starvation and cannibalization probably accounted for the greater death loss, 52%, in tank #1 vs. 36% death loss in tank #2. The squid in tank #1 were the ones being used in experiments with dead silversides. The feeding trials using dead silversides lasted six days, including the first day when the squid were not fed at all, and provided, at the most, only half the amount of fish the squid were consuming when fed live silversides (max. 3.0 dead fish/squid/day vs. an average of 5.3 live fish/squid/day). The lack of food had to greatly increase the stress on the squid and probably led to the eventual death of some of the smaller juvenile squid (which were also more reluctant to attack the dead fish). In addition, two squid were actually seen cannibalized during the feeding trials using dead fish. This cannibalization was probably a direct result of the semi-starved condition of the squid during the feeding trials - the stronger squid simply picked off the weaker ones. Cannibalization might also have been responsible for other deaths during the three week period. Two squid completely disappeared from tank #1 during the first week and it is possible that these squid were eaten by their tankmates. Even though the death loss in both tanks was quite high, juvenile squid accepted both live and dead food readily and overall were fairly easy to maintain in captivity.

Sixty-gal Tank - Small Juveniles

Small juvenile Loligo pealei were also maintained in 60-gal tanks. These squid were caught on the night of August 7, 1981 and averaged in size about 6.0 mm. Eighty-four of these small juveniles were placed in one 60-gal tank, but only seven survived the full two weeks until August 24, 1981 when the project was ended. The main reason for the massive loss of squid was that no one had anticipated catching such small Loligo and so the 60-gal tank was not properly prepared. Small juvenile squid were found to need essentially the same setup as the hatchlings did when they were reared in 60-gal tanks. The water flow had to be circular and a baffle screen had to be placed over the drain. Because the tank was not properly set up until the following day, the majority of small juveniles spent most of their time bumping up against the sides of the tank. Injuries resulting from this bumping were probably what caused most of the deaths. After the circular water pattern was set up, the small squid oriented to the flow and kept away from the sides. In addition to the seven squid that had survived from the initial 84, two more small juveniles were added to the tank on days 11 and 14. These new additions were also still alive on day 14. Day 14 was the last day of the project, and approximate measurements were made

on all nine remaining squid. The estimated average size of the seven squid that had survived the full 14 days was 15.1 mm. This was a 9.1 mm gain over the initial 6.0 mm size of the small juvenile. The average daily gain of the seven juveniles that survived the full 14 days was 0.65 mm/day. The small juvenile squid did readily capture and ingest copepods, larval shrimp, and glass shrimp, but it is really not known how accurate this growth figure is. The average daily gain of 0.65 is probably somewhat inflated because it is also probably true that the larger juvenile squid that were initially caught were also the ones most likely to survive the full 14 days.

Conclusion

Attempts were made to raise Loligo pealei hatchlings in beakers, buckets, and 60-gal fiberglass tanks. At no time, however, was the survival time of the hatchlings extended beyond the control time of three days. Although the rearing attempts were unsuccessful, several important insights were uncovered and these may lead to eventual success in rearing Loligo pealei hatchlings. First, the best results were obtained with black or dark colored rearing containers. Squid hatchlings were attracted to any white or light-colored material and would constantly bump up against it. The constant bumping probably contributed to many early deaths. A second insight was that the water flow should be circular. When the water flow was circular, the squid hatchlings were seen to orient to the flow and kept off the walls of the tank. A final important fact learned was the confirmation that Loligo pealei hatchlings will feed on copepods.

Although no Loligo pealei hatchlings survived past three days, some very small juvenile Loligo pealei were maintained in captivity. The average size of the small wild caught juvenile Loligo pealei was 6.0 mm, but some were as small as 2.0-3.0 mm, barely bigger than hatchling size. Most of these small juveniles died due to trauma from capture and wall bumping, but it was encouraging to note that once the 60-gal tank was properly set up, the small juveniles did feed well on zooplankton and seven survived for a full two weeks.

Larger juvenile Loligo pealei (3.5 cm) were also caught and these proved to be much easier to maintain than either the hatchlings or the small juvenile squid. The tanks they were housed in required little modification and they were found to accept both live and dead food. Of the initial 32 large juveniles, 17 survived the three weeks of feeding trials. The death loss was high, but could probably have been reduced by minimizing trauma during capture, using rearing tanks with larger surface areas, and providing the squid with adequate nutrition during the entire time of the feeding trial.

Overall, it can be seen that as the squid grow older and larger, they become easier to maintain. It is now apparent that once the hatchlings are past those critical first few weeks, it should be possible to raise them to adulthood. The problem, of course, is keeping the hatchlings alive for those first few weeks. I failed, but did learn some valuable information and have some suggestions for future attempts at rearing Loligo pealei hatchlings.

Future attempts at rearing Loligo pealei hatchlings should probably start with the use of a large volume (40-60 gal), large surface area glass or fiberglass tank. With a larger volume system, toxic by-products are less likely to build up and a large surface area would give hatchling squid more room to spread out. Ideally, the walls of the tank should be flat black in color. Either the initial construction materials should be black or the inside walls of the tank coated with tinted resin. A second major modification would be to install an undergravel filter on the bottom together with two inches of gravel. The gravel and filter would eliminate the need for regular siphoning of the bottom and would thereby reduce the stress on the squid hatchlings. A third modification to the rearing tank should be to use an elbow as the seawater inlet and to attach it to the tank wall just underneath the surface of the water. If a center step pipe drain is used together with this setup, a circular water flow pattern will be created.

Once the tank has been properly set up with a circular water flow, the rearing operation should be initiated by suspending a small egg mass in the rearing tank itself and allowing the squid to hatch out directly into that tank. This would make it difficult to calculate the initial number of squid hatchlings starting the experiment, but has the advantage of greatly reducing the stress on the newly hatched squid. - Part of my problem, I now feel, was to use a plain white bucket as the hatching tank. It is possible that even the few hours the newly hatched squid spent in the white bucket could have been enough time for them to lethally injure themselves against the sides of the tank. Even if a hatching tank with dark walls is used (as was done towards the end of my project), the mere act of catching and transferring could also have been extremely stressful to the young squid.

After roughly the desired number of squid have hatched out into the rearing tank, the egg mass can then be removed and copepods added to the tank. The optimal concentration of copepods is not known at this time, but probably a fairly high concentration (0.05-0.50 copepods/ml) is needed. The purpose of maintaining a high copepod concentration is to ensure that squid hatchlings encounter copepods often enough so as not to starve to death, but not so high a concentration as to pose a pollution problem. For my rearing attempts, adequate numbers of copepods were collected with plankton nets from the wild. Future attempts at rearing Loligo pealei hatchlings may want to artificially rear copepods instead. Artificial rearing of copepods was not done with this project because it was thought to be complicated and not capable of generating large enough numbers of copepods. I have since, however, learned that large numbers of Acartia can be raised fairly easily in glass vessels with only minimal care and attention. Artificial rearing of copepods would be advantageous in that it would provide a constant source of pure copepods, reduce the risk of introducing some outside contaminant or pathogen into the system, and greatly reduce the labor involved in supplying copepods to the squid hatchlings.

A final consideration in future attempts at rearing Loligo pealei hatchlings should be to use a closed recirculated water system instead of an open fresh seawater system. It seems ridiculous to suggest that a closed system be

used if a constant source of seawater is available, but a closed system would eliminate many of the problems that I encountered with an open system. With a closed system, there would be no problem of silt building up in the pipes and being flushed into the tanks; there would be no problem of phytoplankton coming in and clogging the tank filters; and there would be no problem of pathogens and other contaminants coming in from the outside. In addition, a closed system has already been used at the Marine Biomedical Institute in Texas to raise Loligo pealei hatchlings with some success.

Loligo pealei has proven over the years to be one of the most difficult species of squid to rear artificially in the lab. I feel, however, that success is very close. I have already shown that juveniles (even very small ones) can be maintained for fairly long periods of time and it is only a matter of time before someone puts together the right system and is able to keep hatchlings alive for an extended period of time.

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