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Optimizing rearing conditions of hatchling loliginid squid

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Abstract Eggs laid by the California market squid (*Loligo opalescens*) were collected from spawning grounds and reared in the laboratory. The eggs were maintained in a rearing tank that was part of a closed, recirculating system. The system included seven 220-l circular tanks with attached filtration. Five experiments were conducted to test hatchling survival. One of them evaluated survival on three different food types: (a) enriched *Artemia* sp. nauplii, (b) wild zooplankton and (c) a mixture of a and b plus mysid shrimp. This mixture of food types (c) was offered to the hatchlings in the other four experiments. High mortality occurred in all experiments between days 1 and 15 post-hatching. However, survival over the entire time span of the experiments (45–60 days) was between 36% and 60%. These survival rates are well above previously reported survival rates for the same time period, and overall are up to 35% better than any survival results ever attained for the routine culture of *Loligo* spp. squid. Results suggest that high survival can be achieved by: (1) rearing hatchlings in a recirculating system consisting of small round tanks designed to maintain water quality and pH within narrow limits (8.1–8.4), (2) maintaining low current speed (1.0–1.4 cm s^{-1}) to reduce skin damage and to enhance hatchling–prey interactions, (3) increasing feeding rate by feeding small amounts of food at regular intervals (every 2–3 h) during day time hours and keeping prey densities above 50 prey l^{-1}, (4) feeding hatchlings with enriched *Artemia* nauplii during days 1–30 post-hatching and (5) feeding a variety of prey types and sizes to match the different sizes and hunting abilities of same-aged but heterogeneously developing hatchlings. The results from this study will enhance future culturing efforts for the commercially important loliginid squid.

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Introduction

During the past 20 years considerable effort has been made to improve laboratory maintenance, rearing and culturing of loliginid squid with the main purpose of providing a reliable supply for neuroscientists (LaRoe 1971; Boletzky and Hanlon 1983; Hanlon 1987, 1990; Hanlon et al. 1987, 1989, 1991; Lee et al. 1994, 1998, 2000). Nonetheless, most major attempts to culture squid species of the genus *Loligo* have been unsuccessful (Hanlon et al. 1979, 1987; Yang et al. 1980, 1983b; Turk et al. 1986). The reasons for failure have been attributed to extremely high mortality rates (80–99%) during the first weeks after hatching (Turk et al. 1986; Yang et al. 1986; Hanlon et al. 1987, 1989), caused by starvation and inadequate tank design that led to skin damage and infection (Hanlon et al. 1983, 1991). The first completion of a squid life cycle was accomplished by Yang et al. (1983a, 1986) using *L. opalescens*. However, mortality was still quite high and the best survival rate obtained during the first 2 months after hatching was 25%. Advances have been made in squid culture using the loliginid species *Sepioteuthis lessoniana*. This species has been cultured through multiple generations in the laboratory (Lee et al. 1994, 1998). Improvements in the culture system resulted in improved survival, mating and fecundity in captivity. The major advantage to culturing *S. lessoniana* is the extremely large size of the hatchlings (~1 cm at hatching), making them easier to feed.

The advances described above led to renewed interest in the culture of small-egged, planktonic-hatching species such as *L. opalescens*. Understanding the many morphological and ecological processes that contribute to optimum survival and growth of hatchling squid is challenging. Thus, this study was designed to optimize survival of squid hatchlings, especially through tank design and feeding regimen. The objectives were: (1) to grow the hatchlings in a closed, recirculating tank system, modifying past tank system design; (2) to correlate morphological development of the hatchlings to feeding, thereby matching hunting capability with prey types; and (3) to quantitatively mix and feed a specific suite of prey types and sizes to same-aged but heterogeneously developing hatchlings.

Materials and methods

Experiments

Eggs of *Loligo opalescens* Berry, 1911 were collected from spawning grounds in California, USA, and air-shipped to the National Resource Center for Cephalopods in Galveston, Texas, USA. Five rearing experiments were conducted between 1998 and 1999. Details of the experimental design are listed in Table 1.

Experiments 1, 2, 3 and 5 tested hatchling survival on a mixture of food types composed of enriched *Artemia* sp. nauplii, wild zooplankton (mainly composed of various developmental stages of copepods) and mysid shrimp (*Americamysis almyra*) (Table 1). Experiment 4 (EX4) was designed to test hatchling survival on three food treatments: (a) enriched *Artemia* sp. nauplii, (b) zooplankton and (c) mixed: a mixture of a and b plus mysid shrimp (Table 2).

Tank system design

Squid eggs and hatchlings were kept in a closed, recirculating seawater system (Yang et al. 1989) consisting of seven 220-l cylindrical rearing tanks (0.95 m diameter×0.4 m height), an activated carbon filter (1.2 m length×0.61 m width×0.22 m height) and a submerged biofilter bed (1.9 m length×0.8 m width×0.4 m height) (Fig. 1a, b, c). The entire system held a volume of approximately 2,300 l. In the center of each rearing tank, a plastic core (15 cm diameter) covered with Nytex mesh netting (150 μm) was suspended 7 cm above the bottom of the tank to keep hatchlings and food organisms from being siphoned out of the tank (Fig. 1c). Water flowed out the core of the seven rearing tanks and was delivered through two pipes to the activated carbon filter (upper tank in Fig. 1b). Then the water went through a fine mesh netting (50 μm) positioned between two layers of a 5 μm polyester filter. Next the water flowed up through 21 l of activated carbon for reduction of suspended matter, dissolved organics and turbidity. This fluidized upwelling carbon bed increased the volume of activated carbon used by >40 times compared to previous rearing experiments (Yang et al. 1983a,b, 1986; Hanlon et al. 1987, 1989). Then, the water flowed by gravity through an oyster shell biofilter bed (5 cm deep) with an established bacterial flora to oxidize nitrogenous waste (lower tank in Fig. 1b). Water was then pumped from below the shell substrate through a 40 W ultraviolet (UV) sterilizer and a water chiller (Fig. 1b). The chilled water was returned to the rearing tanks via a 0.4 m long, 2.5 cm diameter spray bar (length equal to the radius of the tank) evenly perforated on one side (2.0 mm holes spaced 15 mm apart) and suspended slightly (2 cm) above the water surface (Fig. 1c). The spray bar generated a gentle counterclockwise current to the entire tank water column, allowing an even distribution of hatchlings and prey organisms.

The interior of the rearing tanks was painted flat black (Yang et al. 1983a). A rectangular glass window (18×9 cm) on the tank sidewall allowed visual observation. Hatchlings were exposed to both natural and artificial light at low intensities. Light intensities from the surface to the middle of the tanks ranged from 6.0 to 1.0 lx, respectively. During cloudy days, fluorescent bulbs covered with red plastic filters reproduced similar light conditions. Daily photoperiod was maintained consistently at 10 h light:14 h dark.

Water quality

Temperature and salinity were monitored daily. Nitrogenous compounds (ammonia NH_4-N, nitrite NO_2-N, nitrate NO_3-N) and pH were monitored weekly. Ammonia was determined using the method of Solorzano, nitrite by the Shinn method (Strickland and Parsons 1972) and nitrate with pre-packaged reagents (Hach Nitro Ver, Loveland, Colo.). Temperature was held at 16°C (±0.5) and salinity was kept between 30 and 35 PSU. Only natural sea water collected from offshore locations was used in the system. A 80–90% water change was executed after every two experiments to ensure that eggs and hatchlings were exposed to the proper types and concentrations of trace elements. Additionally, Wimex trace elements (Hawaiian Marine Imports, Houston, Tex.) were added to the system once every month (0.1 ml of trace elements l^{-1} water) to restore those removed by water filtration.

To control pH and nitrogen compounds the inflow of the water into the filter bed was covered with a fine mesh netting (50 μm) (Fig. 1b) to reduce the amount of organic matter added to the system. When heterotrophic production of CO_2 was reduced, it helped prevent variations in pH and nitrogen compounds. The mesh was cleaned daily to avoid clogging.

Table 1 Rearing details of the five experiments (*EX1–EX5*) conducted with *Loligo opalescens* hatchlings. Further details on EX4, see Table 2. The mixed food treatment was composed of zooplankton, enriched *Artemia* sp. nauplii and mysid shrimp (*Americamysis almyra*)

Date	EX1 Jun–Jul 1998	EX2 Jul–Aug 1998	EX3 Oct–Nov 1998	EX4 Jan–Feb 1999	EX5 Jul–Aug 1999
Duration (days)	45	60	60	50	60
Number of replicate tanks	5	6	7	6	3
Initial number of hatchlings $tank^{-1}$	1,000–2,000	1,000–3,000	800–1,500	1,000–2,000	1,000–2,000
Maximum number of hatchlings l^{-1}	9.0	13.6	6.8	9.0	9.0
Survival range (%)	45–57	51–59	42–55	36–45	48–60
Food treatment	Mixed	Mixed	Mixed	(1) Enriched *Artemia* sp. nauplii, (2) Zooplankton, (3) Mixed	Mixed

Table 2 Rearing details for experiment 4 (EX4) with *Loligo opalescens* hatchlings. The experiment was conducted between January and February 1999. The mixed food treatment was composed of zooplankton, enriched *Artemia* sp. nauplii and mysid shrimp (*Americamysis almyra*)

Food treatment	Enriched *Artemia* sp. nauplii		Zooplankton		Mixed	
Replicates	A	B	A	B	A	B
Initial number of hatchlings tank^{-1}	1,400	1,000	1,700	1,000	2,000	1,000
Survival at 30 days (%)	52	45	0	0	49	41
Survival at 50 days (%)	46	40	0	0	45	36

Fig. 1a–c Recirculating seawater system designed to optimize survival in hatchling squid consisting of: **a** seven 220-l circular tanks; **b** filtration, cooling and sterilization system. The tanks empty through two pipes into a polyester batting material and a 50 μm Nytex mesh netting (*nm*). Water then upwells through an activated carbon bed (*ac*) and lastly flows down through a oyster shell biological filter bed (*os*). While in this area, the water passes through an water chiller (*wc*) and a UV sterilizer (not pictured) before returning to the tanks. **c** The water outflow from the spray bar imparts a counterclockwise rotation to the entire water column within the tank. The center core is covered with 150 μm mesh netting

Egg care

During embryonic development, the eggs were exposed to stable temperature, salinity, pH and levels of nitrogen compounds. Low light intensities were maintained during the day with a consistent photoperiod. Egg strands were kept in one of the seven rearing tanks until hatching.

Survival

After hatching the squid were distributed, one-by-one, among the other tanks via a small round glass flask, 6–16 h after hatching. This distribution procedure was time-consuming; however, it allowed for quantification of the initial number and age of the squid with minimal stress and mortality. Estimating the initial population based on the number of embryos per egg strand was highly inaccurate due to the three-dimensional arrangement of the eggs in the egg strand, to the variable egg case length and to the hatching rate per strand.

Every other day a small diameter tube (0.5 cm) was used to siphon dead squid and uneaten food from the Nytex mesh at the center core and the bottom of the tanks. Mortality was estimated by counting the number of dead hatchlings among this debris. Daily survival was calculated as the percentage of the number of live hatchlings that were left in each tank compared with the number alive on the first day of the experiment. At the end of the experiments, errors in survival counts were $<7\%$.

Current speed and inflow rate

In preliminary experiments with these smaller rearing tanks, high current speeds were observed to be deleterious; therefore, before the experiments started three intermediate values were tested to determine which one would support optimum survival. Three groups of newly hatched squid were kept in three different tanks with inflow rates of 4, 8 and 12 l min^{-1} that generated current speeds of 0.8, 1.7 and 2.5 cm s^{-1}, respectively. Current speed was measured with a flowmeter (model 2000 Flo-Mate, Marsh-McBirney, Frederick, Md.).

Food and feeding

The *Artemia* sp. nauplii were enriched with SUPER SELCO (SELCO, Gent, Belgium) before feedings at 12 and 24 h after hatching. Two to three times a week, mysids and wild zooplankton were collected from Galveston Bay (Texas) and acclimated to the temperature and salinity of the tanks.

In previous rearing attempts squid were fed several times a day (Yang et al. 1980; Turk et al. 1986; Hanlon et al. 1987), but actual effects of feeding frequency on survival were not quantified. Because survival rates were low during these reports, we quantified the effects of feeding frequency. A feeding incidence (*FI*) was calculated and defined as:

$$FI = \frac{\text{number of hatchlings with food in their arms}}{\text{number of hatchlings in the tank}}$$

Feeding incidence counts were performed 30 min before (pre-feeding counts) and 30 min after (post-feeding counts) the first feeding of the day for experimental days 16–39 during EX2. Each hatchling passing the viewing window was counted and categorized as either with or without food in its arms. Each counting period lasted 2 min, which was the mean time required for a hatchling to complete a full circuit of the tank. Ten 2-min circuits were averaged to constitute one feeding incidence count. Feeding incidence data were arcsine-transformed for statistical analysis (Zar 1996).

Prey organisms: composition, size and density

Prey density was also determined before and after the first feeding of the day and was done a few minutes before the feeding incidence counts. To determine zooplankton and *Artemia* sp. nauplii density, five 100-ml aliquots from the rearing tanks were sampled at random, the number of zooplankton and nauplii in the aliquot were counted, and the counts were averaged.

To determine mysid density, the number of mysids in the tanks (pre-feeding count) was added to the number of mysids added during feeding (post-feeding count). The average of the five zooplankton and *Artemia* sp. nauplii counts was added to the mysid count to obtain the total prey density.

During all experiments, special care was taken to keep prey density between 50 and 200 prey l^{-1}. If prey density appeared high before any feeding, counts were made; if the values obtained were approximately 150 prey l^{-1} or more, then no food was added until the next feeding. Mean prey density ranged from 70 to 100 prey l^{-1}. These values were obtained a few minutes after feeding. Therefore, they may slightly underestimate prey density at feeding because the hatchlings promptly captured prey as soon as they were added into the tanks.

By observing the hatchlings during prey capture it was possible to distinguish some prey types and copepod species by their size, shape and swimming behavior. When a hatchling pursued and captured a prey that could not be identified, the hatchling was taken from the tank and held in a small glass container. In this container, the squid usually released the prey that was then identified under a compound microscope.

Results

Water quality

Ammonia levels obtained were between 0 and 0.02 ppm (recommended <0.10 ppm), nitrite between 0.002 and 0.008 ppm (recommended <0.10 ppm) and nitrate between 2.0 and 5.5 ppm (recommended <20 ppm) (for tolerance levels see Yang et al. 1989; Lee et al. 1994). The pH remained between 8.1 and 8.4 (recommended >8.0).

Egg care

Excellent hatching success was obtained by carefully tying egg strands in bunches of 10–20 with cotton string, then suspending them in the water underneath the spray bars. This exposed the eggs to adequate levels of dissolved oxygen that, in conjunction with proper current speed ($\sim$1.0 cm s^{-1}), ensured good aeration between the strands. High current speeds damaged the eggs and at late embryonic stages provided mechanical stimuli that caused premature hatching. Low speeds reduced aeration between the egg strands and led to death of the embryos.

During EX2 and EX5 (Table 1), some eggs collected in the wild were infested with the polychaete worm *Capitella ovincola* (Fig. 2). These worms are present in the sand where the eggs are laid, but they are not clearly visible when the squid eggs are in early embryonic stages. The worms migrate from the sand and spread among the egg strands, there they grow very fast while probably feeding on microorganisms in the intermediate gelatinous envelope of the egg strands. At first the worms did not seem to disturb the embryos. However, over time the thickness of the external gelatinous envelope was greatly reduced, leading to its deterioration, which exposed the eggs and caused premature hatching. These squid hatched with visible external yolk sacs and limited swimming ability. They died shortly after hatching. Such high incidence of premature hatching was never observed in non-infested egg strands.

The easiest way to avoid contamination was to remove any vestige of sand from the egg strands. This was done by carefully cutting the apical tip of each egg strand, and then tying them in bunches with cotton string. If some contamination occurred the contaminated egg strands were removed to avoid further spread of the worms.

Survival

Survival ranged from 36% to 60% (Table 1). Highest survival occurred during summer months (June–August) (EX1, 2, 5) and lowest during winter (January, February) (EX4) ($P = 0.003$, t-test) (Table 1).

Survival for the experiment that tested three food treatments (EX4; Tables 1, 2) is shown in Fig. 3. Highest mortality occurred within 10–15 days after hatching (Fig. 3a, c). During this period, many hatchlings died before capturing their first prey (Vidal et al., in press). Survival typically leveled off after this period and thereafter remained high until days 45–50, when it again

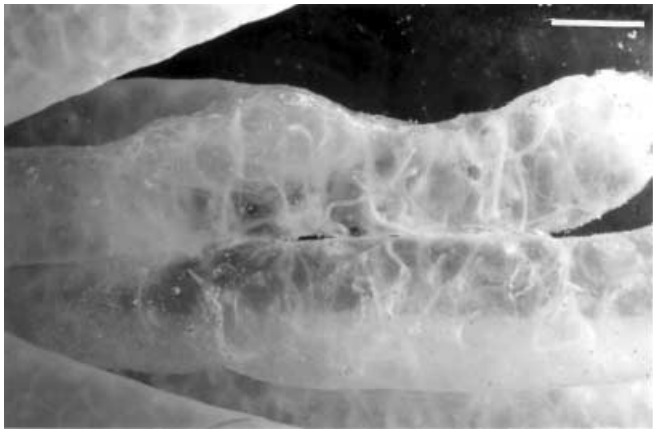

Fig. 2 Polychaete worm (*Capitella ovincola*) occupying the external envelop of the egg strands of *Loligo opalescens*. *Scale bar*: 1 cm

decreased. This pattern was obvious during all the experiments.

During EX4, the food treatment composed exclusively of enriched *Artemia* sp. nauplii was responsible for the best survival, 46% and 40% to day 50 (Fig. 3a; Table 2). The zooplankton treatment produced the lowest survival, with high mortalities from days 1 to 20; no hatchling survived pass day 30 (Fig. 3b). The mixed food treatment yielded the second highest survival, 45% and 36% (Fig. 3c; Table 2). However, because better survival (up to 59%; Table 1) was obtained with the mixed food treatment in the other experiments (EX1, 2, 3), this food treatment was used in EX5.

Current speed and inflow rate

A low-speed current layer caused by friction and minimum angular velocity occurred close to the tank walls. In this layer, even early hatchlings held their position against the current, but in so doing constantly rubbed their skin and fins against the wall.

After a period of 30 days, survival results from the current speeds of 0.8, 1.7 and 2.5 cm s^{-1} were 72%, 57% and 31%, respectively (Fig. 4). From the examination of dead hatchlings collected from the experimental tanks, it was observed that squid exposed to the highest current speed (2.5 cm s^{-1}), generated by an inflow of 12 l min^{-1}, had the highest incidence of fin damage. Initial damage on the fins was noticed from 7 to 12 days after hatching. The flow generated by the spray bars was counterclockwise; therefore, the right fin showed greater abrasion, sometimes being rubbed completely off (Fig. 5). In some cases, the right tentacles and the longest pair of arms (third) also were abraded (Fig. 5). Hatchlings under these conditions could not maintain their normal swimming orientation and, consequently, could not properly capture food. These hatchlings died of either starvation or infection.

Best survival was obtained at an inflow rate of 5.7 l min^{-1} ($\pm$0.44 SE, $n = 10$), producing a current speed of 1.0–1.4 cm s^{-1}. A complete rotation of water in the tank occurred every 2 min ($\pm$0.4 SE, $n = 10$). This promoted a relatively even distribution of hatchlings and their prey, and efficient predator–prey interactions. The water surface was kept as undisturbed as possible because turbulence reverberated from the walls, affecting the hatchlings' swimming behavior. To obtain such an effect, the inflow water was delivered through spray bars that were positioned very near to the water surface (2–3 cm) at intermediate angles (40–50°) (Fig. 1c). Air bubbles should be avoided because they adhered to either the surface of the egg strands or to the hatchlings' skin, which leads to death.

In general, two scenarios resulted from the extremes of current speeds. At high current speed (>2.5 cm s^{-1}), the hatchlings concentrated near the surface of the tank. Thus, when they passed by the water outflowing from the spray bars they were strongly propelled towards the bottom. This process was repeated almost twice every minute, and damaged their skin and interrupted prey detection and capture. At low current speed (<0.8 cm

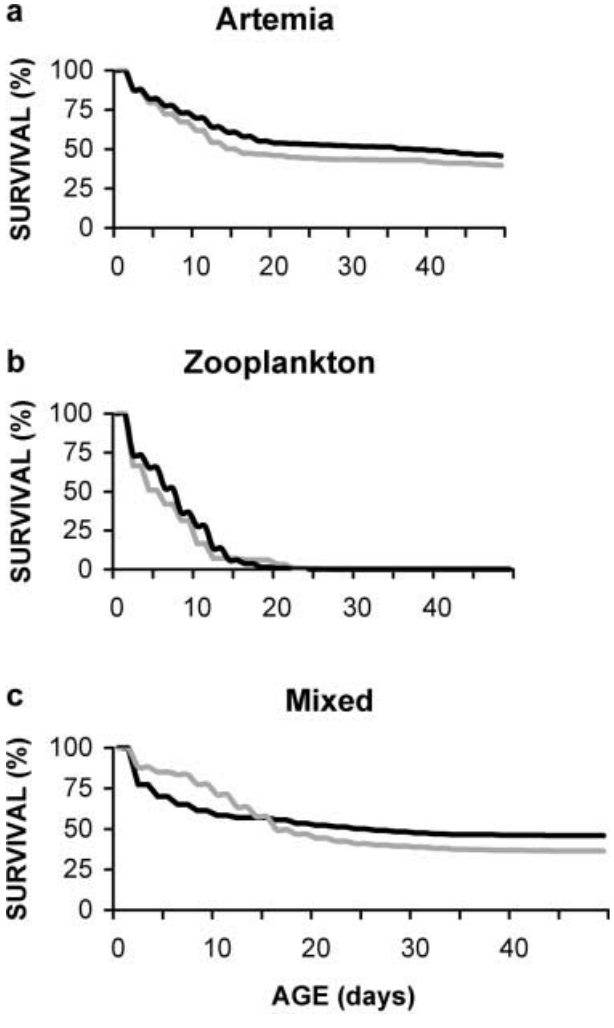

Fig. 3a–c *Loligo opalescens*. Survival of hatchlings raised for 50 days on the following food treatments: **a** enriched *Artemia* sp. nauplii, **b** zooplankton and **c** mixed: a mixture of a and b plus mysid shrimp. *Each line* represents one replicate

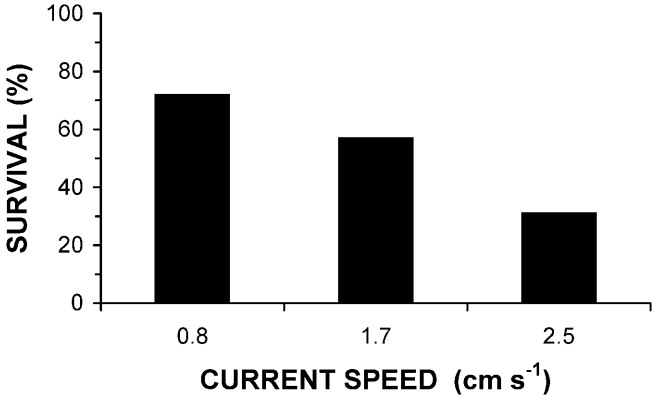

Fig. 4 *Loligo opalescens*. Survival of hatchlings raised for 30 days at three current speeds

Fig. 5 *Loligo opalescens*. Twenty-day-old hatchling showing the right fin and the right tentacle abraded due to constant friction against the tank wall. *Scale bar*: 1 mm

s^{-1}), the hatchlings either swam against the current parallel to the walls of the tanks or went to the bottom of the tank. The main problem caused by low current speed was segregation of hatchlings and their food. While the hatchlings concentrated close to the bottom, the prey organisms (*Artemia* sp. nauplii and zooplankton) displayed a patchy distribution near the surface. This reduced the encounter probabilities between squid and prey.

Food and feeding

During the EX1, EX2, EX3 and EX5 enriched *Artemia* sp. nauplii supported hatchling survival when availability of zooplankton and/or mysid shrimp was low. Owing to its easy preparation in the laboratory, enriched *Artemia* sp. nauplii was the food type present at all times during the 30–40 days post-hatching, when hatchlings clearly showed preference for larger prey sizes.

Post-feeding incidence counts were significantly higher than the pre-feeding counts (Mann–Whitney, *U*-test, $P < 0.05$) (Fig. 6). Addition of food several times a day stimulated feeding even when prey density was already relatively high (Fig. 6). Once the feeding rate was increased, survival improved.

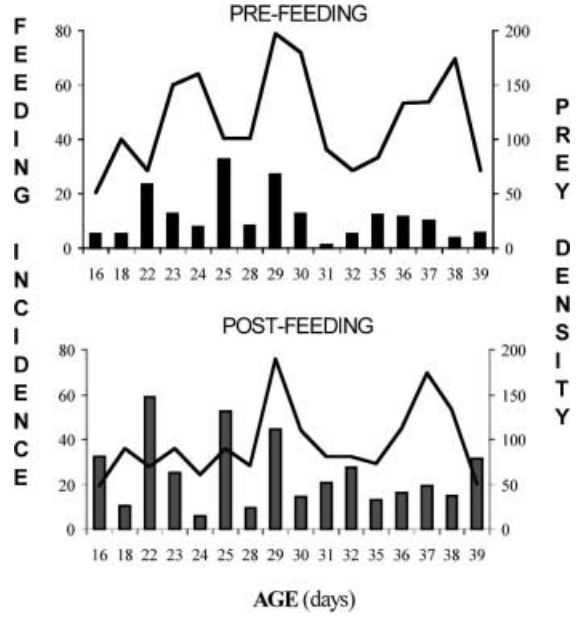

Fig. 6 *Loligo opalescens*. Feeding incidence of hatchlings from days 16–39. The *histograms* represent feeding incidence (%), and the *line* represents prey density (number of prey l^{-1}). Pre-feeding incidence was different than post-feeding incidence ($P < 0.05$, $n = 32$)

Prey organisms: composition, size and density

Prey items ingested by the hatchlings during the experiments are listed in Table 3. Hatchlings captured and ingested prey of a wide range of sizes, shapes and swimming abilities; including relatively small prey, such as enriched *Artemia* sp. nauplii, other crustacean nauplii, copepod nauplii and copepodites. These smaller organisms (0.2–1.0 mm total length) were preyed upon heavily by newly hatched squid, that ranged in size from 2.3–2.8 mm mantle length (Vidal et al., in press). These hatchings were also able to capture large prey, such as mysids of about 5.0–7.0 mm total length.

Discussion

Tank system design

The major modifications in rearing tank design compared to previous rearing systems were: (1) reduction of the tank size from 1,300 l (1.8×0.75 m) to 220 l (0.95×0.4 m), (2) utilization of a finer mesh netting in the center core (150 μm instead of 333 μm) and (3) raising the center core off the tank bottom.

One of the major challenges in culturing squid hatchlings is to provide food in the necessary quantity, quality and consistency. A smaller tank requires less food to maintain appropriate food density, and a relatively low-speed current to homogeneously distribute the food organisms. These advantages make a small tank

Table 3 Composition, size and developmental stage of some prey organisms ingested by *Loligo opalescens* hatchlings

Phylum	Class	Subclass	Order	Species	Developmental stage	Size (mm)
Arthropoda	Crustacea	Branchiopoda		*Artemia* sp.	Nauplii	0.3–0.7
			Cladocera	*Evadne* sp.	Adults	0.6–1.0
		Cirripedia			Nauplii	0.2–0.5
		Copepoda		*Acartia tonsa*	Nauplii and copepodites	0.2–0.5
					Adults	0.8–1.2
				Acartia lilljeborgi	Adults	1.0–1.3
				Anomalocera ornata	Adults	2.0–3.0
				Calanopia sp.	Adults	1.2–1.5
				Centropages velificatus	Adults	1.4–1.8
				Corycaeus spp.	Adults	0.8–1.1
				Eucalanus pileatus	Adults	1.7–2.1
				Euterpina acutifrons	Adults	0.5–0.7
				Labidocera aestiva	Copepodites	0.8–1.2
					Adults	1.5–2.5
				Paracalanus spp.	Adults	0.5–1.0
				Pontella spp.	Adults	3.3–4.1
				Temora styilifera	Copepodites	0.4–0.6
					Adults	1.2–1.6
				Temora turbinata	Nauplii and copepodites	0.3–0.7
					Adults	1.1–1.6
		Malacostraca	Mysidacea	*Americamysis almyra*	Hatchlings, juveniles and adults	2.0–11.0
			Decapoda (Anomura)		Zoea	7.0–10.0
			Decapoda (Brachyura)		Zoea and mysis	2.0–5.0
Chaetognatha						9.0–13.0

adequate for the hatchlings as long as it has a minimum height of about 30 cm to provide swimming space. Additionally, even if availability of food was not a problem, a larger tank (Yang et al. 1983a,b, 1989; Turk et al. 1986; Hanlon et al. 1987, 1989) required a higher current speed and thus a larger mesh size in the center core to accomplish reasonable exchange rates. These conditions strongly affected *Loligo opalescens* hatchling survival (see the following sections).

The size mesh to cover the center core determines the minimum prey sizes retained in the tanks. A 150 μm mesh size proved to be small enough to retain small prey within the tank, yet large enough to avoid rapid clogging. The mesh was also cleaned almost daily to avoid clogging. Major problems such as raising water level, altering current speed and reducing water exchange rate can result from clogging.

Suspending the core above the bottom of the tank reduced mortality. Previous designs made it difficult to siphon dead squid from the bottom without siphoning healthy hatchlings that were swimming nearby. A zone of reduced current was produced underneath the suspended core. Thus, most detritus and mortalities settled in this zone, making cleaning easier and reducing mortality imposed by cleaning.

The homogeneous flat black color used on the tank walls improved the hatchlings' feeding. As reported before (Yang et al. 1983a), the black color reduced reflectance of light and increased the visual contrast of prey organisms against the walls.

Water quality

All species and ages of cephalopods require pristine water quality, and this is particularly true for eggs and hatchlings. Past rearing experiments reported pH values as low as 7.4 (Hanlon et al. 1983, 1987, 1989; Yang et al. 1983b; Turk et al. 1986). In preliminary experiments we observed that pH values outside of the 8.1–8.4 range increased mortality. Besides, the addition of sodium bicarbonate to regulate the pH, used in several previous reports (Yang et al. 1980, 1989; Hanlon et al. 1987), can promote uncontrolled fluctuations in the pH, stressing the hatchlings and leading to mortality. Other water quality parameters in the present study were kept within the limits proposed by other researchers (Yang et al. 1989; Lee et al. 1994, 1998, 2000).

The use of large amounts of activated carbon in an upwelling system may have been one of the major changes that promoted good water quality. The water welled up through the carbon bed causing the carbon to fluidize to some extent. Gentle agitation within the bed is most significant because it resulted in: (1) self-cleaning of the carbon particles by gentle agitation; (2) an increase in the useful life of the carbon, decreasing frequency of replacement; and (3) an increase in the adsorptive surface area of unpacked carbon particles. All the previous work on squid hatchlings had used closed plastic cylinders of carbon (Yang et al. 1983a,b, 1986; Hanlon et al. 1987, 1989; Turk et al. 1986). These carbon cylinders caused carbon packing, trapping of suspended solids,

formation of microbial assemblages and a decrease in water flow (Turk and Lee 1991). The present study was the first to incorporate a gently agitating, high-volume (3.4×10^{-3} kg carbon l^{-1} system volume), open carbon bed. Improving water quality with a high-volume, upwelling carbon bed likely improved survival.

Egg care

Egg care was the first step in rearing success. Temperature, salinity and nitrogen compounds should be kept as stable as possible during embryonic development. Abrupt changes in luminosity caused premature hatching during late embryonic stages (stages 28–30, Arnold 1965). Since hatching occurs mainly at night or in the dark, sometimes taking several days, exposing late stage embryos to 24 h of constant light, even at low intensities, can postpone hatching for a few days. Then, massive hatching will occur when the illumination is reduced. This is not advisable since squid will hatch with their internal yolk reserve almost depleted, resulting in higher mortality rates (Vidal et al., in press).

During embryonic development, the eggs produce large amounts of ammonia and require high levels of dissolved oxygen (Hanlon 1990). Thus, the practice of suspending the egg strands in highly aerated water was critical for the survival of the embryos.

Eggs collected in the wild were sometimes infested with the polychaete worm *Capitella ovincola* (Fig. 3). Infestation of egg strands with capitellid polychaetes has been reported before (McGowan 1954; Fields 1965). However, infestation was never before related to deterioration of egg strands or to premature hatching and subsequent high mortality. A similar deterioration of egg strands was reported for cultured *Sepioteuthis lessoniana* (Lee et al. 1994). This egg deterioration was believed to be caused by protozoans and microinvertebrate epibionts, not by capitellid polychaetes. It is likely that in both cases deterioration occurred as a result of microbial degradation of the gelatinous matrix of the external egg envelope. Microbes can convert the gelatinous matrix into a rich source of food for these worms and for epibiont organisms.

Survival

Survival of the hatchlings was closely related to food availability. Highest survival was obtained during the summer when a variety of food types were readily available (Table 1). Highest mortality (0–15 days after hatching) coincided with the critical transition between full yolk absorption and successful prey capture (Vidal et al., in press). The cause of the second mortality period that typically occurs after days 45–50 had not previously been determined (Hanlon et al. 1983). The present study suggests that it is caused primarily by the lack of the appropriate-sized food and by acute fin damage. By day 45–50, hatchlings in the current experiments had a mean mantle length of about 7–8 mm and required higher daily food intake and larger prey such as fish larvae.

Results from the present study utilizing a food treatment exclusively of enriched *Artemia* sp. nauplii were contrary to several previous studies that reported *Artemia* sp. nauplii as unsuitable prey for squid hatchlings (Hanlon et al. 1979; Yang et al. 1980, 1983b; Boletzky and Hanlon 1983). The positive results obtained with enriched *Artemia* sp. nauplii may be due to improvement of the nutritional content of the nauplii by enrichment with highly unsaturated fatty acids, as well as the increase in the number of feedings per day and in prey density. The low survival rates obtained with the zooplankton food treatment were partially due to the low availability of zooplankton during some period of the experiment.

Based on the results reported in the current experiments survival was improved to 60% during the first 60 days post-hatching. This is a dramatic improvement when compared with the best previous results of 25% for *L. opalescens* and <20% for the other *Loligo* spp. during the same time period (Fig. 7a). Figure 7b illustrates that even limited survival of the hatchlings in the laboratory has not always been possible. In previous rearing attempts, survival rates of only 1–10% were common during the first 15 days post-hatching (Fig. 7b). Survival in the least successful of the experiments of this study was not only better than the best survival results from previous rearing experiments with *L. opalescens*, but it was better than any survival results

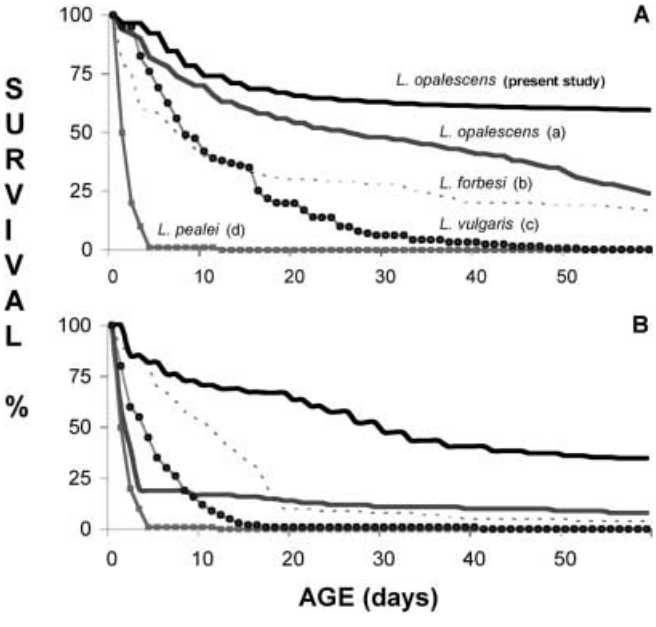

Fig. 7a, b *Loligo* spp. Survival of hatchlings during 60 days of mariculture. **a** Most successful rearing attempts. **b** Least successful rearing attempts [*(a)* Yang et al. 1986; *(b)* Hanlon et al. 1989; *(c)* Turk et al. 1986; *(d)* Hanlon et al. 1987]

ever attained for any experiments with *Loligo* spp. squid (Fig. 7b). While the emphasis of this study was survival through the critical post-hatching phase, population growth rates were around 7.4% and 6.4% body dry weight day^{-1} for the mixed food treatment during the first 20 days after hatching at 16°C (Vidal et al., in press). Mantle length increased at a rate of 2.4% day^{-1} for the same time period.

Current speed and inflow rate

Once appropriate prey of requisite size and type are offered in adequate densities, the key factor that ensures good interaction between the hatchlings and their prey is proper current speed and low surface turbulence. Current speed is a function of both water inflow and outflow rate and was one of the most important factors determining distribution of prey, feeding success and, thus, survival of the hatchlings.

Although previous studies recommend agitation of the water surface as a mandatory precaution to avoid trapping hatchlings by surface tension (Hanlon et al. 1979; Yang et al. 1989), our results indicate that this can increase mortality and should be avoided. Additionally, it has been shown that intermittent variations in turbulence trigger an escape response by copepods that will influence prey capture (Hwang and Strickler 1994). In this study, low surface turbulence conditions were achieved with current speeds of 1.0–1.4 cm s^{-1} and inflow rates of about 5.7 l min^{-1}. In previous rearing attempts larger diameter tanks were used, calling for the utilization of stronger inflow rates (>16 l min^{-1}), current speeds and surface agitation (Hanlon et al. 1983, 1987; Yang et al. 1983b, 1986, 1989). These factors alone can explain low survival rates due to skin and fin damage. Skin and fin damage caused by friction against the tank walls is still the major cause of mortality. Although this problem is not completely solved, it can be greatly reduced based on the above considerations.

Food and feeding

Hatchlings were not attracted to uneaten prey surviving in the tanks. Thus, the addition of excess food reduced subsequent feedings, thereby decreasing survival. Accordingly, when prey density values were above 150 prey l^{-1}, subsequent feeding incidence values were reduced (Fig. 6). This underscores the importance of feeding small amounts of food several times a day. It was not well understood why hatchlings would preferentially capture fresh prey added to the tanks instead of the surviving prey. One possible explanation is the visual stimulus provided by the addition of prey organisms concentrated in a patch before dispersing. Another possibility is the condition of the prey. Unless food was provided to prey organisms, they would be near starvation after a few days in the rearing tank and have lower nutritional value. It was shown that *Acartia tonsa* copepods significantly lose their nutritional value after being deprived of food for a few days (Saiz et al. 1998). The same is probably true for enriched *Artemia* sp. nauplii; if not eaten 12 h after being harvested (36 h after hatching), they would contain significantly less nutritional value. Hatchling squid may distinguish healthy from starved prey based on several cues, such as swimming patterns or chemoreception. Nonetheless, it was observed that mysids and some pontellid copepods fed on *Artemia* sp. nauplii. Uneaten mysids in the tanks grew larger, making their capture by the hatchlings less likely. Thus, starvation of the prey organisms was not always the case, but must not be overlooked. Prey availability is essential, but prey quality is probably just as important for larval growth and could partially explain our higher survival rates compared to the previous reports.

Prey organisms: composition, size and density

Information essential to understanding and projecting larval survival includes the optimal size, type and concentration of prey organisms (Pechenik et al. 1990; Rumrill 1990). However, this information is limited for wild hatchling squid. The few studies undertaken have indicated that copepod and crustacean larvae may represent important components of the squids' diet (Vecchione 1991; Vidal and Haimovici 1998), and this has also been supported by laboratory studies (Boletzky and Hanlon 1983; Yang et al. 1986; Hanlon et al. 1991; Chen et al. 1996; Villanueva 1994). It is well known that both adult and juvenile squid are able to capture prey much larger than their own size (Hanlon and Messenger 1996). However, as a result of variable size at hatching (Vidal et al., in press), there was a wide variation in the hatchlings' ability to capture prey (Yang et al. 1986). Within the same cohort some hatchlings grew quickly and were able to capture larger prey (mysids), while others grew slowly and depended upon smaller prey (i.e. enriched *Artemia* sp. nauplii and developmental stages of copepods and other zooplankton), ranging in size from 0.2 to 1.0 mm (Table 3). Therefore, it was essential to supply smaller prey during the first weeks after hatching. Later, feeding a variety of prey of different sizes and species was important so as to match the different sizes and hunting abilities of same-aged but heterogeneously developing hatchlings.

While high prey densities can accelerate feeding and growth, there is a threshold of prey density at which growth reaches a maximum (Boidron-Metairon 1995). We believed that the prey densities offered to the hatchlings were close to this threshold. Even though the number of hatchlings per tank diminished during the duration of the experiments, prey density was maintained above 50 prey l^{-1}. Decreasing prey density would lessen the encounter probabilities between squid and prey, thus reducing feeding. Previous rearing attempts with *L. opalescens* have reported prey density values

between 12 and 24 prey l^{-1} (Yang et al. 1983a, 1986) that were >50% below the minimum value recommended (50 prey l^{-1}) in the current study.

Recommendations for optimizing squid hatchling survival

The results of the present study significantly improved rearing success for squid hatchlings and offered insight into their early life history. Recommendations derived from the present study and previous research with squid hatchlings include:

1. Keep eggs well aerated and carefully monitor development. Reducing aeration between the egg strands causes death to the embryos.
2. Use a small circular tank. A small circular tank (about 1.0 m diameter and 150–400 l volume) with a minimum height of 30 cm is best for optimizing survival of squid hatchlings.
3. Optimize current speed. Current speeds between 1.0 and 1.4 cm s^{-1} appear to be ideal.
4. Match the core's mesh size to the species' hatching size as well as the prey. A 150 μm mesh is ideal for intermediate-sized hatchlings.
5. Suspend the tank's core. Suspend the center core above the bottom of the tank to improve cleaning and to decrease mortality caused by cleaning.
6. Maintain pH within narrow limits (8.1–8.4). This can be accomplished by removing organic matter from the recirculating system using a fine mesh particle filter, siphoning frequently or employing a protein skimmer.
7. Stabilize water quality parameters (i.e. pH, temperature, salinity and nitrogen components). During embryonic development, water quality parameters should be held as stable as possible to avoid low hatching rates and/or premature hatching. Water quality continues to be important during hatching and through the first 2 months.
8. Control lighting. Light levels should be low (<6.0 lx) and constant during the day, and the photoperiod should be consistent.
9. Color the tank bottom and inside wall flat black. To reduce reflectance of light and increase the visual contrast of prey organisms.
10. Keep prey density above 50 prey l^{-1}. Increasing prey density increases encounters between squid and prey, improving feeding and survival.
11. Feed small, frequent amounts of prey. Feed small amounts of prey at regular intervals (every 2–3 h) during the day instead of overloading the tanks once a day.
12. Feed a variety of prey. Feeding a variety of prey is important so as to provide prey to heterogeneously growing hatchlings with different hunting abilities.
13. Feed enriched *Artemia* sp. nauplii during days 1–30. To overcome problems of prey availability, the utilization of enriched *Artemia* sp. nauplii is of major importance. The nutritional content of the nauplii must be improved by enrichment with highly unsaturated fatty acids.

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