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ASPECTS OF FEEDING, GROWTH AND SURVIVAL OF THE EUROPEAN SQUID *LOLIGO VULGARIS* LAMARCK, 1799, REARED THROUGH THE EARLY GROWTH STAGES

P.E. TURK, R.T. HANLON
L.A. BRADFORD and W.T. YANG

The Marine Biomedical Institute,
The University of Texas Medical Branch,
Galveston, TX 77550-2772, U.S.A.

CALMAR
NUTRITION
CROISSANCE
ELEVAGE

RÉSUMÉ. — *Loligo vulgaris* a été élevé en laboratoire jusqu'à une taille maximale de 75 mm (longueur dorsale du manteau) et un poids frais de 28,2 g. Les densités et préférences de nutrition ainsi que le taux de croissance ont été observés pendant des expériences durant jusqu'à 140 j. La mortalité après l'éclosion est très haute : 50 à 100 pour cent des Calmars morts sont dépourvus des statolithes indispensables à la nage et à l'orientation. Le taux de croissance observé est compatible avec la longévité d'une année de cette espèce.

SQUID
FEEDING
GROWTH
CULTURE

ABSTRACT. — *Loligo vulgaris* was reared to a maximal size of 75 mm dorsal mantle length and 28.2 g wet weight. Experiments lasting up to 140 days permitted observations on feeding densities, feeding preferences and growth rate. Hatchling mortality was very high; between 50 and 100 percent of the dead squids were missing statoliths which are needed for swimming and orientation. The growth results are compatible with the concept of a one-year life cycle for this species.

INTRODUCTION

The neritic squid *Loligo vulgaris* is an important experimental model (e.g., Naef, 1923; Spyropoulous, 1965; Marthy, 1982) and is harvested in substantial quantities for human consumption (Worms, 1938b). However, the life cycle is incompletely known (Worms, 1983a). Boletzky (1974, 1979) reared the hatchlings for several months and reported preliminary data on feeding and growth. We report herein our attempts to culture this species in recirculating seawater systems for biomedical research. These data should be useful in elucidating unknown aspects of the early stages of the life cycle.

MATERIALS AND METHODS

Three experiments were conducted between April 1982 and September 1984. All eggs were collected in the vicinity of the Laboratoire Arago in Banyuls-sur-Mer, France and shipped by air to Texas. The eggs were maintained through their developmental period at temperatures ($\sim$ 13 °C) approximating the natural environment. After hatching, a slow gradual rise in temperature ($<$ 1 °C per day) was allowed until culture system temperatures reached ambient laboratory temperatures. Dead squids were counted daily, and the dorsal mantle length (DML) of a representative sample measured. Growth rates were

estimated from the day hatching began using freshly dead or dying squids. Live squids were not sacrificed for growth measurements in these experiments because the objective was to raise as many squids as possible for as long as possible. Ten to 50 dead squids per day were examined for statoliths.

Squids were reared in 3 000 l closed (recirculating) culture systems previously described by Yang *et al.*, (1983). The rearing tank was circular, measuring 1.80 m in diameter and 0.75 m deep. The squids in Experiment 2 were reared in this system for 112 days then transferred to a 13 000 l raceway (Yang *et al.*, 1983) for the remaining 38 days of the experiment. In Experiments 1 and 3, two physically identical culture systems were stocked with egg capsules. One system had artificial sea water (Instant Ocean^R brand) while the other had filtered natural sea water collected 50 to 80 kilometers offshore. A single culture system utilizing the artificial sea water was used in Experiment 2. In all the experiments the initial squid stocking density was more than 2,000 hatchlings per system. The tank bottom was cleaned daily by siphoning. One to four percent of the water volume was added weekly to replace water removed by siphoning. A mixture of trace elements (Wimex^R brand) equal to 0.005 per cent of the system volume was added biweekly. Temperature and salinity were measured daily, and pH every other day. Precise colorimetric analyses for ammonia, nitrite and nitrate were performed weekly (Strickland and Parsons, 1972). Deionized water was added periodically to replace water lost to evaporation, and sodium bicarbonate was added periodically to maintain pH above 8.0 (Spotte, 1979).

Food organisms for the squids included zooplankton (mostly estuarine or neritic copepods, e.g., *Acartia tonsa, Labidocera aestiva,* and *Centropages velificatus*), mysid shrimps (*Mysidopsis* spp.), palaemonid shrimps (*Palaemonetes* spp.) and several species of small fishes (primarily *Menidia beryllina, Poecilia latipinna, Gambusia affinis, Cyprinodon variegatus* and *Fundulus* spp.). Food organisms were collected from the field several times a week, treated for parasites or pathogens, size-sorted and transferred to holding tanks. They were acclimated to the temperature and salinity of the squid system over a 24-hour period prior to each feeding. Food organisms were concentrated in a measured volume of water and their number estimated from an aliquot sample. Zooplankton and small mysids were fed to newly hatched squids, and larger foods were added as the squids grew. Food organisms were fed in excess during the entire experiment.

Observations

1. ***Water Quality*** (Table 1)

Table 1. — Summary of culture system levels for temperature, salinity and pH, in the three experiments

Parameter		Experiment 1	Experiment 2	Experiment 3
Temperature (°C)	$\bar{x}$	15.60	17.50	18.50
	range	13.30 - 24.00	11.80 - 23.00	13.10 - 21.90
Salinity (ppt)	$\bar{x}$	35.10	34.50	34.00
	range	34.00 - 37.00	33.00 - 36.50	33.00 - 36.00
pH	$\bar{x}$	8.03	8.20	8.22
	range	7.85 - 8.20	8.20 - 8.30	8.10 - 8.29

Water quality was good. The highest measured levels of ammonia (0.071 mg.l^{-1}), nitrite (0.005 mg.l^{-1}) and nitrate (9.1 mg.l^{-1}) in our experiments were below the upper limits suggested by Spotte (1979) for most marine animals (i.e., 0.10, 0.10 and 20.0 mg.l^{-1}, respectively).

2. ***Feeding***

Food concentrations in the rearing tank were maintained by observation so that food organisms were within attack range of the squids. Hatchling squids seldom oriented toward or attacked food organisms more than a few millimeters away. As the squids grew, the distance they would stalk prey increased and, therefore, densities were allowed to drop. The number of daily feedings and the amount of food added was occasionaly high to compensate for food organisms that did not survive well or tended to concentrate at the top (e.g., zooplankton) or bottom (e.g., mysid shrimp) of the water column away from the squids. Table 2 summarizes the number of feedings daily and the estimated rearing-tank density of food added daily. The highest densities (i.e., 24 and 45 organisms per liter) represent figures for zooplankton. The actual density of food in the rearing tanks was not estimated to avoid disturbing the squids.

Table 2. — The mean number and range of daily feedings, and the mean and range of rearing-tank densities of food added daily

		Experiment 1	Experiment 2	Experiment 3
Number of feedings	$\bar{x}$	4	2	4
	range	1 - 10	1 - 4	0 - 8
Estimated rearing-tank density of food added daily (No.ℓ$^{-1}$)	$\bar{x}$	8	4	7
	range	0 - 24	0 - 45	0 - 21

Figure 1 illustrates the feeding regimen of Experiment 2 (starting on the first day of hatching), which produced the largest, oldest squids. Zooplankton were fed for 22 days, mysids and *Palaemonetes* spp. larvae were fed an additional 30 days. Small *Palaemonetes* spp. post-larvae were started on day 53 and fed throughout the remainder of the experiment even though the squids were most frequently ob-

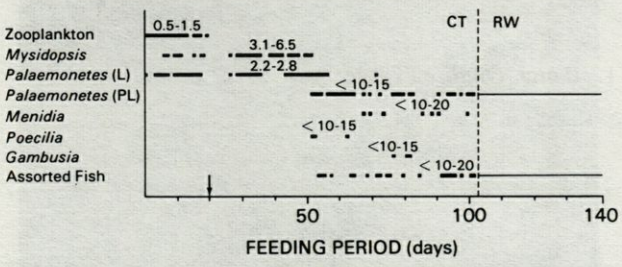

Fig. 1. — Food organisms fed during Experiment 2. Vertical dashed line separates the period squids were reared in the circular tank (CT) from the time in the raceway (RW). Arrow indicates experimental day that hatching ended. *Palaemonetes* spp. shrimp were fed as larvae (L) and post-larvae (PL). Numbers above the food bars show the range in body length (mm). The "assorted" fish were a mixture of acceptable species. The raceway was pre-stocked with *Palaemonetes* spp. and fish and required no additional feeding.

served feeding on fish during that period. The raceway, where two squids were reared for the last 38 days of the experiment, was pre-stocked with hundreds of small fish and *Palaemonetes* spp. that were not preyed upon heavily enough to require restocking during that part of the experiment. The silverside fish *Menidia beryllina* was preyed upon most aggressively, but was least available during the experiment. *Gambusia affinis, Poecilia latipinna* and a mixture of unidentified juvenile fish were routinely fed upon in the absence of *Menidia beryllina.* In Experiments 1 and 3, zooplankton and mysids were used throughout the entire experiment; the squids did not grow enough to permit a phase-out of zooplankton or the introduction of *Palaemonetes* spp. post-larvae and fishes.

Ad libitum feeding made it difficult to quantify food preferences or feeding rates. During observations lasting up to 15 minutes, it was rare for more than one or two percent of the squids to be seen with food. The number of feeding squids increased when the tank lids were opened, and also following the addition of new food. For example, shortly after *Palaemonetes* spp. larvae were added to the tank, most of the squids observed with food had these shrimp larvae even though preexisting food organisms (zooplankton or mysids) were already abundant in the tank.

3. Growth and Survival

Mean hatching size was 3.45 mm DML (range 2.92 – 3.85 mm; taken from 30 samples over the hatching period). In Experiment 1, the largest squid grew to 27.0 mm DML and 1.17 g wet-weight (WW) within 123 days after the onset of hatching. A second squid grew to 11.0 mm DML and 0.33 g WW within 64 days. One squid in Experiment 2 grew to 75.0 mm DML and 28.2 g WW within 112 days and another grew to 74.0 mm DML and 25.5 g WW within 140 days. Experiment 3 produced squids of 6.2 mm DML (no weight taken) and 9.2 mm DML (0.08 g WW) within 54 days. Growth data were limited in all experiments due to poor survival; most data were gathered from Experiment 1, where a fairly consistent series of dorsal mantle lengths was available for the first 44 days post-hatching. These data along with the mantle lengths available from Experiments 2 and 3 were plotted against time in Figure 2, with additional data from Boletzky (1979) superimposed from comparison. Linear regression analyses were used to fit squid DML data from Experiments 1 (n = 21) and 3 (n = 2) to different growth models : linear, exponential, logarithmic and power. These data best fit (r^2 = 0.9529) the exponential growth model DML = ae^{bt} where a = the projected y intercept (DML at hatching), e = the natural 2.7183, b = the slope or growth rate and t = time in days (Fig. 2). The estimate for growth rate was 2.28 percent of DML per day. When DML data for the faster growing squids in Experiment 2 (n = 7) and in Boletzky's experiments (1979; n = 3) were combined and used in the exponential regression, the estimated growth rate was 3.07 percent per day (r^2 = 0.9001). This growth rate estimate may be low due to the cooler temperatures in Boletzky's culture system (17.5 °C declining to 13.0 °C).

Experiments 1, 2 and 3 lasted 123, 140 and 54 days, respectively, from the day of first hatching

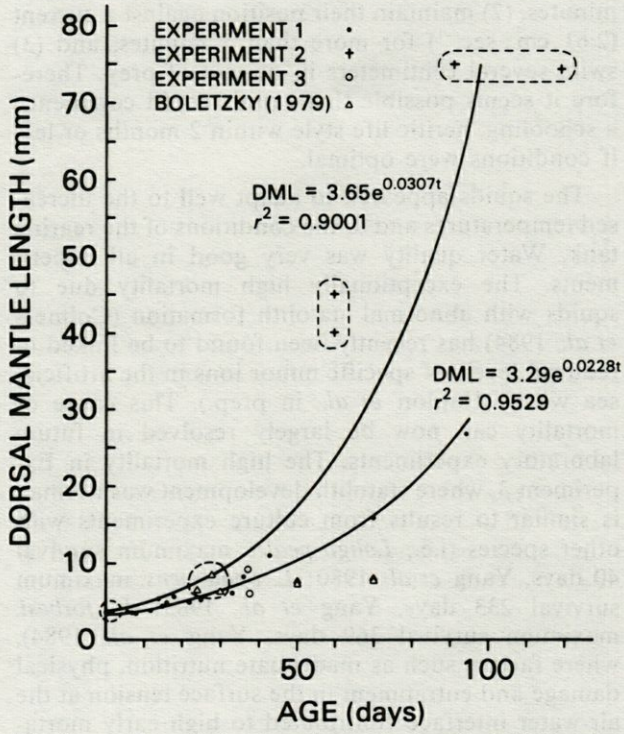

Fig. 2. — Growth in dorsal mantle length (DML). The lower growth curve was fitted to data from Experiments 1 (n = 21) and 3 (n = 2). The higher growth curve was fitted to data from Experiment 2 (n = 7) and Boletzky (1979; n = 3) shown within the dashed lines.

until the last squid died. Complete hatching took 30, 21 and 17 days, respectively; thus the age of the oldest squid in each experiment may have been younger than we report. Survival was very poor in all three experiments. Of more than 2,000 squids hatched per experiment only 503, 14 and 528 hatchlings, respectively, were alive on the last day of hatching. Fifty percent or more of these were dead within the next two days. The best survival was in Experiment 1 where about 50 squids (10 percent) lived at least 12 days after the end of hatching (day 42), and the last squid died on day 123. Fifty percent of the squids in Experiments 1 and 2 were missing one or both statoliths, rendering them unable to swim properly and capture food (Colmers *et al.*, 1984). In Experiment 3, only one percent of the squids were missing statoliths in the system with natural sea water.

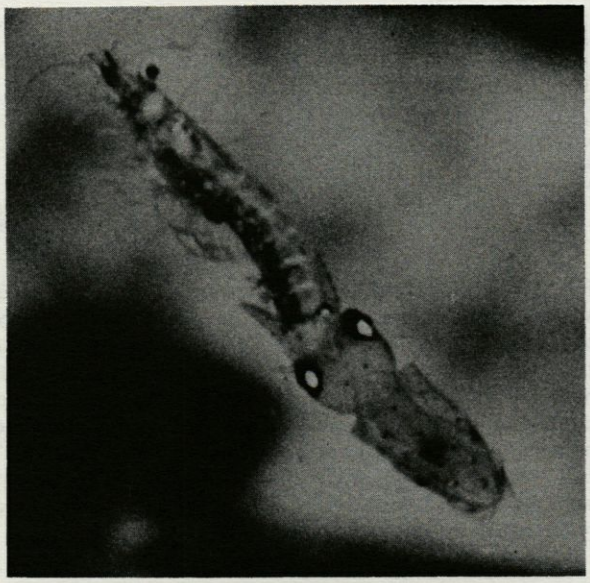

Fig. 3. — *Loligo vulgaris* hatchling feeding on a mysid shrimp.

DISCUSSION

Loligo vulgaris was reared longer (140 days) and to a larger size (75 mm DML) than reported previously (75 days, 7.5 DML; Boletzky, 1979). Worms (1983a) estimated the duration of planktonic (vs. nektonic) life at about 2 months. We observed that within 20 days post-hatching some squids were able to : (1) swim in a horizontal position for several minutes, (2) maintain their position against a current (2.61 cm . sec^{-1}) for more than 5 minutes, and (3) swim several centimeters in pursuit of prey. Therefore it seems possible that squids could commence a schooling, neritic life style within 2 months or less if conditions were optimal.

The squids appeared to adapt well to the increased temperatures and to the conditions of the rearing tank. Water quality was very good in all experiments. The exceptionally high mortality due to squids with abnormal statolith formation (Colmers *et al.*, 1984) has recently been found to be linked to reduced levels of specific minor ions in the artificial sea water (Hanlon *et al.*, in prep.). This cause of mortality can now be largely resolved in future laboratory experiments. The high mortality in Experiment 3, where statolith development was normal, is similar to results from culture experiments with other species (i.e., *Loligo pealei,* maximum survival 40 days, Yang *et al.*, 1980; *L. opalescens,* maximum survival 233 days, Yang *et al.*, 1983; *L. forbesi,* maximum survival 369 days, Yang *et al.*, 1984), where factors such as inadequate nutrition, physical damage and entrapment in the surface tension at the air-water interface contributed to high early mortality.

Newly hatched squids (3.45 mm DML) fed upon a large size range of prey, from copepods 0.8 mm to mysid shrimp 6.5 mm long (Fig. 3). From our observations, mysids were the easiest food organisms for the squids to capture. Copepods were often able to avoid capture by "jumping" out of reach as the squid attacked. *Palaemonetes* spp. larvae were easily captured and appeared to be a preferred food. Long, thin silvery fishes such as *Menidia beryllina* also appeared to be preferred foods of juvenile squids. These types of food organisms are available in the natural environment of *L. vulgaris.*

Growth of the largest squids was very fast. Since we have few data, we plotted the fastest and slowest growth curves (Fig. 2). Our faster growing squids (3.07 percent per day) and slower growing squids (2.28 percent per day) grew 1.8 and 1.3 times faster respectively, than laboratory-cultured *Loligo opalescens* (Hixon, 1983). Worms (1983a) estimated the average growth of *L. vulgaris* in nature at 5-20 mm per month, while our largest squids (74 and 75 mm DML) grew at a rate of 20-25 mm per month. Post-mortem examination revealed these two largest squids to be immature males; a penis was evident, but there was no sign of spermatophore production. Worms (1983a) could sex wild-caught squids at 50 mm DML and some males had spermatophores at 100 mm DML. We attribute the faster growth rate of our squids (versus the results of Boletzky, 1979) to the variety and higher density of food organisms, and to the warmer culture temperatures we were able to provide the squids. These high growth rates are compatible with the concept (Worms, 1983a) that *L. vulgaris* could complete its life cycle within one year given the best conditions.

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