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Preliminary Investigations of Laboratory Growth Studies of the squid *Loligo pealei* (LeSuerer)

by

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INTRODUCTION

Preliminary laboratory investigations leading to studies of the growth of the squid, Loligo pealei (LeSueur), from the Southern New England area of the Northwest Atlantic have been conducted at the Northeast Fisheries Center (NEFC). These investigations were designed to determine the procedures and techniques needed to maintain juvenile and adult L. pealei in aquaria while growth, food ration and behavior are monitored. Aquaria of different sizes and structures were tested as were various types and quantities of food.

Appropriate methods of anesthetizing the squid for length and weight measurements and of marking individuals for future observations were also determined. The techniques tested and results obtained in these investigations are summarized here.

METHODS AND MATERIALS

Specimens

Squid were obtained at two different times during the experiment. Initial observations were begun on 25 August with 24 juvenile Loligo pealei obtained after the completion of a previous study. These squid ranged in size from approximately 5 to 40mm; they were not measured. Nine of these L. pealei were 20mm or less (Group A) and were maintained in separate tanks from the larger individuals (Group B). On 24 September three large L. pealei (112-150mm), which had been kept in a large outdoor pool for several days after capture by the Supply Department of the Marine Biological Laboratory (MBL), were introduced to the experiment (Group C). The small squid were of undetermined sex, while the large group consisted of two males and one female.

Holding Tanks

Initially, all of the squid (Groups A and B) were maintained in three cylindrical fiberglass tanks 0.6m in diameter by 0.75m deep with a water level of about 0.6m. These tanks were partially covered and completely coated on the outside with black plastic to reduce the amount of light in the pools and to prevent excess disturbance from outside activities. Water flow was maintained at about 2ℓ per minute at the surface, along the perimeter, and water level was maintained through a center post, constructed of a pipe within a pipe so that water entered from the bottom.

Six of the Group B squid were moved from the small tanks to a 1.2m diameter tank, lined with neutral colored carpeting to prevent abrasion from bumping the surface of the tank. We felt the textured surface would be more visible to them and that if the squid did hit it, they would suffer less damage than they would against a hard fiberglass surface. Water flow to and from this tank was from along the surface perimeter.

The three large squid (Group C) were maintained in a tank with 0.75m of water depth, with a surface fed water supply, as in the other tanks. However, a larger cone shaped center drainage post was used to produce a moderate circular flow in the tank. This had the effect of increasing available jetting distance, since the squid would orient into the flow and not across the diameter of the tank. Black plastic sheeting, pressed against the wetted outer surface of the tank wall resulted in a black and white reticulated pattern on the inner surface.

Temperature

Seawater was initially supplied at ambient temperatures ranging from 21.6 to 15.8°C (Figure 1,A). However, when temperatures dropped to below 17.2°C

the squid became sluggish and did not feed. Beginning on 1 October the water in the tank was heated and recirculated to maintain temperatures above about 17°C (17°-19°C), afterwhich the squid resumed feeding and normal activity. After 26 days a circulating pump was added to the tank containing Group C to maintain current in the tank while allowing a reduction of input of heated seawater. The vibrations from this pump agitated the squid causing them to run into the side of the tank and resulted in severe fin damage. The pump was removed within 24 hours.

Feeding

Feeding varied for the different sized *L. pealei*. The very small individuals (Group A) were initially fed unmeasured quantities of fresh caught zooplankton, but within a few days several small *Fundulus* (0.2 - 0.4g each) were also introduced. As the squid learned how to capture this prey, it became the preferred food. Grass shrimp (*Hippolyte* sp.) were kept in the tank with the small *L. pealei* and were consumed occasionally when no *Fundulus* were available. The larger (Group B) squid from the initial group were fed about three *Fundulus* averaging about 0.3g each (2-3cm) per squid per day. These were generally consumed within about 30 minutes. After transfer to the large tank, 3-4 *Fundulus* weighing about 0.5g - 0.6g each, were introduced daily, per squid. Small *Fundulus* were not available for a period after mid-September and the squid were fed grass shrimp.

The three large *L. pealei* (Group C) were fed *Fundulus* on all but 2 days when grass shrimp were substituted for a portion of the *Fundulus* ration. Each squid consumed an average of 10.3 ± 4.8g of *Fundulus* per day (Table 1), although in many cases the heads of the *Fundulus* were not eaten. This estimated amount varied during the course of the experiment. Initially we may

not have fed these individuals enough, as they ate everything shortly after it was introduced. By the 10th day, however, food supply was no longer restrictive and there were Fundulus remaining on subsequent days. Consumption declined substantially during the last 5 days of the experiment.

Handling

The smallest individuals were thought to be too fragile for handling, and were kept in the same tank primarily to determine long term maintenance techniques. When the Group B individuals were moved to the larger lined tank they were also weighed and measured. To accomplish this with a minimum of disturbance, the small tanks were drained to about 30cm depth and the squid were netted out to a 10 by 15" aquarium enclosed in a box to prevent outside stimuli, and containing about 10cm depth of seawater and anesthetizing solution. Individuals (5) from the first tank were removed to the aquarium all at one time, to prevent repeated excitement of remaining individuals as each successive squid was removed. They were submerged in a solution of 150g urethane/5ℓ seawater, (O'Dor 1977) and immediately transferred to a container of fresh seawater and rinsed. They were then carefully weighed, measured and placed in the large, lined tank. All these individuals died within a short period, so the anesthetizing solution was diluted. The six individuals in the second tank were removed, one at a time to the enclosed aquarium. The first three were anethetized in a 50g urethane to 5ℓ seawater solution, and took between 10 and 17 seconds to cease movement. These were drained, weighed, measured, flushed with fresh seawater and placed in the large tank. As these individuals began to revive, they became very active, charging the surface, displaying continually and flexing their tentacles and arms. Each appeared to return to normal within 5-10 minutes. The last three individuals from the second

tank were anesthetized individually in a 25g urethane/5ℓ seawater solution prior to measuring, and although they took longer to narcotize (47 to 80 seconds), their recovery appeared to be less violent.

When the three large individuals were transferred from the outside pool to the 1.2m tank, they were narcotized in a 30g urethane/5ℓ seawater solution. These individuals were effected in 20 seconds and after weighing and measuring, recovered after about 2 minutes of rinsing with fresh seawater.

Marking

In futher studies, the ability to make routine observations of growth, feeding and behavior on individual L. pealei, will be important. Therefore a method of distinctly marking each squid is needed. O'Dor (et.al 1977) found that patterns of large dots tattooed onto the fins with India ink were effective. To test this approach, one of the three large L. pealei was marked with three small dots of India ink, injected by syringe into the left fin, prior to placement in the 1.2m tank. These marks were not noticeable after release in the tank.

This method was attempted again on large squid maintained for short periods by MBL. Squid (5) were removed from their circular holding tank (containing about 75 squid), and held on a wooden platform while several subcutaneous injections of India ink were made into the fins and the Dorsal mantle surface. One squid was returned to the tank containing the other squid while the other four were placed in a separate larger tank with various other fish specimens. Since the main purpose of this task was to determine if the marks would be visible, only minimal care was taken in handling the specimens. Consequently three of the four squid released in the large tank died within an hour of release. However, all marks remained visibile, even on the dead squid,

and the individual returned to the squid tank was easily distinguished from the other squid for the two days that it remained in the tank.

RESULTS

Survival

Maximum survival times ranged from 72 days for Group A to 34 days for Group B and 29 days for Group C. It should be noted, however, that Group A and B individuals were obtained from another experiment and had been in captivity from 1 to 2 weeks prior to the beginning of this project, and that Group C individuals had been maintained in an outdoor pool for several days prior to transfer to the experiment. Minimum estimates of mean survival time for these groups, during the experiment, were 16.5 days for Group B, and 28.7 days for Group C. Four Group A individuals survived an average of 50.0 days, but early mortality from this group was not noted. The longest surviving individual (72 days) was from Group A. Severe fin and anterior mantle damage resulting from continual abrasion with the side of the tank, was noted prior to the death of most individuals. In several instances squid were observed laying motionless, tentacles spread out on the bottom of the tank with the mantle slightly raised, for a period of time (up to several days), prior to death. However, other individuals which appeared healthy one day were found dead on the bottom the next. Mortality for the Group C individuals may have been partly the result of injuries sustained when agitated by a circulating pump introduced to the tank to maintain water flow. However, it should also be noted that these mortalities may have been related to the onset of sexual maturation, where the last two individuals to die were males which had just reached sexual maturity and the squid which died one day earlier and

which had suffered more extreme fin and mantle damage, was a female. It is generally believed that L. pealei die after spawning, and although there was no evidence that the female had spawned, both males were mature enough to have copulated, and the greater degree of damage to the female could have been caused during fertilization.

Growth

Initial lengths (dorsal mantle length) are not available for individuals from groups A and B, but we estimate that these ranged from about 5 to 20mm and from 40 to 50mm, respectively. Only the longest surviving individual from group A (72 days) was measured. This was a 49mm female which, given our initial size range estimates, grew between 29 and 44mm. Length and weight measurements of group B individuals were taken when they were transferred to the large tank, but final measurements were not taken since most individuals died at night and were too deteriorated to be remeasured once they were found.

Initial and final length and weight measurements obtained for the three L. pealei of group C are presented in Table 2. These individuals grew an average of 1.5 ± .22mm and 2.5 ± .19g per day, based solely on initial and final measurements.

DISCUSSION

The observations reported here agree with those reported by O'Dor (et. al 1977) for Illex illecebrosus, that a major cause of mortality for captive squid is skin damage. However, with proper circulation in the holding tanks, this damage can be minimized. Infact, L. pealei can survive and recover from relatively extensive skin loss if they are protected from further damage with adequate tank circulation. Maintaining a continuous circular flow in the tank

allows the squid to orient parallel to the side of the tank. Even when the squid jet forward for great distances, this flow should keep them away from the side, preventing abrasions.

Summers and McMahon (1974) found that densities of up to 16 squid/m^3 were feasible, indicating that perhaps 20 L. pealei could be maintained in the 1.2m tank in future growth studies. The smaller tanks (0.8m diameter) are probably adequate for 10-15 small individuals while the larger (1.5m) tank should hold 25-35 L. pealei without crowding.

Availability of food is another critical factor in maintaining L. pealei. Although very small individuals initially depend on zooplankton, as soon as they are able to capture larger prey, preference apparently shifts to the larger items. When both grass shrimp and Fundulus were provided, Fundulus was always the prefered prey of both larger and very small L. pealei. Quantities of food consumed will vary as a function of the size of the squid, but will also be dependent to some extent on the water temperature. When temperature drops below some minimum threshold, feeding appears to slow. In fact, when the temperature fell to below about 17°C the squid remained essentially dormant on the bottom and did not feed. Recognition of a temperature threshold in the activity of tank held squid is an important observation of this preliminary study and its implications toward behavior, distribution and migration of wild populations should be analyzed.

In future growth studies food ration should be determined by feeding two groups of L. pealei at different rates (g. prey per g. squid) to establish maximum and minimum growth. Rations of these two groups could then be compared to provide an estimate of the range of growth in nature.

Tattooing of captive L. pealei with unique patterns of dots of India ink, injected via syringe into the dorsal surface of the fins will provide a simple

means by which to record individual observations of growth, feeding and behavior throughout future experiments. The use of anesthesia whenever *L. pealei* are handled for measurements or tattooing has been found effective in reducing the detrimental reactions to these procedures. However, while Urethane anesthesia was used during this study, recent discussions, with O'Dor (pers. comm. R. O'Dor, Dalhouise University) indicate that a 1.5% ethanol in seawater solution is much safer to use and is just as effective in narcotizing the squid, as was the urethane.

SUMMARY

The preliminary study described here has allowed determination of effective methods of maintaining *Loligo pealei* in a laboratory situation, for at least 72 days. Effective methods of handling and marking individuals have also been determined. This information will be used in future studies designed specifically to determine growth functions and feeding rations of *Loligo pealei*.

LITERATURE CITED

O'Dor, R. K., R. D. Durward and N. Balch. 1977. Maintenance and Maturation of squid (Illex illecebrosus) in a 15 Meter circular pool. Biol. Bull., 153: 322-335.

Summers, W. C., and J. J. McMahon. 1974. Studies on the maintenance of adult squid (Loligo pealei). I. Factorial survey. Biol. Bull., 146: 279-290.

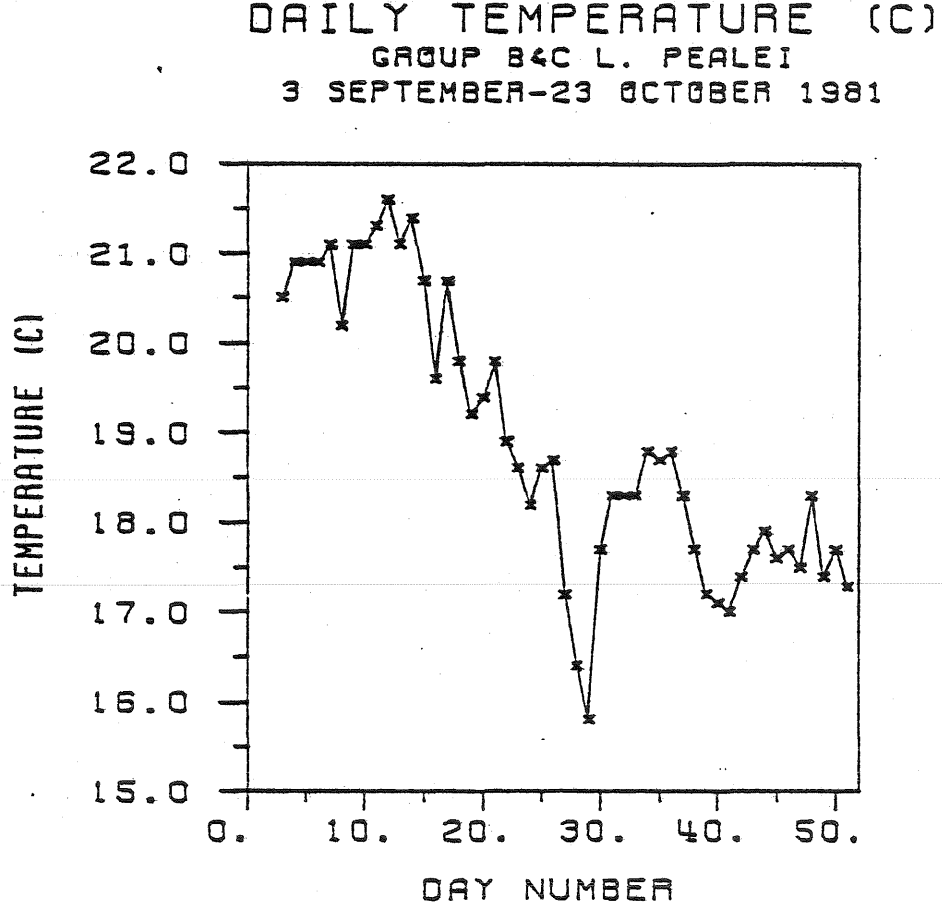
Table 1. Daily feeding (*Fundulus heteroclitus*) data for Group C *Loligo pealei* (3 squid).

Day	Total Weight (g)	Total Number	Average/squid (b Weight (g)	Average/squid (b Number	Number (a Remaining	Temp. °C
1	14	9	4.7	3		18.9
2	9	8	3	2.7		18.9
3	17	10	5.7	3.3		18.2
4(c	16+12	11+8	9.3	6.3		18.6
5	38	22	12.7	7.3		18.7
6	38	14	12.7	4.7		17.2
7	47	20	15.7	6.7		16.4
8	41	21	13.7	7.0		15.8
9	44	20	13.2	6.0		17.7
10	38	16	14.1	6.0	2	18.3
11	41	23	13.7	7.7		18.3
12	41	16	12.8	5.0		18.3
13	40	43	14.2	14.7	1	18.8
14	42	43	12.0	12.3		18.7
15	31	40	10.3	13.3	6	18.8
16	33	40	12.6	14.7	6	18.3
17	27	13	3.5	1.7	2	17.7
18	40	18	17.5	8.0	10	17.2
19	38	17	14.1	6.3	4	17.1
20	42	14	13.0	4.3	2	17.1
21	47	21	11.2	5.0	3	17.4
22	40	20	20.0	9.7	9	17.7
23	38	18	0.7	.3		17.9
24	-	-	9.8	5.0	17	17.6
25	25	22	10.4	8.3	3	17.7
26	16	21	5.3	7.0		17.5
27	30	36	4.4	5.3		18.3
28	13	19	9.7	13.0	~20	17.4
29	15	19	5.0	6.3		17.7
30	9	10	3	3.3		17.3

a) Number of prey items remaining from the previous day not including remains (as heads) of individuals partly consumed.
b) Excluding those remaining the following day (Number and estimated weight.
c) Squid were fed twice to determine if they needed more food then they were being given; ratio was subsequently increased.

Table 2. Initial and final lengths (mm) and weights (g) of *Loligo pealei* from Group C, including increases, and total number of days survived.

Length (cm)		Weight (g)		Growth		Days Survived
Initial	Final	Initial	Final	Length	Weight	
112	161	60	129	49	69	28
146	184	77	143	38	66	29
150	192	81	158	42	77	29



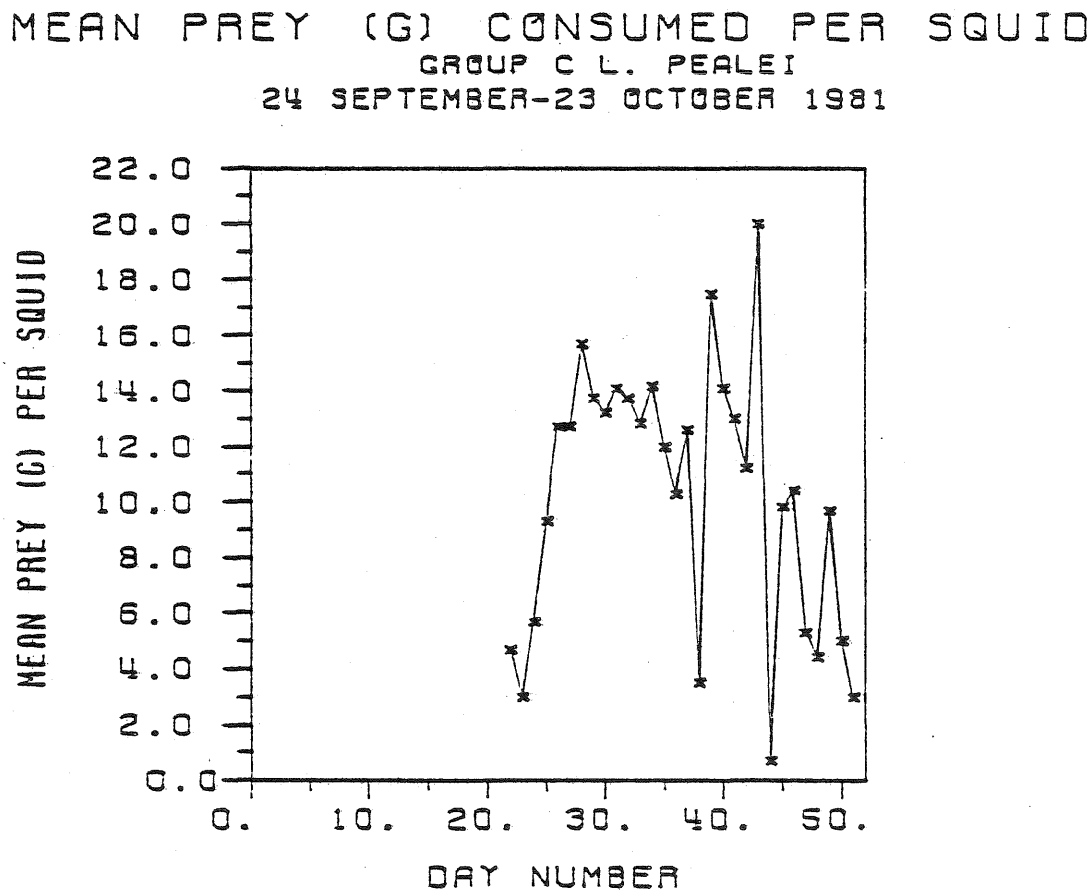


Figure 1. Daily temperature and mean prey consumption for group B anc C and group C *Loligo pealei*, 3 September - 23 October, 1981.