GROWTH, BEHAVIOR, AND SEXUAL MATURATION OF THE MARKET SQUID, *LOLIGO OPALESCENS*, CULTURED THROUGH THE LIFE CYCLE

W. T. YANG, R. F. HIXON, P. E. TURK, M. E. KREJCI, W. H. HULET, AND R. T. HANLON[1]

ABSTRACT

Loligo opalescens, a commercially important species of the eastern Pacific, is the first pelagic cephalopod to be cultured through the entire life cycle. Squid were cultured twice to viable second generation progeny in closed seawater systems using artificial and natural seawater. The reasons for success compared with previous attempts were 1) increased depth in the culture tank, 2) improvements in water conditioning methods, and 3) an increase in availability, density, and species diversity of food organisms. The diet consisted of live zooplankton (predominantly copepods), mysid and palaemonid shrimp, and estuarine fishes. Mean daily group feeding rates of subadults and adults were 14.9% and 18.0% of body weight. Growth was fast, increasing exponentially the first 2 months of the life cycle (8.35% increase in body weight per day) then slowing to a logarithmic rate thereafter (5.6-1.6% increase per day). Growth rings in statoliths corresponded to one per day for the first 65 days. Maximum life span was 235 and 248 days in the two experiments, with a maximum size of 116 mm dorsal mantle length. Viable eggs were produced within 172 and 196 days, respectively. Eggs developed in 30 days at 15°C. Survival through the life cycle was low, with the highest mortality occurring in the first few weeks when squid made the transition from feeding on yolk to active predation on fast-moving plankton. Fin or skin damage and senescence after reproduction accounted for late mortality. The laboratory life cycle of less than a year is compatible with existing field data that propose either a 1- or 2-year life cycle, depending upon the season of hatching.

Since 1975 we have been studying loliginid squid to develop methods of providing a consistent supply for neuroscience research. These studies include aspects of fishery biology (Rathjen et al. 1979; Hixon 1980a, b, 1983; Hixon et al. 1980), capture and maintenance methods (Hanlon et al. 1978, 1983; Hulet et al. 1979; Hanlon and Hixon 1983), behavior (Hanlon 1978, 1982), and mass-culture methods (Hanlon et al. 1979; Yang et al. 1980a, b, 1983a, b). Much of the baseline information acquired through these controlled culture experiments will also be important to the fisheries biology of commercially exploited loliginid squids (cf., Roper et al. 1983).

About 20 major attempts have been made to culture loliginid squids through the life cycle, but none have been successful (see review in Yang et al. 1980b), even though wild-caught mature females of *Loligo* and *Doryteuthis* spawn readily in captivity (Hamabe 1960; Fields 1965; Takeuchi 1969, 1976; Hurley 1977; Arnold et al. 1974; Hanlon et al. 1983). Fields (1965) attempted unsuccessfully to culture *Loligo opalescens* as early as 1947. Hurley (1976) reared *L. opalescens* for 100 d to a mantle length (ML) of 13 mm. Hanlon et al. (1979) reared this species to 17 mm ML in 79 d and, based upon that work, reared *L. opalescens* from hatching to subadults (Yang et al. 1980b, 1983a). We have now improved previous culture methods by increasing the rearing population density and by improving the space requirements for young and adult squid. With a more consistent supply of foods and improvement of water management, we have now successfully cultured this squid twice from egg to second generation, thus closing the life cycle.

MATERIALS AND METHODS

Two culture experiments are reported herein: L.O. 1981 (full life cycle partly published in Japanese by Yang et al., 1983b); and L.O. 1982 (full life cycle). A third experiment, L.O. 1980, was published by Yang et al. (1980b, 1983a) and is referenced for comparison in the Discussion and figures.

For L.O. 1981, freshly laid eggs were obtained from wild-caught squid kept in holding tanks at Sea Life Supply (Sand City, CA 93955). Eggs were collected from spawning grounds in Monterey Bay, CA for experiment L.O. 1982. Eggs were air-shipped

[1]The Marine Biomedical Institute, The University of Texas Medical Branch, 200 University Boulevard, Galveston, TX 77550-2772.

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to Galveston (Yang et al., 1980b; 1983a, b). Only early stage eggs were shipped and cultured (never beyond stage 19, Arnold 1965). The eggs were acclimated gradually to the temperature and salinity of the culture tank water; incubation temperature was maintained around 15°C while salinity ranged between 34 and 36‰. Bundles of a few capsules each were suspended from a rack at the water surface to ensure oxygenation and uniform development of eggs. Styrofoam panels covered the rearing tank and the illumination level was kept below 1 lux to prevent the growth of benthic diatoms on egg capsules.

A circular tank (CT) system consisting of two circular tanks (each 1,300 L) was used for incubation and early rearing of hatchlings and juvenile squid. Water circulation was modified in L.O. 1982 when compared with earlier culture experiments (Yang et al. 1980b: fig. 1, 1983a: fig 1). Prior to L.O. 1982, a laboratory-constructed particle/carbon filter was used with circulation first passing through an ultraviolet (UV) sterilizer. L.O. 1982 used modular type particle and carbon filters, with the UV sterilizer in the last position in the water conditioning process. The raceway (RW) system (RW culture tank volume—10,970 L in L.O. 1981, and 13,180 L in L.O. 1982) was used for final grow-out after transferring the squid from the CT culture tanks. The transfer was necessary to give the squid greater horizontal swimming space. The initial RW system in experiment L.O. 1981 had been modified from previous experiments (Yang et al. 1980b, 1983a) to improve water quality by 1) adding a rectangular, 960 L capacity water conditioning tank (0.46 × 1.22 × 1.83 m, water depth 0.43 m) with water circulation of 54 L/minute, 2) adding another cooling unit, 3) adding three protein skimmers, 4) adding three UV light sterilizers (each 30 W, total 90 W), 5) modifying the water uptake system in the RW with a float near the center to remove near-surface water without sucking up squid or food organisms and to increase the lateral swimming space for the squid, 6) painting an irregular mottled pattern on the sides of the RW to make the walls more visible to the squid, and 7) most importantly, by increasing RW water depth gradually from 24 cm initially to 40 cm (average depth 38.8 cm) to provide swimming space for the squid and to increase the average culture water volume in the RW from 5,990 to 8,610 L.

A further improved RW system (Fig. 1) was used in experiment L.O. 1982. It consisted of two biological filter tanks (A, C) with oyster shell subgravel filters and airlifts for water circulation, a tank for growing macroalgae (B), the RW where the squid were cultured (D), and a separate tank where protein skimmers were operated continuously (E). The surface water was taken from the RW through pipes suspended in a screened floating core. Water within the system was recirculated by three routes. First, water was pumped to filter tank A that contained approximately 0.15 m^3 of oyster shell over a false bottom. Water passed through the filter bed, then flowed through a constant-level siphon to tank B where algae were illuminated by two 400-W metal halide lamps. Water flowed by gravity into the second filter tank C that contained 0.18 m^3 of oyster shell substrate and two 1-hp cooling units, and finally returned by gravity to the RW proper. Second, water was pumped through two sets of six modular filters: four modules containing pleated 20 μm fiber particle filters and two containing activated carbon. From the modular filters, water either flowed directly into the RW or through a 60 W UV sterilizer before returning to the RW. Third, water was pumped at 36 L/minute to a tank that contained five protein skimmers and then flowed back into the RW. The outflow of the three recirculating routes created a clockwise water flow in the RW proper. This motion accumulated dead squid and food organisms in one place on the bottom. The bottom was painted solid black with nontoxic Thixochlor[2] paint and the sides were painted with an irregular mottled pattern. Three 11 × 28 cm windows were mounted in one side of the RW for observing the squid's feeding and behavior. The tanks were insulated with polystyrene sheeting and 2.3 cm thick polystyrene covers.

To ensure activation of the biological filter for both CT and RW systems, filter beds were inoculated 2 to 3 wk beforehand with nitrifying bacteria on oyster shell from other systems. Fish and shrimp were placed in the water conditioning tank to build up the bacterial population. Thus the filter beds were established by organic conditioning methods (Moe 1982) instead of by directly adding ammonia source chemicals.

A set of black silk nets was used to transfer squid from the CT system into the RW system. A triangular lift net was laid on the bottom of the tank while two rectangular net curtains were slowly drawn from the left and right sides of the tank to concentrate the small squid above the lift net. The lift net was gradually raised, a wash tub placed underneath, and both were moved to the RW tank where the squid were gently released into the tank.

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

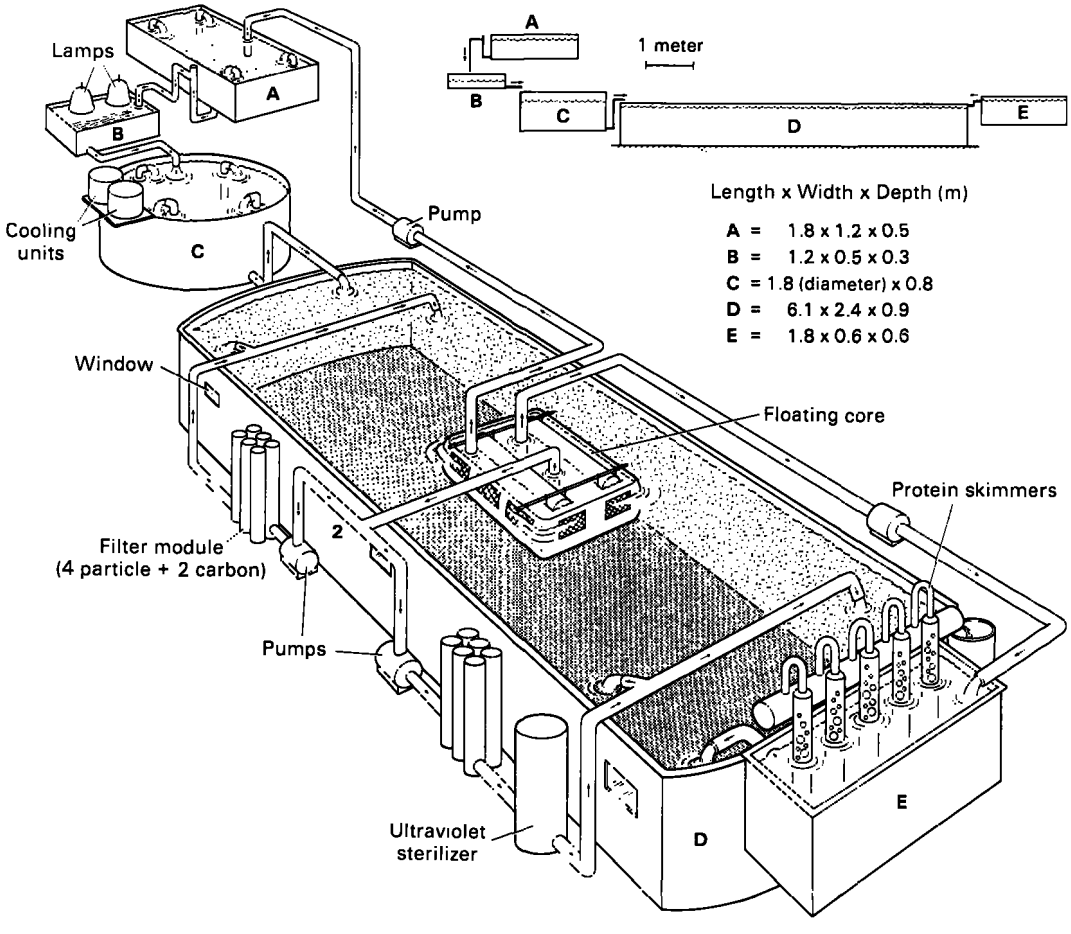


FIGURE 1.—Raceway (RW) system (L.O. 1982) with recirculating culture seawater (17,000 L total) for grow-out of juvenile and adult squid.

In L.O. 1981, 129 squid were not transferred from the CT tank and they continued to grow in the CT, thereby allowing comparisons of temperature tolerance and survival in small versus large culture systems.

Natural seawater and artificial sea salts (Instant Ocean) dissolved in deionized water were used in CT systems for L.O. 1982 and L.O. 1981, respectively, and artificial seawater was used exclusively in the RW system in both experiments. Salinity was maintained between 34 and 37‰. Trace elements were supplemented regularly with Wimex Trace Elements. Temperature was maintained at 15°C unless otherwise noted. The pH was maintained between 7.8 and 8.2, and low pH was corrected by the gradual addition of sodium bicarbonate.

Temperature and salinity were measured daily, pH every other day, and metabolic waste products (ammonia, nitrite, and nitrate) were measured weekly. Ammonia-nitrogen levels were determined by the Solorzano method (Strickland and Parsons 1972), and nitrite-nitrogen was determined by the Shinn method (applied to seawater by Bendschneider and Robinson in Strickland and Parsons 1972). Nitrate-nitrogen levels were determined using a prepacked Hach reagent kit.

Various live food organisms were fed to the squid several times daily throughout the experiments. Live planktonic organisms such as zooplankton (mainly copepods) and small mysidacean shrimp (*Mysidopsis almyra*) were the primary foods during the first 60 d in the CT system. Brine shrimp, *Artemia salina*; larval red drum, *Sciaenops ocellatus*; and mysis stage penaeid shrimp were fed as supplemental foods. Food organisms were added to the CT system four or five times daily. Thereafter

in the RW system, adult mysids; palaemonid shrimp, *Palaemonetes pugio*; and a variety of marine or estuarine fishes were fed to the squid at least twice daily.

Zooplankton were washed carefully in clean seawater. Mysids and palaemonid shrimp were treated overnight with quinacrine, while erythromycin and/or tetracycline were used to treat fish (Yang et al. 1980b, 1983a, b). Before feeding, all foods were counted or weighed and slowly acclimated to the temperature and salinity of the cultured water.

Dead squid and dead food organisms from previous feedings were removed by siphoning once or twice daily from the CT or RW systems. Daily food consumption in the RW was derived by subtracting the weight of uneaten food remains siphoned each day from the weight of food organisms added daily to each culture system. Daily feeding rate (wet weight) is expressed as the percentage of food consumed by the total estimated biomass of the squid. Daily biomass of squid was estimated by multiplying the number of live squid on a given day by the average weight of an individual squid on that day. Daily squid weight estimates were projected from linear regression of the weights of freshly dead squid against time. All measurements and wet weights (WW) were usually made with freshly dead squid although live squid were occasionally used. Badly damaged or partially cannibalized squid were not measured or weighed for this analysis. The initial squid population was derived from the number of dead or sacrificed specimens removed from the culture systems.

Overhead fluorescent lights provided illumination. In the CT systems for both experiments there was constant light that measured 11 to 15 lux in the middle of the water column. In the RW systems there was also constant light although light only filtered in through plastic-covered holes in the polystyrene tops. In L.O. 1981 it measured 17 lux in the center of the RW and 0.5 to 0.7 lux at each end. In L.O. 1982 it measured 4 to 7 lux near the ends under the opaque top and 11 lux near the center where light passed through the clear plastic.

Statoliths from hatchlings of known age in L.O. 1982 were dissected from the squid and decalcified in a 1:1 mixture of 4% EDTA in distilled water and 0.2 H sodium cacodylate buffer (pH 7.4). Decalcification facilitated the counting of rings in statoliths from squid age 65 d or younger, but older statoliths were distorted by the process. The rings were counted from photographs taken with a Leitz Combiphot II and Kodak copy film #4125.

RESULTS

Water Quality

There were no obvious differences in growth or survival between squid cultured in artificial seawater (L.O. 1981) and filtered natural seawater (L.O. 1982). Water quality in the CT systems was maintained in very good condition due to the short culture period, while water quality in the RW system was more difficult to maintain because of the long grow-out period and the greater biomass of squid and food organisms. In L.O. 1981 (Fig. 2) from days 180 to 190 the estimated total biomass reached the maximum peak of 1,706 g (cf., Fig. 7), which is equivalent to 155 g/m^3 of rearing water volume. After the 160th day, food organism biomass increased to between 300 and 400 g/day. As a result, the amount of nitrate-nitrogen gradually accumulated to over 23.0 mg/L during the period from day 180 to day 193 (Fig. 2). On day 164, 1,900 L (17% of total volume) of fresh Instant Ocean was replaced in this system. However, the nitrate-nitrogen level did not drop in proportion to the percent water change. Concurrently, pH dropped to 7.75 by day 169 and dissolved sodium bicarbonate (Atz 1964; Bower et al. 1981) was introduced to the system to adjust the pH above 7.9. The sodium bicarbonate solution required very strong aeration to be effective when it was put into the culture water. A similar trend of slightly increased nitrate-nitrogen and decreased pH occurred (about day 200) in L.O. 1982 (Fig. 2). This was corrected in the same manner.

The vegetative macroalgae, *Gracilaria tikvahiae*, was cultured in the water conditioning tank of the RW system in L.O. 1982 to remove ammonia and prevent the accumulation of nitrate-nitrogen, but its effectiveness was not clear.

Incubation and Hatching of Eggs

Average hatchling size in both experiments was 2.7 mm ML (range 2.3-2.8 mm ML) with a hatching success of over 90%. In L.O. 1981, hatching began on 14 October and lasted until 17 October. Embryonic development required 27 to 30 d at 15°C. The hatching period lasted 4 d, compared with L.O. 1982 that took 5 to 6 d. The period of embryonic development in L.O. 1982 was not precisely known because the eggs were collected in nature. Development of eggs within the same egg cluster was different depending upon the capsule position within the cluster. Moreover, hatching time within the same capsule

differed, since distal embryos usually hatched first. Since we used early stage eggs removed from their habitat in California, no polychaete worms (*Capitella ovincola*) were observed in the egg capsules (cf., McGowan 1954), although we had observed worms in other late stage California egg capsules.

During embryonic development, granules or crystals appeared in the perivitelline fluid of some eggs, but no significance to survival or development could be associated with this condition. The outer tunics of the egg capsules incubated in Instant Ocean were more elastic until the later stages (around stage 27) than those incubated in natural seawater. More bacteria and other benthic organisms grow on the capsules incubated in natural seawater. These differences did not influence development or hatching success. Embryos near hatching (stage 29) generally moved little or were nearly static, but in most individuals the external yolk sac was already broken off within the egg. External yolk sacs were observed on a few hatchlings. In L.O. 1981, bright illumination stimulated hatching in very late stage eggs and therefore light levels were increased during later stages of egg development in L.O. 1982.

Foods and Feeding

The species and size of food organisms were similar in the two experiments. The general progression of food types began with zooplankton, then mysidean shrimps, then palaemonid shrimp larvae and adults, and finally fishes (Fig. 3). The use of brine shrimp has been curtailed since they were found to be unattractive to the hatchlings.

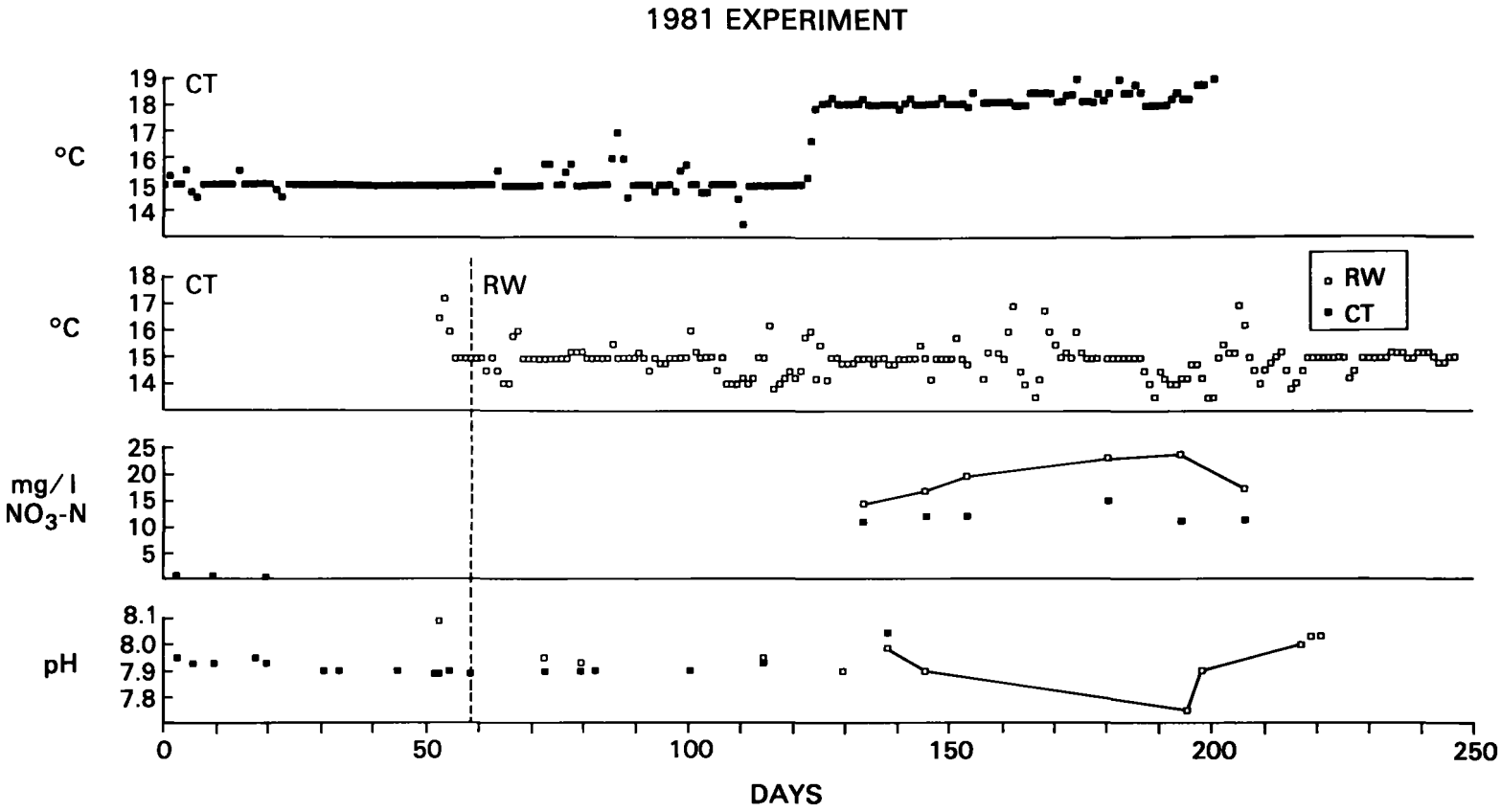
The size range of food organisms fed in the first 30 d is large, especially when compared with the size of 1-d-old hatchlings (2.3-2.8 mm ML, Fig. 4). However, as shown in Figure 4, the hatchlings have only small fins and are not strong swimmers; therefore, feeding on active prey at this stage is not excellent. A summary of the types and quantities of food offered in the experiments (L.O. 1981 is used as an example) is given in Figure 5. Large amounts of food were available to the squid; this was important during the first weeks when hatchlings could only capture food organisms drifting very close to them. The relationship of hatchling to food organism density during the first 59-d period in each experiment is summarized in Table 1. Unfortunately there was no clear relationship between densities and survival. For example, in L.O. 1982, there were twice as many food organisms per squid as in L.O. 1981, but survival (cf., Fig. 13) was not better. Figure 5 shows more specifically the number of food organisms fed daily in L.O. 1981.

The early rearing period in L.O. 1981 and 1982 coincided with the spawning of mysid shrimp in the Galveston estuaries. Therefore, small mysids with a total length of about 2.0 mm (Fig. 4B) were abundantly supplied. This was particularly important since small mysids swim more frequently in the water column than do adults. Young mysid hatchlings were given as food by day 12 in L.O. 1981 and immediately in L.O. 1982 (Fig. 3). Small mysids distribute themselves more evenly in the culture tanks and are easier for hatchlings to capture. *Palaemonetes* spp. were fed to juvenile and adult squid (Fig. 3). Shrimp ranged in size from 2.0 to 25.0 mm. They were graded by size and fed based on size and availability. Daily siphoned remains indicated that only the abdominal flesh was consumed, with the thorax and carapace discarded.

Fish were generally used for juvenile or older squid. However, fertilized red drum eggs were available in L.O. 1981, and larvae up to 13-d old (Fig. 4E) were given to the hatchlings. In the two experiments, a total of over 14 fish species of 10 families were fed (Table 2). To determine the diet preference for fish species, the actual consumption of fish (i.e., total weight of fish put in tank minus total weight of fish remains) was compared for a total of 5 kg fish fed in L.O. 1982 (Fig. 6). The cyprinodont fish were most preferred (consumption of 83%). Only small *Fundulus* spp., smaller than 31 mm (Cyprinidontidae), were fed because large *Fun-*

TABLE 1.—The mean density of squid and food organisms per liter of culture water from days 0-30 and 30-59.

		Day 0-30			Day 30-59		
Exp. No.	Initial hatchling population	Squid No./L	Food organisms No./L	Ratio of food organisms to squid	Squid No./L	Food organisms No./L	Ratio of food organisms to squid
L.O. 1980	864	0.46	14.2	30:1	0.35	9.4	26:1
L.O. 1981	2,061	0.93	24.0	25:1	0.54	12.4	23:1
L.O. 1982	1,704	0.27	14.6	54:1	0.14	5.6	40:1


1981 EXPERIMENT
CT
°C
19
18
17
16
15
14
CT
RW
°C
18
17
16
15
14
RW
CT
mg/l
NO_3-N
25
20
15
10
5
pH
8.1
8.0
7.9
7.8
0
50
100
150
200
250
DAYS

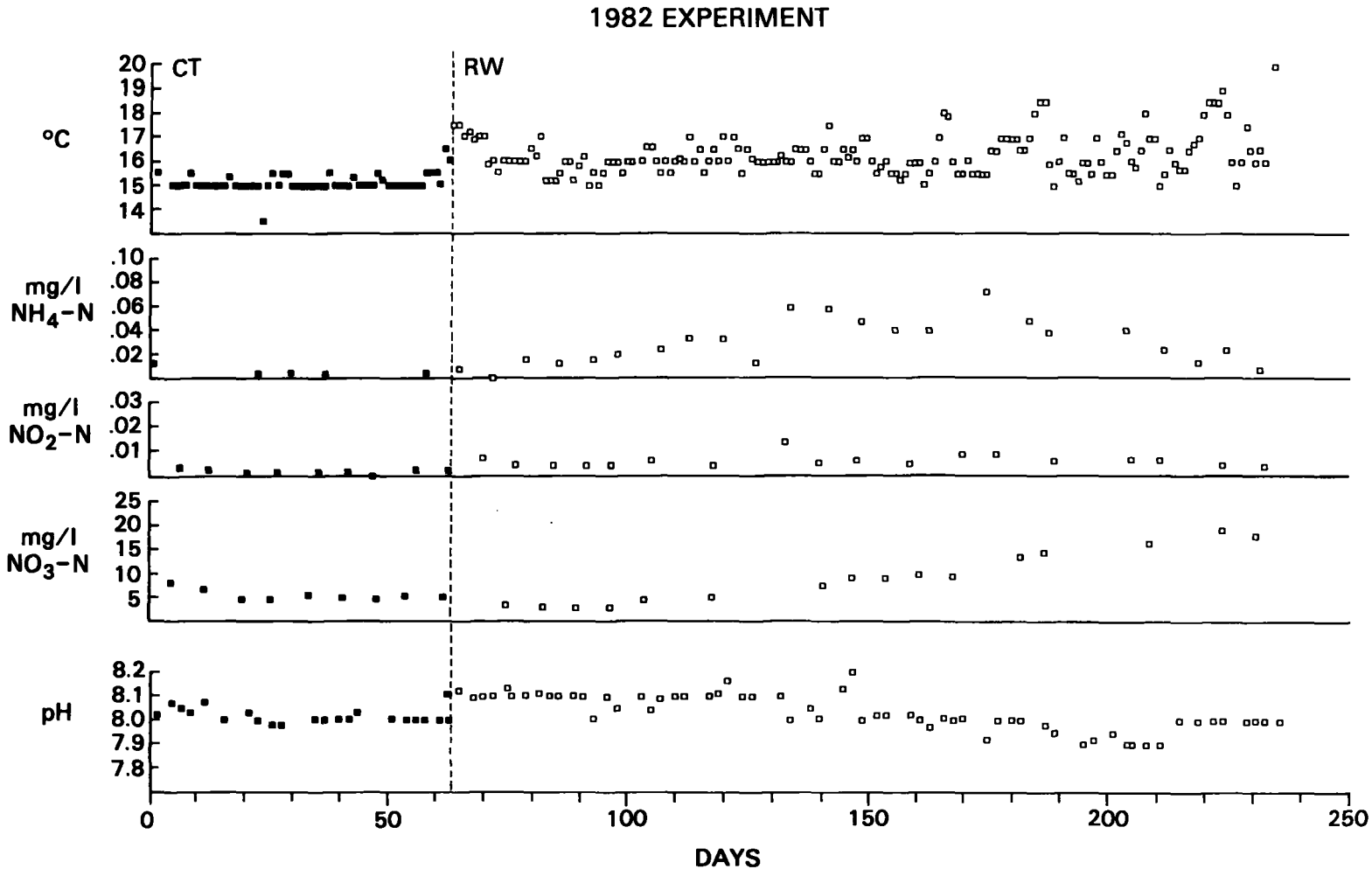


FIGURE 2.—Water quality measurements in CT (circular tank) and RW (raceway) systems throughout the life cycle (L.O. 1981 and 1982). Some squid in L.O. 1981 were kept in the CT system (see top temperatures) while others were moved to the RW system (bottom temperatures).

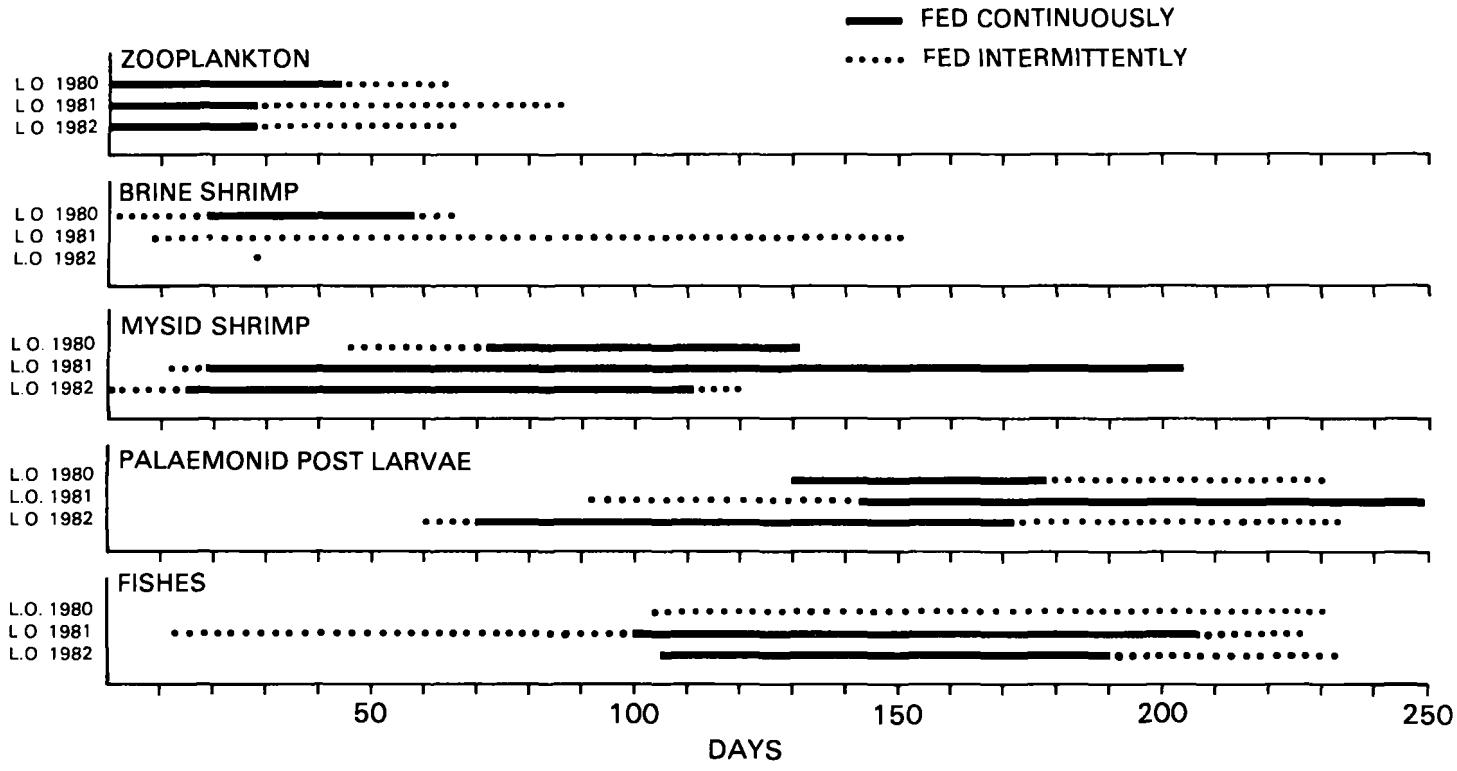


FIGURE 3.—Comparative use of food organisms fed to *Loligo opalescens* during three culture experiments.

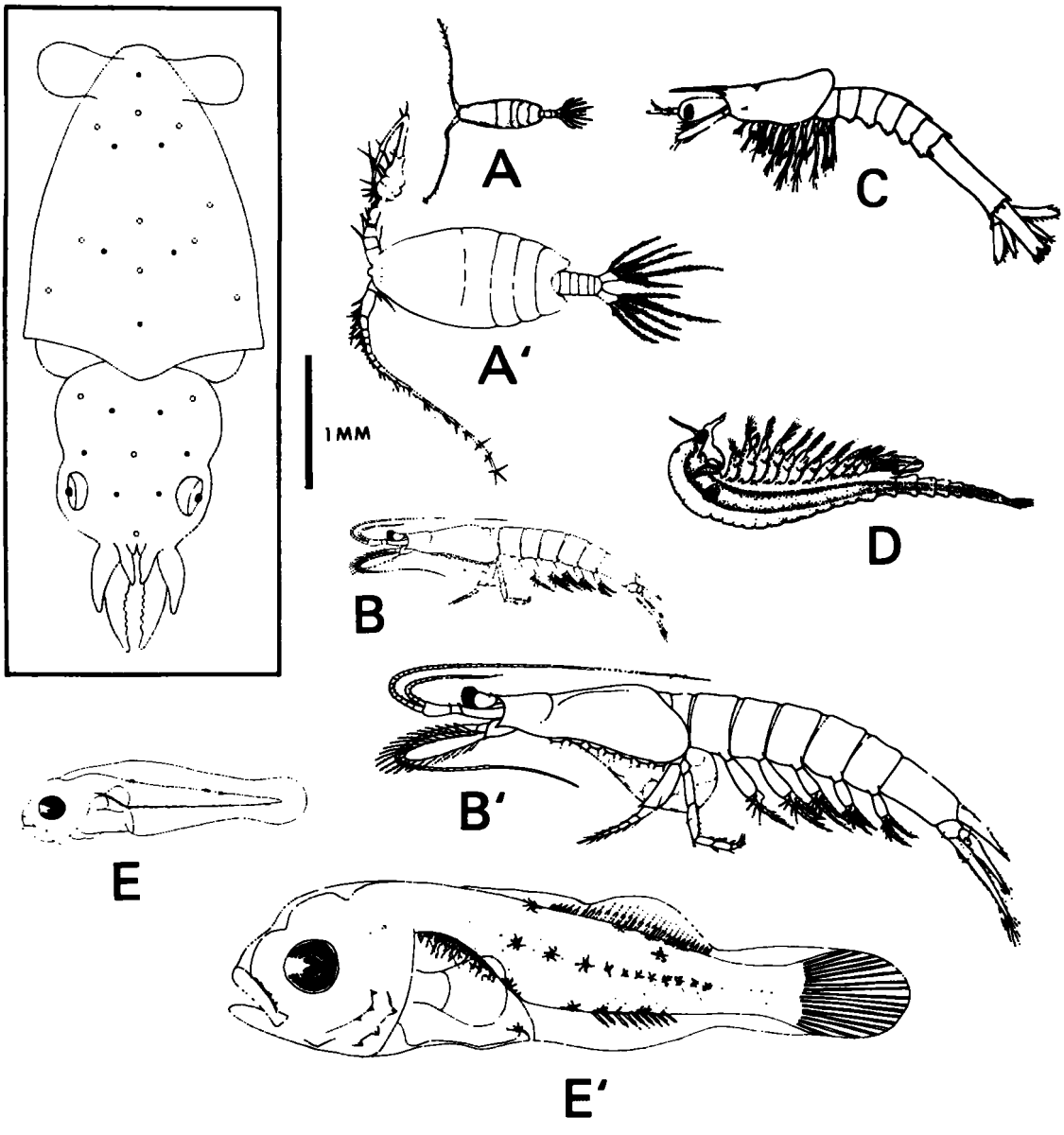


FIGURE 4.—Size relationship of hatchling *Loligo opalescens* and various food organisms fed squid for the first 30-d posthatching. A, copepod *Acartia tonsa*; A', copepod *Labidocera aestiva*. B, hatchling *Mysidopsis almyra*; B', adult *M. almyra*. C, mysis stage of *Penaeus* spp. D, adult *Artemia salina*. E, 1-d-old larva of red drum *Sciaenops ocellatus*. E', 13-d-old larva of *S. ocellatus*.

dulus spp. competed with the squid for crustaceans in the tank. Uneaten mullet (Mugilidae) accumulated to form small schools, that the squid would not approach or feed upon as readily as fish that swam individually. Squid consumed 44% of the mullet even though the amount fed was equal to amounts of Poecilidae and Sciaenidae, which were consumed more (72% and 68%, respectively). The food remains indicated that the squid ate only the flesh of fish, leaving the head and vertebrae.

Figure 7 gives the estimated daily group feeding rate (L.O. 1981) based upon the daily biomass of squid and the daily food consumption from day 108 to day 232. Daily group feeding rate averaged 14.9% (range 4-29%). Squid biomass reached a maximum on day 183 and continued high for 11 d before the

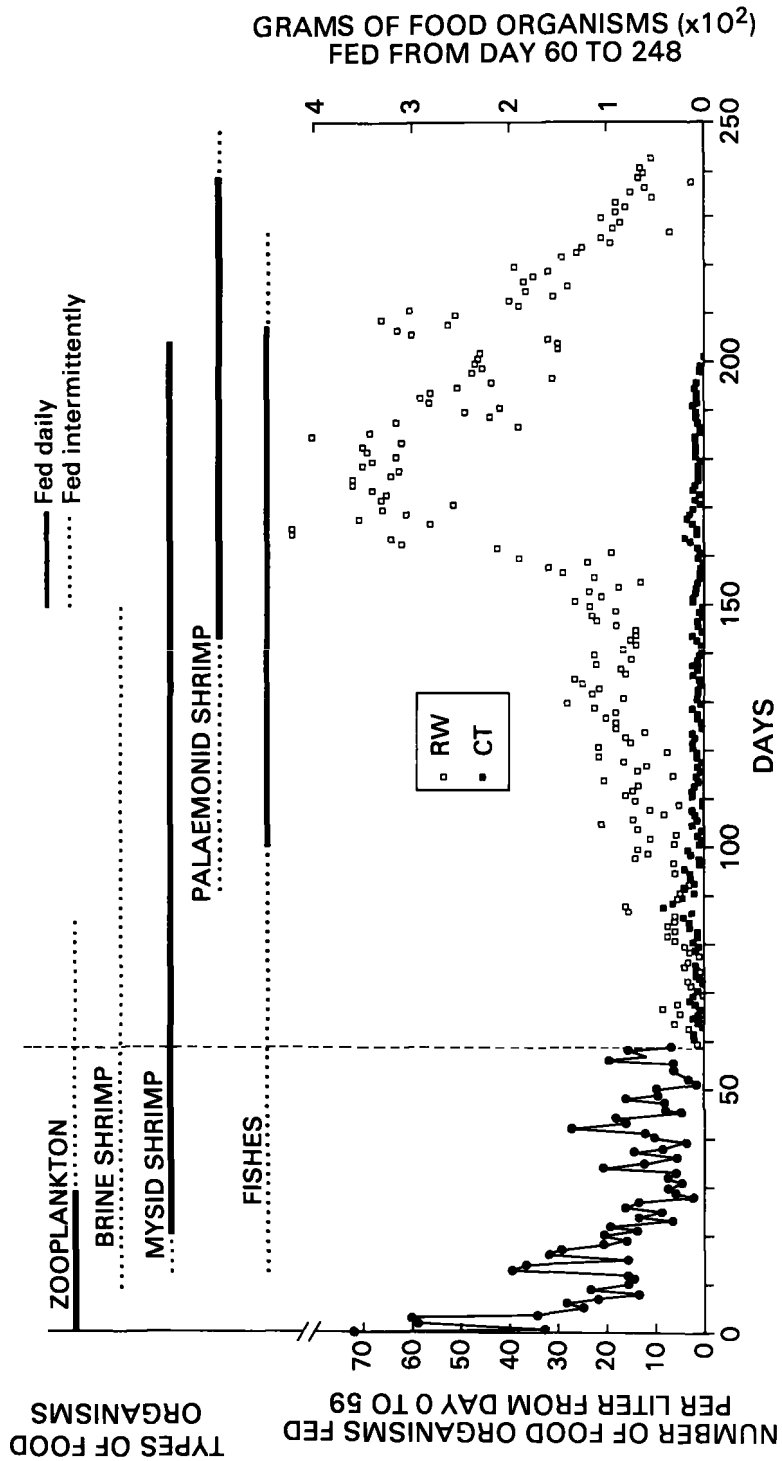


FIGURE 5.—Types, quantities and densities of food organisms fed daily in experiment L.O. 1981. Vertical line represents the culture day 59.

TABLE 2.—Fish species and size range given as food in all three experiments.

Family and species	Size (TL in mm)	L.O. experiment 1980	1981	1982
Family: Clupeidae				
Brevoortia spp.	15.0-31.0	—	—	X
Family: Engraulididae				
Anchoa mitchilli (Valenciennes)	20.0-25.0	—	—	X
Family: Cyprinodontidae				
Adinia xenica	—	—	—	X
Cyprinodon variegatus Lacepede	10.0-28.0	X	X	X
Fundulus spp.	15.0-31.0	X	X	X
Family: Poecilidae				
Gambusia affinis (Baird and Girard)	12.0-28.0	X	X	X
Poecilia latipinna (Lesueur)	22.0-41.0	X	X	X
Family: Atherinidae				
Menidia beryllina (Cope)	18.0-52.0	X	X	X
Family: Carangidae				
Hemicaranx amblyrhynchus (Cuvier)	—	—	—	—
Family: Gerreidae				
Eucinostomus gula (Quoy and Gaimard)	—	—	X	X
Family: Sparidae				
Lagodon rhomboides (Linnaeus)	—	—	X	X
Family: Sciaenidae[1]				
Sciaenops ocellatus (Linnaeus)	1.5-14.5	—	X	X
Pogonias cromis (Linnaeus)	10.0-15.0	—	X	X
Family: Mugilidae				
Mugil spp.	18.0-38.0	X	X	X

[1]There were about six more species of Sciaenidae; minority species were not identified.

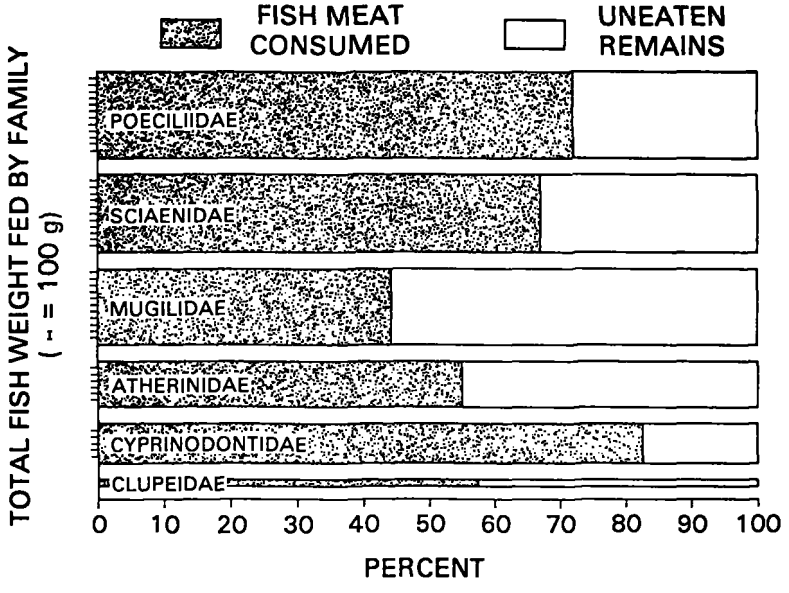


FIGURE 6.—Food preference for fishes by squid in experiment L.O. 1982. Total fish weight fed to the squid was 5.0 kg.

initiation of spawning; biomass then decreased because of the mortality accompanying spawning. Squid in L.O. 1982 were fed ad libitum and daily group feeding rates could not be determined. However, the average group feeding rate calculated weekly for L.O. 1982 allowed an estimate of 18.0% for the daily feeding rate.

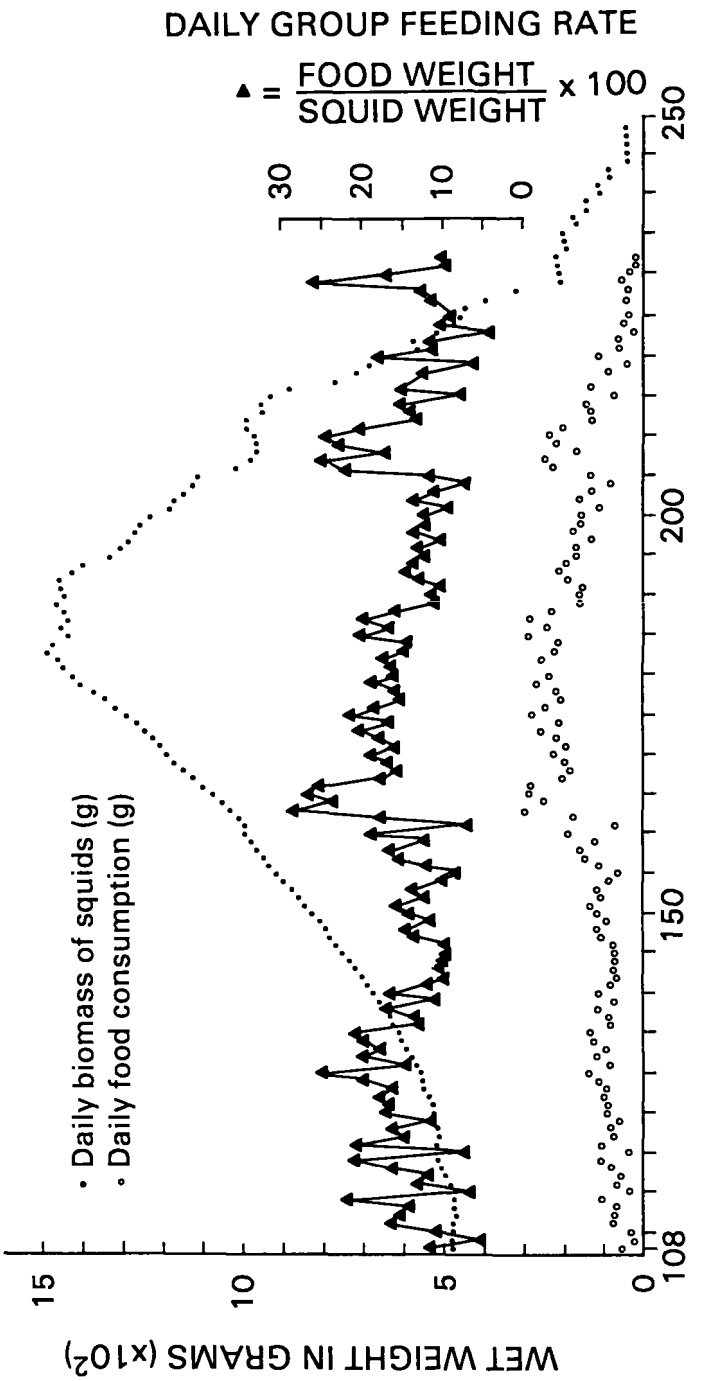


FIGURE 7.—Analysis of the daily group feeding rate of subadult and adult *Loligo opalescens* in the RW system from day 108 to 248 in experiment L.O. 1981.

Growth

Figure 8 illustrates growth data through the life cycle for both experiments. At hatching, *Loligo opalescens* has a mean mantle length of 2.7 mm, a wet weight of 0.001 g, and has approximately 100 chromatophores on its body. In L.O. 1981, the largest reared squid was a male of 113 mm ML and 58 g. In L.O. 1982, the largest reared squid was a female of 116 mm ML and 63 g. Mean sizes for adults from the two experiments were 87 mm ML ($S\bar{x}$ = 2.7) and 23.8 g ($S\bar{x}$ = 1.9) for 35 males, and 83 mm ML ($S\bar{x}$ = 1.9) and 21.2 g ($S\bar{x}$ = 1.5) for 58 females.

Growth equations for the squid in L.O. 1981 clearly describe two separate phases of growth. The mantle length of squid cultured in the CT system (days 1-56) increased at an exponential rate ($ML = 2.121\ e^{0.02398t}$; $r^2 = 0.92$) or 2.4% increase per day, while those cultured in the RW system (days 56-248) grew logarithmically ($ML = 0.2884\ t^{1.495}$; $r^2 = 0.97$). Weights were only measured on squid from the RW

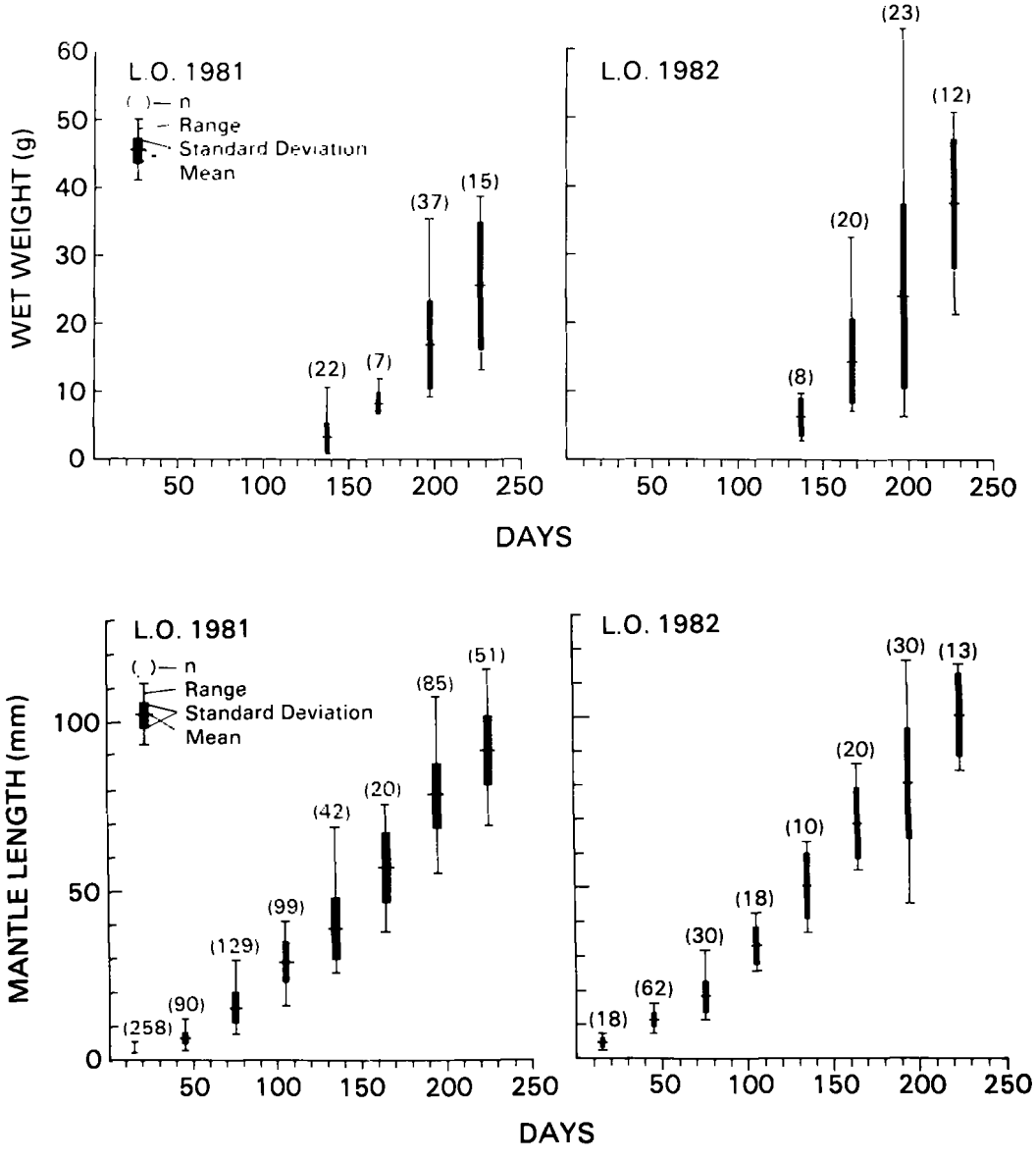


FIGURE 8.—Comparison of growth (wet body weight and mantle length) in experiments L.O. 1981 and L.O. 1982.

system (days 108-248) and the growth curve showed a logarithmic increase ($W = 6.283 \times 10^{-7} t^{3.660}$; $r^2 = 0.92$). Hence, younger squid grew at an exponential rate and growth slowed to a logarithmic rate in older squid.

Squid exhibited fast exponential growth for the first 2 mo in L.O. 1982 and slower logarithmic growth thereafter (Fig. 9). Wet weight data from live animals in L.O. 1982 indicated a mean growth rate of 8.35% increase in body weight per day for the first 2 mo. Mantle length increased 3.19%/day or the equivalent of 8 mm/month. The squid were doubling their weight every 8 d and doubling their length every 21 d. Growth rates declined from 5.6%/day WW at day 60 (and 2.2%/day mm ML) to 1.6%/day WW (and 0.63%/day mm ML) at day 240.

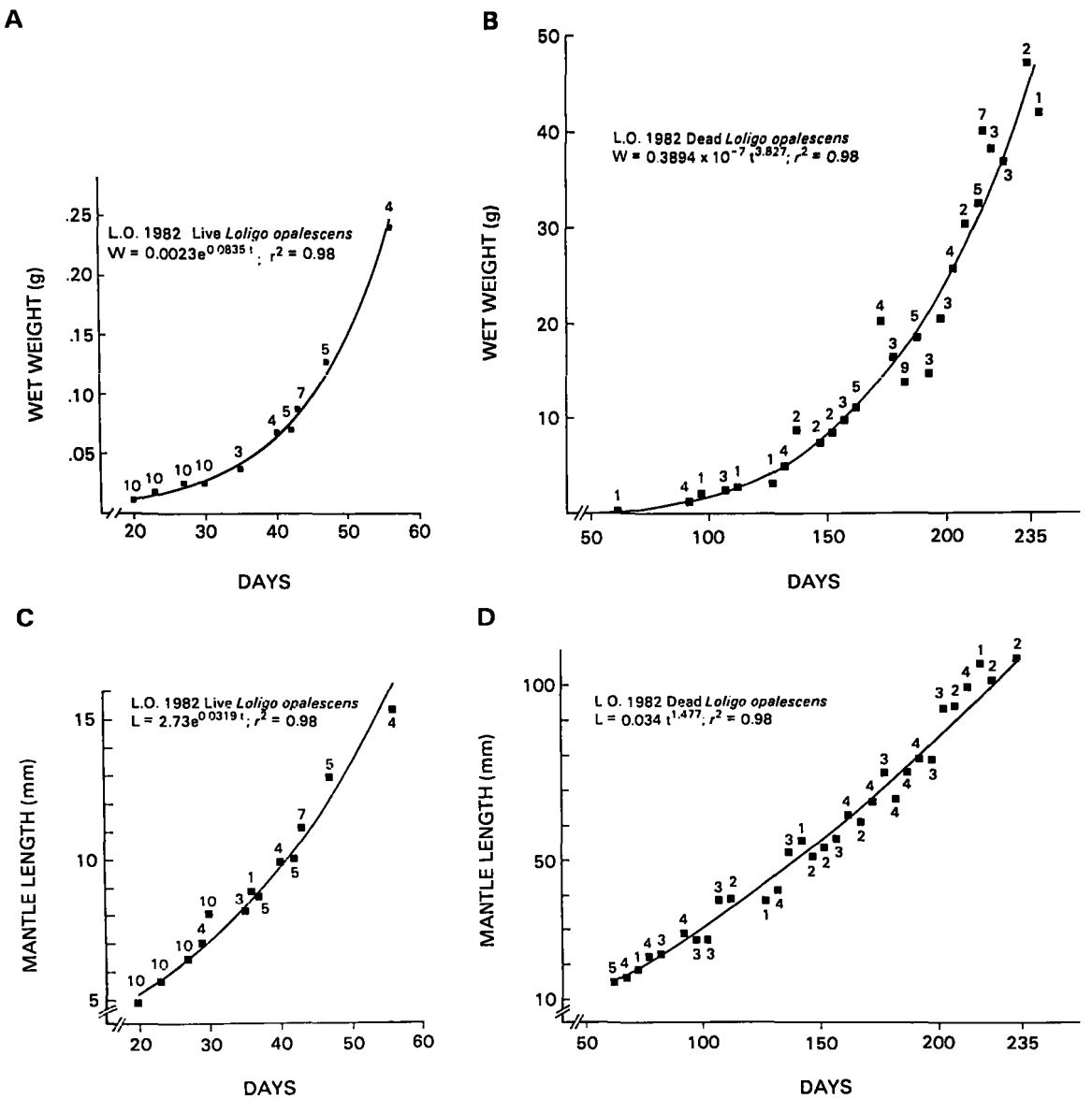


FIGURE 9.—Early exponential growth of *Loligo opalescens* in experiment L.O. 1982: A, Live wet weight, illustrating exponential growth through day 60. B, Dead wet weight, illustrating logarithmic growth from day 60 to maturity. C, Live mantle length measurements, showing exponential growth as in A. D, Dead mantle length measurements, showing logarithmic growth to maturity as in B. Numbers above rectangles indicate actual number of squid measured for that mean.

Mean growth was 16 mm/month for this period. Doubling times for weight increased from 12 d at day 60 to 42 d at day 240, and for length from 31 d at day 60 to 109 d at day 240.

The length-weight relationships of squid in L.O. 1981 and 1982 are illustrated in Figure 10 and are compared with data on wild squid (Fields 1965). The slopes of the curves are slightly higher in laboratory-reared animals, indicating that these squid are heavier per unit length than wild squid. Table 3 illustrates differences in predicted weights for representative mantle lengths from L.O. 1982 data versus Fields' (1965) data. The length-weight relationship for males vs. females in L.O. 1981 is shown in Figure 11; no significant differences between sexes were detected ($P > 0.05$).

Statoliths from 55 early hatchlings (L.O. 1982) aged 21 to 79 d (±5 d) were examined to correlate statolith ring numbers with the age of individual

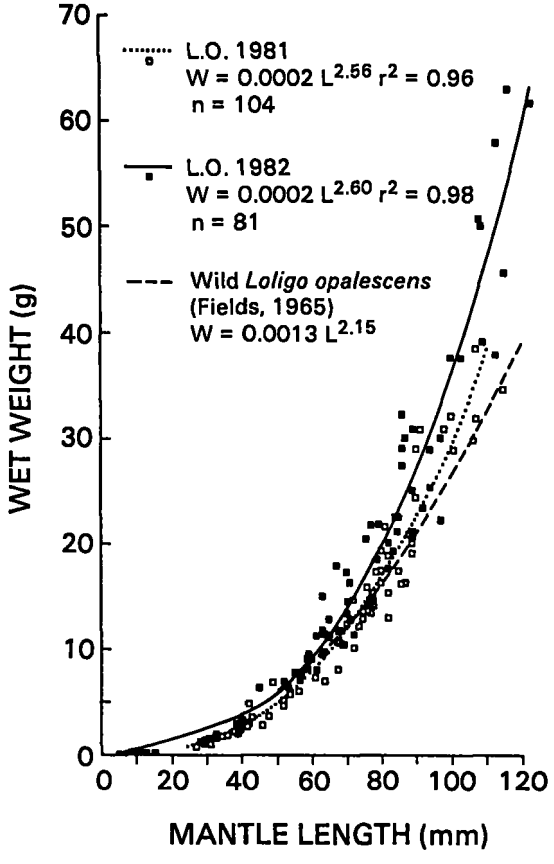


FIGURE 10.—Comparison of length-weight relationship of squid cultured in L.O. 1981 and 1982, and squid collected in the field at Monterey Bay, CA by Fields (1965).

TABLE 3.—Examples of length-weight differences between L.O. 1982 and the data of Fields (1965). Reference Figure 10. ML = mantle length; WW = wet weight.

ML (mm):	25	50	75	100	125
L.O. 1982: WW (g)	0.86	5.22	15.00	31.70	56.60
Fields (1965): WW (g)	1.31	5.80	13.90	25.90	41.90

squid (Fig. 12). The linear relationship between the number of rings (R) and the age in days (D) for 43 statoliths aged 21 to 65 d was $R = -7.24 + 1.13 D$, with an r^2 value of 0.90. Counts of rings differed from the actual age by an average of ±4.2 d (range −12 to +8 d).

Survival

Figure 13 compares survival in the two experiments. The longest lived squid were 248 d in L.O. 1981 and 235 d in L.O. 1982. Survival dropped below 50% on day 15 in L.O. 1981 and on day 2 in L.O. 1982. In L.O. 1982, the early rapid population reduction was due to the removal of newly hatched squid for a different experiment. Mortality rates slowed after the early heavy population reduction; 10% survival occurred on day 120 in L.O. 1981 and on day 49 in L.O. 1982. In all cases, mortality gradually slowed after 60- to 70-d posthatching. Survival reduction after day 180 in both experiments was considered to be related to spawning (Figs. 13A, B).

In L.O. 1981 experiment (Fig. 13A), 50% survival of 391 squid transferred to the large RW system occurred at day 114, but at day 84 for the 129 squid left in the same small CT system. For example, 10 d after transfer the squid in the CT system had 30% mortality whereas those in the RW system experienced only 20% mortality. Thus, transferring squid at about 60 d gave better results by reducing the mortality from fin and skin damage that accrues in the smaller CT system.

In the middle of L.O. 1981 (day 108) cannibalism was observed. The fins and/or posterior mantle were clearly eaten in some squid; these squid differed from those that died from fin damage or from scraping on the bottom of the tank since the latter developed lesions near the tip of the mantle (Fig. 14). From days 108 to 206 there were 16 partly eaten squid in the RW system (7% of the population on day 108), compared with two squid (of 10 total) in the CT system between days 157 and 172. Slightly higher levels of cannibalism (19% between days 97 and 191) were observed in L.O. 1982.

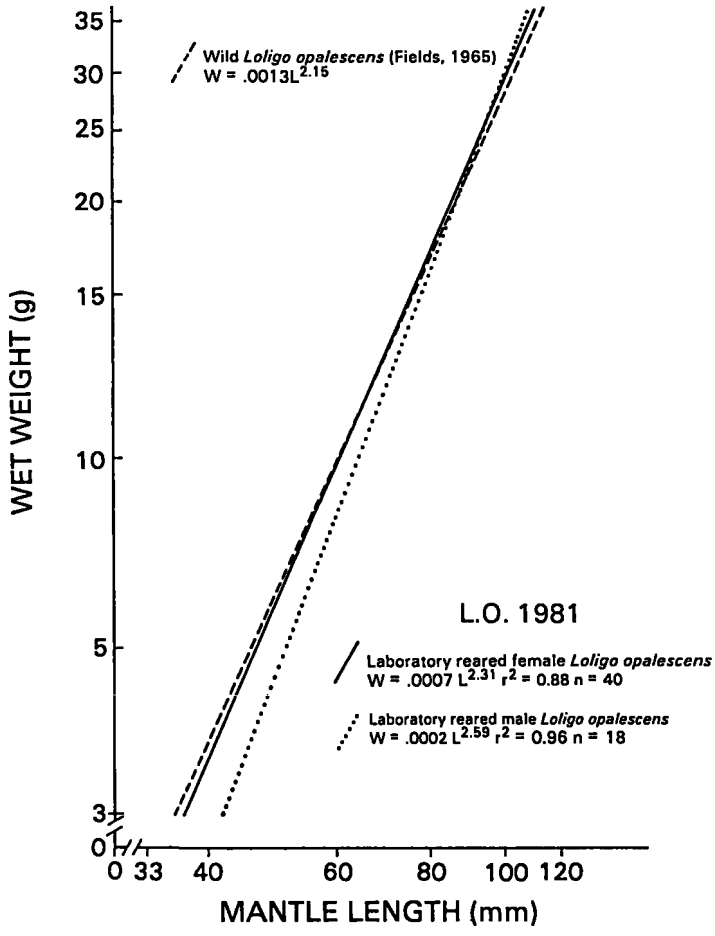


FIGURE 11.—Length-weight relationship of males versus females in L.O. 1981, compared with the data of Fields (1965).

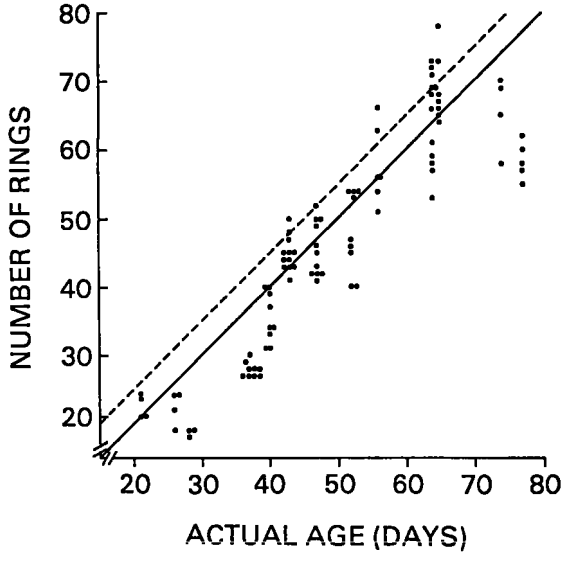


FIGURE 12.—Increase in statolith rings with age (L.O. 1982). Closed circles represent ring counts of 55 statoliths from *L. opalescens* of known-age (21-79 d). Each statolith was counted twice from different photographs. Unclear exposures (16) were not counted. The solid line represents a linear relationship of age and ring numbers. The space between the solid and dashed lines reflects the 5 d of hatching.

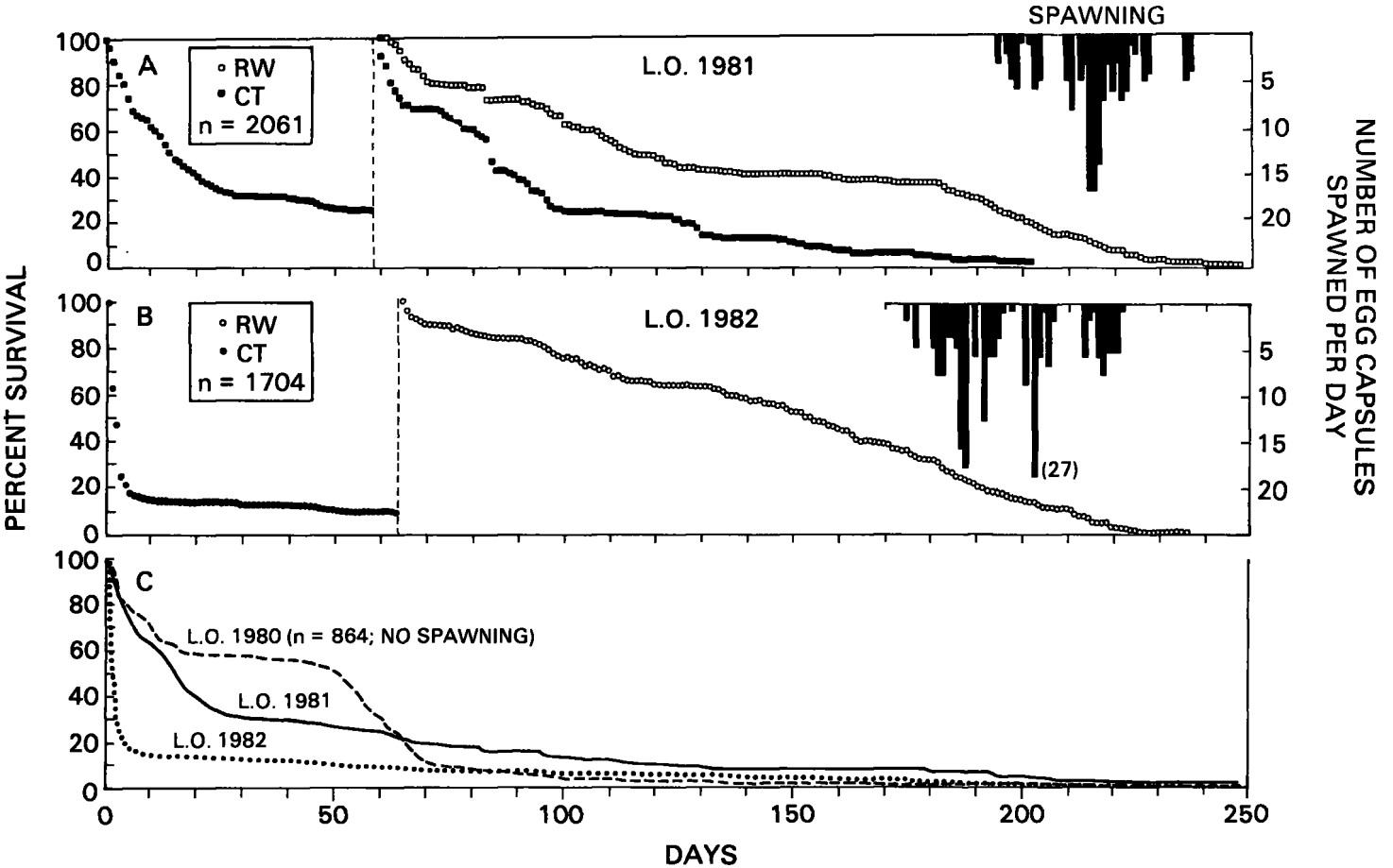


FIGURE 13.—Comparison of daily percent survival and spawning (number of egg capsules in inverted histogram) activity of *Loligo opalescens* in three culture experiments. A, on day 59 (vertical dashed line), 391 squid were transferred to the 10,000 L RW system for the rest of the experiment; the remaining 129 squid were cultured in the CT system. Survival thereafter was calculated as a percent of the number of squid alive on day 59 in each tank system. B, The cultured 147 squid were transferred to the RW system on day 63. Spawning began early on day 173. A maximum of 27 egg capsules were spawned in 1 d. C, Comparison of the daily percent survival data from all three *L. opalescens* culture experiments. CT and RW data were pooled for each experiment for this analysis.

Other causes of injury and death in the later part of RW culture were 1) swimming into the water intake pipes, 2) jetting out of the water and hitting the bottom of the polystyrene tank covers, and 3) colliding occasionally with the walls and slowly accruing fin damage. The resulting abrasions on the body and fins (Fig. 14) were probably the main factor influencing mortality after about 60 d of culture.

1 or 2 d. They usually had some obvious skin damage and were probably unable to maintain disciplined swimming with the school.

Sexual Maturation, Mating, and Egg Laying

In L.O. 1981, the first signs of sexual maturation were when chromatophore patterns associated with

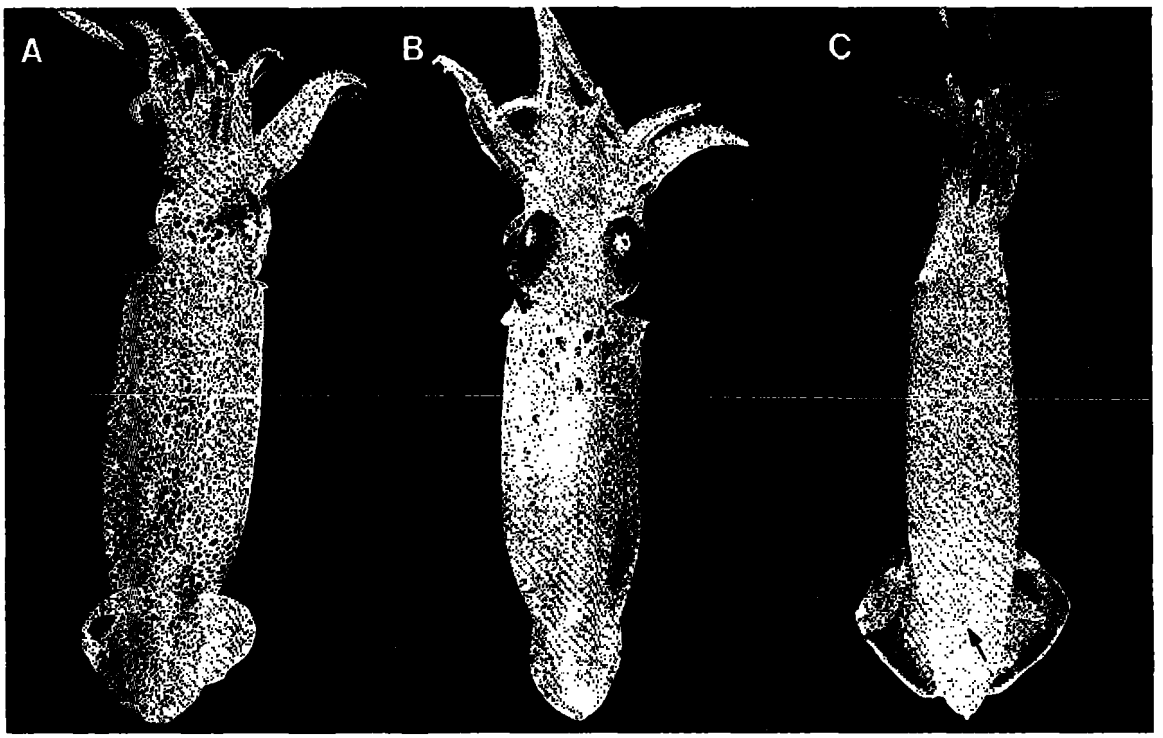


FIGURE 14.—Fin and skin damage that resulted in mortality of cultured squid. A, Epidermis and dermis missing on periphery of fins, with fin margin thickened from scar tissue. B, More extreme case with damage extended to mantle. C, Excessive skin damage on ventral mantle caused by scrapping the tank bottom. A hole (arrow) was produced in the mantle wall and prevented jet-propulsed swimming.

Schooling Behavior

The squid were able to hold a swimming position in the tank between days 41 and 44 in both L.O. 1981 and 1982, corresponding to a mantle length of about 10 mm. In the early phase of RW culture in L.O. 1981 and 1982, squid swam in two or three loose groups throughout the RW. Later, they schooled together at both ends. The reasons for this behavior are unknown, but it may have been related to lower illumination levels at the RW ends or to the well-aerated seawater entering the RW at these points. Individuals not schooling were often found dead in

courting were observed in males. On day 174 two males showed the "Shaded testis" component of patterning similar to that described in *Loligo plei* (Hanlon 1982). Later, other chromatic components of patterns seen in mature males of *Loligo plei* were observed: faint, lateral stripes on the mantle ("Lateral flame"); a discontinuous suture line along the fin margin ("Stitch work fins"); a clear area in the dorsal portion of the mantle above the testis ("Accentuated testis").

Maturation and spawning occurred earlier in L.O. 1982 than L.O. 1981 (Fig. 13). The penis was first recognizable in a 100-d-old male (25 mm ML) and

the nidamental gland was observed in a 101-d-old female (23 mm ML) in L.O. 1981. The penis was first recognizable in a 93-d-old male (29 mm ML, 1.15 g WW), and the nidamental gland was observed in a 92-d-old female (33 mm ML, 1.71 g WW) in L.O. 1982. Figure 15 shows that females become mature at approximately 60 mm ML. This maturation index is based upon reports by Hixon (1980a) and Macy (1982) in which the ratio of nidamental gland length to mantle length is >0.20. Squid this size could produce fully formed egg capsules. The smallest male with spermatophores was 71 mm ML in L.O. 1982.

In L.O. 1981, first mating activity was observed on day 193. A pair was swimming together, a second male interrupted, and a third male grasped the female in the midmantle area but she jetted away. On day 197 another pair was swimming together at the end of the RW and a brief head-to-head mating was observed. They separated for about 1 min, then the male grabbed the female by the arms for a second time. Drew (1911) illustrated this copulating position in *Loligo pealei*. A second mating position was observed on day 226. A male grasped a female's mantle one-third of the way from the posterior tip of the mantle, then he gradually moved to the female's head near the mantle opening and then let go. This was done very swiftly and it was impossible to see if a spermatophore was passed. Freshly dead females had spermatophores attached around the sperm receptacle below the mouth after head-to-head matings. Mating activity was not as closely monitored in L.O. 1982, but first observations were several weeks earlier (before day 175).

Spawning started on day 196 and lasted till day 239 in L.O. 1981. Of 151 egg capsules, 24 (16%) were unfertilized (Table 4). Squid kept in the CT system (3°C higher temperature from day 125) spawned first on day 185, 11 d earlier than the RW system, but none were fertile. Spawning occurred earlier in L.O. 1982, beginning day 175 and ending day 222. All of the 199 spawned capsules were infertile. The maximum number of spawned capsules in a single day was 27 on day 203 (Fig. 13B). Most eggs were collected in the morning indicating that spawning

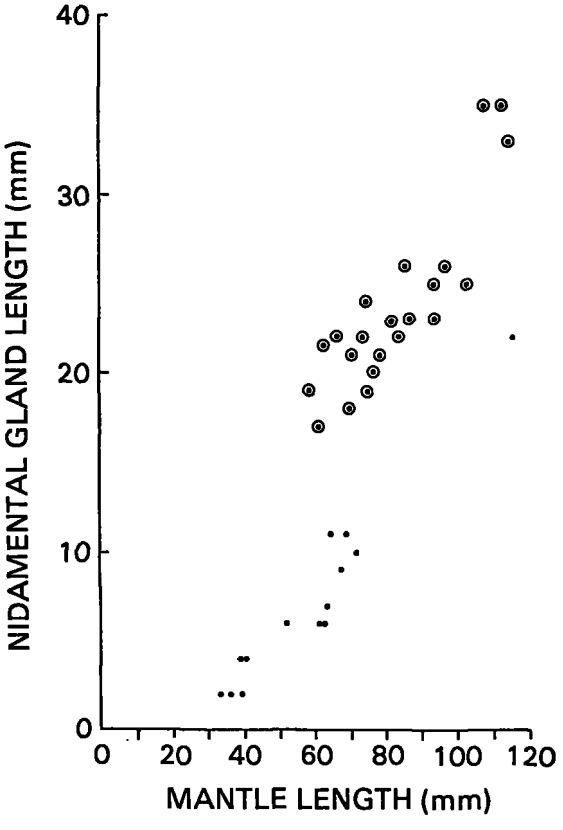


FIGURE 15.—Maturation index for females from pooled data of L.O. 1981 and 1982. Dots with circles indicate sexually mature females in which the ratio of nidamental gland length to mantle length is >0.20. See text.

TABLE 4.—Spawning date and number of egg capsules spawned in the raceway system (L.O. 1981).

Month/ day	Age/ day	Capsules spawned	Capsules without eggs
05/01	196	3	0
05/03	198	2	0
05/04	199	5	0
05/05	200	6	0
05/06	201	1	0
05/08	203	1	0
05/09	204	6	0
05/10	205	5	0
05/16	211	5	1
05/17	212	8	0
05/19	214	5	2
05/20	215	3	3
05/21	216	17	8
05/22	217	17	2
05/23	218	14	0
05/24	219	7	0
05/25	220	3	0
05/26	221	6	0
05/27	222	3	0
05/28	223	7	2
05/29	224	6	1
05/30	225	1	0
05/31	226	2	1
06/02	228	5	2
06/03	229	4	2
06/11	237	5	0
06/12	238	4	0
	Total	151	24 (16%)

took place mainly at night, but some individuals spawned during the day. Egg capsules in the early portion of the spawning period were small, with a length of 2.2 to 4.7 cm when laid. Superficially there were no differences with normal capsules, but usually the early ones contained only a few eggs while a few had none. Typical newly laid egg capsules were between 6.0 and 9.0 cm and contained an average of 156 eggs (range 107-199). These egg capsules were normal in length and egg number compared with *L. opalescens* in nature (Hixon 1983).

A large number of typical egg capsules were incubated and a normal second generation hatched. The average mantle length of second generation hatchlings was 2.3 mm ML (range 1.9-2.7 mm ML, n = 13). This was smaller compared with first generation hatchlings (average 2.7 mm ML) but there was no difficulty in rearing them on copepods for 10 d. Since initial survival was confirmed, further rearing ceased.

In L.O. 1982, three patches of artificial egg capsules made of silicon glue were placed on the bottom of the RW tank to stimulate spawning. The squid spawned 15 fertilized egg capsules around the artificial capsules (Fig. 16).

DISCUSSION

Water Quality and System Design

Water quality was consistently good throughout both experiments and was probably a major contributor to culture success. The CT systems were particularly clean (Fig. 2) because the water volume was relatively large for the small biomass of animals. In the large RW system, water quality changed only slightly when the biomass of squid and food organisms reached its maximum from approximately days 150 to 220 (Figs. 2, 5, 7). The highest total biomass level was 1,706 g between days 180 and 190 in L.O. 1981, which is equivalent to approximately 155 g/m^3 of water. At this point, the nitrate-nitrogen level reached 23 mg/L, which is still low [Spotte (1979a) gave a conservative safe level of 20 mg/L for most marine organisms]. Ammonia-nitrogen and nitrite-nitrogen levels always stayed below the recommended safe level of 0.1 mg/L (Spotte 1979a) in both experiments. We know from our recent unpublished data that a drop in pH (which accompanies nitrogen level increase; Hirayama 1966) is more dangerous to squid; therefore, addition of sodium bicarbonate was necessary to keep the pH near 8.0. Several improvements in system design helped improve water quality over our *L. opalescens* experiment in 1980 (Yang et al. 1983a), when nitrite-nitrogen reached 1.22 mg/L and nitrate-nitrogen reached 39.20 mg/L. These included increased culture water depth and volume in the RW (5,990 to 8,610 L), increased number of protein skimmers from 2 to 5 and generally more oyster shell substrate area for increased biological filtration. Furthermore, regular addition of trace metals assured high levels since losses occur through foam fractionation in protein skimmers (Spotte 1979b) and metabolism of filter bed bacteria, squid, and food organisms.

Growth and Survival

Growth in *L. opalescens* is very fast (Figs. 8, 9) and conforms to a general trend among cephalopods in which the early life cycle is characterized by rapid exponential growth, followed by slower logarithmic growth until reproduction and death (Boyle 1983; Forsythe and Van Heukelem in press).

Egg development is temperature-dependent and takes 19 to 25 d at 16.5°C (Fields 1965), 27 to 30 d at 15°C (L.O. 1981, this report) and 30 to 35 d at 13.6°C (McGowan 1954). Hatching success was high, and young squid survived several days on internal yolk. Many squid will feed before internal yolk is absorbed (Boletzky 1975). The young will feed on a variety and wide size range of crustaceans and fishes (Fig. 4). Zooplankton, but especially copepods, are readily attacked and eaten by very young squid. It is noteworthy that relatively large mysids could be fed successfully to hatchlings within the first week (Fig. 3: L.O. 1982) and for 3 to 4 mo thereafter as a primary food. Mysids are easier to collect and acclimate to laboratory conditions and are thus attractive to the culturist for pragmatic reasons. *Loligo opalescens* hatchlings (2.3-2.8 mm ML) are much larger than those of *L. pealei* (1.7 mm ML) or *L. plei* (1.6 mm ML) (McConathy et al. 1980) and are consequently easier to rear because larger food organisms can be used immediately. Larval fish were attractive to young squid but are difficult to provide.

Major mortality occurred within 10 d posthatching. Although high food densities and variety were provided (Tables 1, 2; Figs. 3, 4, 5), many squid appeared to have difficulty making the transition from passive yolk absorption to active feeding on live organisms. A learning process may be involved, because capturing copepods was initially difficult (squid have been observed to miss 40 times consecutively) and improved when squid attacked from behind. Past experience (cf., Yang et al., 1983a) suggested that increasing food abundance relative to

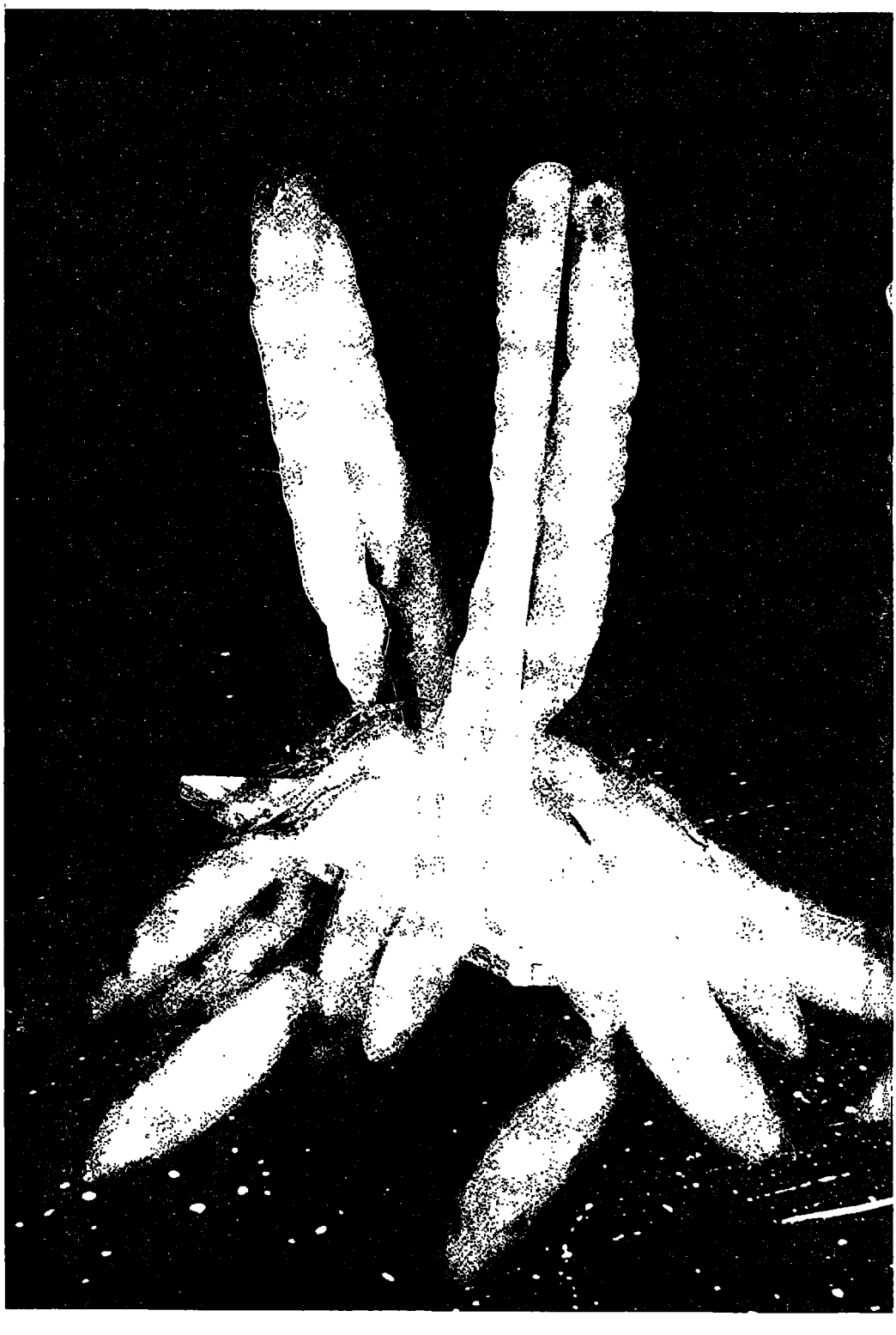


FIGURE 16.—Fertilized egg capsules laid at the base of artificial silicon egg capsules (erected).

squid abundance would enhance survival, but no change has been observed. Further experimentation is required, but a central question is whether many squid are genetically unfit to survive or whether we have not yet provided the proper foods and environment for good survival. Although the former prospect seems unlikely from the evolutionary viewpoint, our experimental design has certainly promoted outstanding growth in surviving squid.

With the growth data from live squid in L.O. 1982, we confirmed that squid grow exponentially both by weight and length during the first 2 months (Fig. 9). Weight increases at a rate of 8.35% body weight/day (doubling their weight every 8 d) and this compares very favorably with octopods (4-7%), other squid (5-7%), and cuttlefishes (5-12%) (Forsythe and Van Heukelem in press). Logarithmic growth during the rest of the life cycle also conforms generally to other cephalopods, except that some cephalopods have a longer exponential growth period up to one-half their life cycle (Forsythe and Van Heukelem in press). The length-weight relationship (Figs. 10, 11) generally conforms to those of wild-caught squid, but indicates that laboratory-reared squid weigh more per unit length (Table 3), possibly, as a result of reduced swimming. The slopes of the lines (all <3.0) indicate allometric growth (Forsythe and Van Heukelem in press). The estimated feeding rates of 18.0% body weight/day (days 121-176) in L.O. 1982 and 14.9% (days 108-230) in L.O. 1981 compare well with the estimate of 14.4% (on a dry weight basis) for *L. opalescens* of a similar size in the natural population (Karpov and Cailliet 1978). Younger *L. opalescens* (48-56 d) fed on *Artemia* were estimated by Hurley (1976) to feed at rates of 36 to 80%/day (dry weight). Another loliginid squid, *Sepioteuthis sepioidea*, had feeding rates of 20 to 25% (wet weight) between days 70 and 105 (La Roe 1971). Other squids of similar size show comparable rates: *Loligo plei*, 10 to 18% (Hanlon et al. 1983); *L. pealei*, ca. 11% (Macy 1980); *Illex illecebrosus*, ca. 10% (Hirtle et al. 1981); and *Todarodes pacificus*, ca. 24% (Soichi 1976).

Maximal survival and size in our three major experiments were L.O. 1980 - 233 d, 77 mm ML (Yang et al. 1983a); L.O. 1981 - 248 d, 113 mm ML; L.O. 1982 - 235 d, 116 mm ML. Figure 13 illustrates survival throughout these experiments and shows that there was a long, steady mortality after the initial high mortality of the first 2 wk. Once in the RW systems (i.e., after 2 mo) most mortality was attributed to fin and skin damage (Hulet et al. 1979; Fig. 14) that accrued slowly from colliding with the sides of the tank. The painted designs on the walls were clearly helpful in reducing wall collisions but damage over time was lethal in many squid. Cannibalism accounted for a minor number of deaths (ca. 7-10%) Most mortality after day 170 in L.O. 1981 and L.O. 1982 was due to 1) sexual maturation and spawning and 2) an unusual situation where fully mature females scraped the bottom of the tank often enough to wear a large lesion through the ventral mantle (Fig. 14C).

It should be noted that survival rate was greater where large tanks such as the RW were used. In L.O. 1981 (Fig. 13A), 50% survival of squid left in the smaller CT system occurred only on day 84 compared with day 114 for those transferred to the RW.

In summary, growth was excellent, indicating that estuarine foods were sufficient and that system design and water quality were conducive to growth, especially in the first 2 mo. Survival was good from the historical perspective (cf., Arnold et al. 1974; Yang et al. 1983b) but rather poor from the production standpoint. A recent hypothesis concerning temperature effects on growth (O'Dor and Wells in press) indicates that higher temperature in the first half of the life cycle and lower temperature in the latter half may enhance growth and survival of laboratory-reared squid. In future work it would be desirable to enhance growth during the latter half of the life cycle and to provide an environment in which somatic growth continues for a longer period before sexual maturation occurs.

Behavior

Squid are generally sensitive laboratory animals, responding very quickly with their sophisticated sensory systems to any fast environmental change. They habituate to many daily disturbances in the tank system (e.g., tank cleaning, etc.) provided everything is done slowly. Later in the life cycle they become slightly less sensitive.

Hatchlings were positively phototaxic and often swam at the water surface. In nature, young squid have been caught mainly by plankton nets mounted on a sled and towed along the bottom (Recksiek and Kashiwada 1979). It is not possible at this time to explain the movements of hatchlings in nature based upon laboratory observations of positive phototaxis.

A key component in feeding behavior was movement by the prey, regardless of the size or age of the squid or food organisms. Young squid preferred copepods but ate a variety and a very wide size range of organisms (Fig. 4). In general, the squid preferred crustaceans over fish, but the relatively

restricted diet offered to them may have influenced that. Fields (1965) and Karpov and Cailliet (1978) agreed that *L. opalescens* adults prefer fish over crustaceans but there was no clear-cut preference in younger squid. It is clear from laboratory observations that squid learned to associate certain events with feeding (e.g., opening the tank top), and the general level of activity increased markedly during these periods. We were also able to stimulate feeding in the CT systems by dimming and brightening the lights to attract the planktonic food organisms into the water column near the squid.

Schooling behavior was correlated with size. Larger body size and growth of the fins were required before squid could swim in place against a current; this occurred at about 10 mm ML (41-44 d in L.O. 1981 and 1982). Hurley (1976) reported that *L. opalescens* 4 to 5 mm ML could briefly form loose schools when disturbed, but this may have been in static water. At 15 mm ML, *L. opalescens* were powerful enough to form distinct schools (Yang et al. 1983a), indicating the size at which one could expect schooling to appear in nature. How and why squid begin schooling in nature has not been investigated.

Cannibalism was not seen in L.O. 1980 (Yang et al. 1980b, 1983a) and accounted for 7 to 19% of mortalities in experiments L.O. 1981 and 1982. Lack of food did not precipitate this behavior. On the spawning grounds in Monterey, CA, mature squid often have cephalopod remains in their stomachs (Loukashkin 1977; Karpov and Cailliet 1978); in one case as many as 75% of males had squid remains in their stomachs (Fields 1965). This could be a behavioral response to overcrowding (Fields 1965) or to restrict prey organisms on the spawning grounds. We anticipate that cannibalism in tanks would be a significant problem only during prolonged food shortage or if squid of a very wide size range were in the same system (cf., Hanlon et al. 1983).

Body patterning was not studied in great detail but several observations are noteworthy. Young animals are capable only of simple chromatic expression such as "All dark" or "Clear". When excited, *L. opalescens* of all sizes show some degree of darkening; this is similar to other loliginid squids (cf., Hanlon 1982; Hanlon et al. 1983). By the time the squid are approximately 80 to 100 mm ML they can show a repertoire that includes about a dozen chromatic components of patterning (e.g., Dark arm tips, Ring on the mantle, etc.). This places *L. opalescens* in a category of rather simple patterning, making it comparable to *L. pealei* and *L. vulgaris*, slightly more complex than *Lolliguncula brevis* (Dubas et al. 1986), but simpler than *Loligo plei* (Hanlon 1982; Hanlon et al. 1983). Further analysis is warranted because much behavior is expressed through patterning and may yield important behavioral clues.

Social behavior was first manifest in schooling (see above) then much later in mild intraspecific aggression. Occasionally two squid would fight over one fish, but the first firm observations came at the time of sexual maturation when mating was seen. As Hurley (1977) noted, there were no obvious interactions among males to form a dominance hierarchy for mate selection. Mating was initiated by males, and both typical forms of mating were observed: "head-to-head" matings in which spermatophores were stored in the *bursa copulatrix*; and male-underneath matings in which spermatophores were deposited in the mantle near the oviduct (cf., Drew 1911; McGowan 1954; Hurley 1977). Females mated promiscuously as they do in nature, and females were also stimulated visually to lay eggs around artificial facsimiles of egg mops (Fig. 16). Males were not observed to guard or defend egg capsules as described by Hurley (1977), but this may have been because relatively few egg capsules were left in the tank each day.

Reproduction

In L.O. 1980 only the subadult stage was reached in 233 d (Yang et al. 1980b, 1983a). Full sexual maturity was achieved in L.O. 1981 and 1982 and spawning of viable eggs occurred from days 196 to 239 and 175 to 226, respectively (Fig. 13). Relatively few egg capsules were laid per female, and these capsules were generally shorter and contained slightly fewer eggs per capsule than those reported from natural populations, but this was probably due to the smaller size of these spawning females (Hixon 1983).

Laboratory cultured *Loligo opalescens* matured precociously and since they are terminal spawners this prevented attainment of full adult size. In the laboratory, males as small as 71 mm ML had fully formed spermatophores and females became sexually mature beginning at about 60 mm ML (Fig. 15). In nature, the average adult size is 150 mm ML for males and 140 mm ML for females, although size at onset of maturity is variable and can be as low as 72 mm ML for males and 81 mm ML for females (Fields 1965; Hixon 1983). Precocious maturation has also been reported in other squid maintained in the laboratory (cf., Durward et al. 1980; Hanlon et

al. 1983). The stimuli (or stressors) that cause this are unknown.

Van Heukelem (1979) reviewed environmental factors that influence maturation in cephalopods and reported that light, temperature, and nutrition are the key stimuli. In our experiments, light was constant (24 h on), temperature was consistent (ca. 15°C) and food was relatively constant and highly available compared with natural populations. However, all three conditions are different from nature. The most interesting result concerns light, which is thought to have a major effect on maturation through the light-optic gland-gonad pathway (cf., Mangold and Froesch 1977; Wells and Wells 1977). Long daylength of high intensity is thought to delay maturation; in our experiments daylength was 24 h but intensity (ca. 4-17 lux) was low compared with full sunlight. However, we do not know what light intensity subadult *L. opalescens* are subject to in nature. Clearly, long daylength alone does not delay maturation in *L. opalescens*. Future experimentation will be necessary to identify the combinations of environmental factors that affect maturation in the laboratory.

Life Cycle Comparisons: Laboratory vs. Fishery Data

In general, five major rearing attempts have been successful in varying degrees: 1) Hurley (1976), to 100 d; 2) Hanlon et al. (1979), to 79 d; 3) L.O. 1980, to 233 d and subadult stage (Yang et al., 1980b, 1983a); 4) and 5) L.O. 1981 and 1982, to sexual maturity and egg laying within 8 mo (this report). From this it is clear that the life cycle can be <1 yr under laboratory conditions.

Fields (1965) stated, based upon fishery data, that "Almost all females spawn at the age of 3 years...." However, more recent field (cf., Recksiek and Frey 1978) and laboratory studies of *L. opalescens* (above) indicate that life span estimates beyond 2 years are excessive. Furthermore, recent books on cephalopod life cycles (Boyle 1983, in press) indicate that few squid live beyond 2 years.

Growth information on laboratory populations is now quite good. The present data allow an accurate assessment by weight from hatching onwards (Fig. 9) and firmly verify that young squid are capable of dramatically fast, exponential growth when food is not limiting. This indicates that in nature squid are capable of exploiting plankton blooms and other instances of greater food availability; the highest feeding rates we estimated (29%) also confirm field observations that squid will eat large quantities of food when available and when necessary. Field estimates of growth by Fields (1965) and Spratt (1978) are compared with laboratory data in Figure 17. Field's data are very conservative (averaging 4 mm/month) and based only upon monthly modal length-frequency diagrams from squid on or near spawning grounds. Spratt (1978) estimated growth from statolith rings and hypothesized that growth is rapid during the first few months then decreases with age. Laboratory growth was much faster, but animals were not subject to environmental fluctuations. We estimate that growth in nature approximates something between the laboratory data and Spratt's data, and that date of hatching, seasonal temperature fluctuations, and food availability result in life cycle variations between 1 and 2 years. One would expect to observe exponential growth of young squid during spring and summer when temperatures and food availability are high, slower logarithmic growth in fall and winter, and spawning the following spring.

Field evidence (McGowan 1954; Fields 1965) and reproductive physiology studies (Grieb and Beeman 1978; Knipe and Beeman 1978) indicate that *L. opalescens* is a terminal spawner (Hixon 1983), and our laboratory observations verify this since all animals died shortly after spawning (Fig. 13).

Rings in statoliths may eventually be used as a reliable age marker to determine growth rate and life span. Our preliminary results in this paper from 43 statoliths of known age support Spratt's (1978) conclusion that ring deposition occurs roughly on a daily basis during the first 65 d. However, our laboratory data indicate that the relationship does not hold well beyond that age, although Spratt suggested that daily ring deposition occurs up to 150 d. Thereafter, Spratt (1978) hypothesized lunar (monthly) rings on statoliths but there are no laboratory data for comparison. Daily, fortnightly, or monthly growth rings have been hypothesized in the squid *Gonatus fabricii* (Kristensen 1980), *Todarodes sagittatus* (Rosenberg et al. 1981), *Illex illecebrosus* (Hurley and Beck 1980), and *Loligo forbesi* (Martins 1982), but there are no hard data to confirm these estimates. The mechanism of ring formation is unclear but may be related to feeding, since in this part of our laboratory study the squid received food during 12 h and none for the next 12, while concurrently there was constant light and no temperature fluctuation (Hixon and Villoch 1984). Hurley et al. (1985) and Dawe et al. (1985) found evidence of daily rings in statoliths by inoculating squid with tetracycline or strontium. Further work is required to

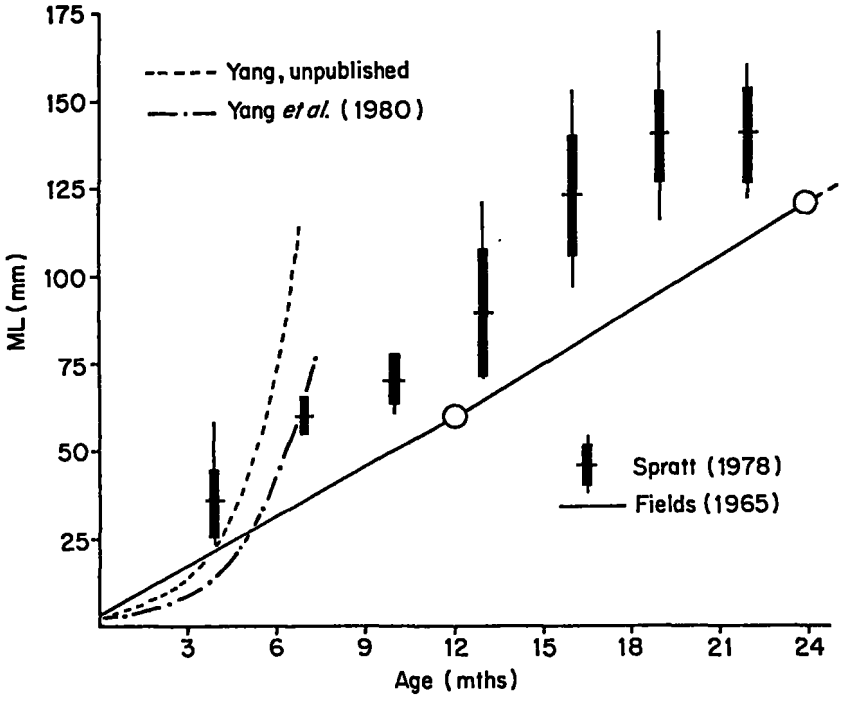


FIGURE 17.—From estimates of growth rate in mantle length of *Loligo opalescens*. Fields (1965) used population data. Spratt (1978) combined age (statolith ring counts) and ML data and calculated a mean (horizontal line), range (vertical line) and standard deviation (vertical bar) values for 3-month intervals throughout the life cycle. Yang et al. data are from laboratory rearing studies (1980b, 1983a, this report) (modified from figure 7.1, Hixon 1983).

determine if and how statolith rings are correlated with age.

A major gap in fisheries studies concerns where the hatchlings go from the spawning grounds. Very few young squid have been captured (Okutani and McGowan 1969; Recksiek and Kashiwada 1979) even in the vicinity of spawning grounds. Hatchlings are positively phototaxic and this may serve to disperse them immediately from the spawning grounds. Thereafter their movements are unknown, although rarely young squid 3.5 to 7.0 mm ML have been caught in neritic plankton samples, usually at depths of 25 to 40 m nearshore in water between 12.5° and 21.0°C (Okutani and McGowan 1969). Detailed knowledge of water currents between spawning grounds and nearshore, combined with monitoring of plankton abundance (especially copepods and larval fish) by surface, bottom and oblique tows may provide important clues about movements and feeding patterns of young-of-the-year squid. Laboratory studies indicate that squid can swim well enough to hold their position against a current by 10 mm ML, or about 40 to 45 d posthatching. By 15 mm ML (ca. 60-80 d) they can form and maintain well-formed schools. The functions of schooling in nature probably relate to defense, feeding and migratory behavior.

The California squid fishery has nearly collapsed since El Niño of 1983, and the squid population has been generally displaced northward as far as southern Canada. Some small spawning populations are still present in southern and central California. It may be rewarding to investigate feeding and migratory patterns of young and adult squid to better understand population recruitment into this ecologically and economically important fishery resource.

Biomedical Research Applications

Loligo opalescens has proved to be a suitable model for giant axon preparations (e.g., Llano and Bezanilla 1980). However, for most axon experiments the largest axons (>400 μm diameter) are needed; this requires the largest squid taken in the fishery, usually 150 mm ML and larger. Our largest squid, 116 mm ML, had an axon about 240 μm in

diameter. Unknown factors in our laboratory environment resulted in precocious sexual maturation and thus smaller animals. Therefore, we are now evaluating the culture potential of *Loligo forbesi*, a much larger squid from the eastern Atlantic, since precocious maturation in that species would still result in axons >500 μm. Preliminary experiments bear out this proposition as we have recently cultured *L. forbesi* to 140 mm ML and 400 μm diameter axons. However, *L. opalescens* would be an excellent model for the giant synapse preparation in which smaller squid are most suitable. Therefore, *L. opalescens*, with a now substantial amount of culture information, may be a highly suitable species in the United States for providing squid on a consistent basis for neuroscience research. Moreover, the recent disappearance of *L. opalescens* (1983-85) from traditional fishing grounds in California make laboratory culture an attractive alternative for animal supply.

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Note: We dedicate this paper to our coauthor and dear friend Dr. Raymond F. Hixon, who passed away on 19 March 1984 as he valiantly fought to recover from chronic myelogenous leukemia.

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