FACILITIES AND METHODOLOGY FOR THE CULTURE OF THE SOUTHERN SEA BASS (*Centropristis melana*)[1]

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ABSTRACT

A closed system was designed for mass culture of marine finfish larvae. Southern sea bass (*Centropristis melana*) brood stock were collected in the Gulf of Mexico during January-March, 1975, their natural spawning season. Brood stock were maintained in a simulated spawning environment of 17-19°C temperature and 10 hr L:14 hr D photoperiod. Vitellogenesis of ovarian oocytes remained in the tertiary yolk globule stage in females held under these conditions and males would release milt freely upon manual stimulation. Final oocyte maturation and spawning were induced with Human Chorionic Gonadotropin and manual spawning techniques. Approximately 2,500 larvae were reared in three trials using 2,000-liter silo tanks with biological filtration. Laboratory reared rotifers (*Brachionus plicatilis*) were solely used as food the first 4 days after feeding began. Wild plankton, rotifers, *Artemia nauplii*, and prepared flake were also used as larvae developed. Greatest larval survival from hatching was 10.0%. Larvae grew from 2.78 mm to 9.0 mm in 24 days; the greatest growth rate occurred after day 14. Physiological edema was observed in two trials affecting 30-50% of the larvae. Physiological edema appears to be the principal constraint to successful rearing of sea bass larvae.

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INTRODUCTION

In the current period of renewed interests in marine finfish propagation, numerous researchers have stressed the importance of the timely development of the art. Many approaches to technological advancement have been described in the literature. Techniques are usually developed for the species in point; that is, systems are designed to simulate the necessary parameters of a desirable species.

The approach presented in this investigation employs the concept of versatility in design. The larval system described herein will test the mass culture potential of various species of estuarine, coastal, and offshore pelagic spawning finfish. Contributory information on spawning and larval rearing of the southern sea bass [Centropristis melana (Linné)] is also presented.

Many investigators have successfully induced spawning and reared the larvae of marine finfish in various systems, primarily for taxonomic studies. Few reports, however, deal specifically with mass culture techniques. Hoff et al. (in press) present an adequate review of gonadal maturation and spawning under laboratory conditions, as well as defining optimum spawning technique for the Florida pompano, [Trachinotus carolinus (Linné)] and Centropristis melana. May (1975) describes a method of collection, induced maturation, and spawning of already ripe Gulf croaker [Bairdiella icistia (Jordan & Gilbert)]. Kuo et al. (1974), in a series of sixteen experiments, describe effects of temperature and photoperiod on oocyte development in captive grey mullet, [Mugil cephalus (Linné)]. Nash et al. (1974) outline a method for rearing larvae of the grey mullet in mass using Kriesel tanks. Houde and Ramsay (1971) describe a Kriesel rearing tank (1,326.5-liter polyethelene container) used to successfully rear a list of species; however, no data was presented. Frakes et al. (in press) describe a single successful rearing attempt using a silo tank with biological filtration to mass rear larvae of southern sea bass.

This report describes additional research and application of techniques in rearing marine finfish developed and described by Hoff et al. (in press) and Frakes et al. (in press). The present study incorporates modifications and improvements in equipment and technique. The experiments were designed to test systems and techniques involved in rearing sea bass from the embryo through the larval period (approximately 24 days after hatching).

LABORATORY FACILITIES

Introduction

The Florida Department of Natural Resources maintains mariculture facilities at its Marine Research Laboratory located on Bayboro Harbor in St. Petersburg, Florida. Bayboro Harbor, a finger of Tampa Bay, is subject to

considerable salinity fluctuations and has a large quantity of suspended material in the water column. For these reasons, the mariculture facilities are quasi - closed systems using limited water exchange.

Facilities include one fiberglass solarium and five temperature-photoperiod controlled tank rooms with self-contained filtration systems. Hoff et al. (in press) give a complete description of these systems.

Larval Rearing Laboratory

The 31.82 m^2 larval fish laboratory (Figures 1 and 2) is of masonry interior and concrete flooring. Wall construction is stucco, applied in two coats with a viscane vapor barrier below the stucco. Ceiling height is 3.66 m permitting the use of tall tanks. All plumbing is polyvinylchloride (PVC). There is overhead incandescent lighting for room illumination and experimental lighting is fluorescent full-spectrum Duro-Test Vita-Lites.[1] Photoperiod is controlled by Intermatic electric timers. The laboratory has ample sea, fresh, and deionized water supply lines. Electrical and aeration outlets are conveniently located adjacent to the tank and filter sites. Aeration is supplied from the main laboratory air supply, consisting of two Sutorbilt positive blower units (Model 3M) with a blower speed of 1,458 rpm. The units are driven by 2 hp electrical motors equipped with inlet filters and silencers. Each system is serviceable separately and is removable while the other is in operation. In case of a malfunction of one unit, the other unit automatically starts. Temperature is controlled by one 24,000 BTU reverse cycle air conditioner located near the ceiling. Working areas are accessible by a boardwalk surrounding the four filters and tank area. The boardwalk is built in removable sections for easy access to floor-level plumbing and tanks.

Filtration

Filtration consists of four basic units (Figures 2, 3). These units can be operated as single filters, as filters in parallel, or as a series of two or more. The basic unit is a concrete vault 3.5 x 0.96 x 0.5 m. Filters are drawn by conventional airlift and by pump. Airlift consists of a network of 2.54 cm PVC pipe below the false bottom. The pipe is drilled (1.0 cm) along its entire length. Water is drawn through the network and filter bed respectively. The filter bed consists of 1.25 cm grid fluorescent light louver material covered with 800 µm mesh plastic screen. Above this is 30.0 cm of 98% aragonite (particle size 1 mm^3-1 cm^3) which serves as biological bed and buffering medium. Volumetric capacity is 1,000 liter per unit. Flow rate by airlift is 36.0 liter min^{-1} and by pump is 24.0 liter min^{-1}, with total flow rate being approximately 0-60 liter min^{-1}.

[1]Reference to trade names does not imply endorsement by the Florida Department of Natural Resources Marine Research Laboratory.

This rate varies with filter-tank configuration. Filtered water flows from the filter to either a large manifold where it is distributed to the appropriate tank, or to a series line where it is delivered to the next filtration component. Flow rate into each tank can be adjusted by a valve. In minimal flow situations unused water from the filter is allowed to pass through an overflow standpipe and back into the filter delivery line. Initial rearing attempts employed only biological filtration. Filter bed cultures were inoculated initially from active filter bed media and were fed inorganic ammonia at an initial concentration of 12.0-20.0 mg liter^{-1}. As soon as nitrite reached 0.0 mg liter^{-1}, water was changed and filter beds were maintained on fish, squid, trout chow, and live invertebrates. Initial experiments used a one filter to one rearing tank configuration.

Rearing Environments

Rearing system design is similar to the silo described by Houde and Ramsay (1971) and Frakes et al. (in press). The aluminum silos used in this system (Figures 4, 5) employ a fiberglass inner liner 0.32 cm thick and a fiberglass cone bottom with a bottom slope of 15°. There is a detrital siphon (1.9 cm I.D.) fixed near the center drain. Water depth is maintained at approximately 1.8 m with an exterior overflow standpipe. Total capacity is 2,000 liters. Filtered seawater enters the silo from an overhead delivery manifold. Flow rate is controlled by a 2.54 cm ball valve, and interchangeable delivery designs are used. During one rearing attempt, filtered water entered the bottom of the silo in a circular path through a single delivery line. Another attempt used the same method with water delivered at surface level. Subsequently, the delivery line was reduced from 2.54 cm to 1.14 cm. This delivery line is divided into two lines using a "T" PVC connection with each line delivering a jet of water in opposite directions perpendicular to the water column. This apparatus is centrally located about halfway down the water colmn. The effect seems to be most gentle to the larvae, and it produces an even circular flow throughout the water column.

Tall drain filters 190.0 cm x 5.08 cm are used to increase surface area of the drain and thus reduce entrapment of larvae. The drain filter consists of one piece of 5.08 cm PVC pipe drilled with 1.27 cm holes. This is wrapped with a layer of PVC mesh surrounded by a layer of plastic or nylon screen. The mesh size of the nylon and plastic screen is increased as larval and food particle sizes increase. The entire unit is threaded on one end for attachment to the bulkhead fitting in the silo bottom. Window ports are located along the wall of the silo throughout the depth of the water column. All tanks and filters are equipped with overflow drain stand pipes to prevent spillage in case of a pump malfunction or clogged line.

Experimental lighting is suspended from the ceiling. Full-spectrum fluorescent lights are used to simulate daytime, and incandescant flood

lights are used intermittently. The height of the light source above the water surface is adjustable so that light intensity can be varied.

Food Culture System

A food culture room 8.0 x 3.73 x 3.68 m is located adjacent to the larval laboratory. Photoperiod and temperature are controlled by Intermatic timers and two reverse cycle air conditioners.

The room contains four identical food rearing systems (Figures 6, 7). Each system consists of an algal supply bank (eight 20-liter glass carboys) supported by a 269.0 x 25.0 cm waterproof trough. Approximately 1.0 m below the troughs are located two 269.0-liter aquaria 133.5 x 61.5 x 55.0 cm for the rearing of zooplankters. The zooplankton aquaria have front illumination from six bulbs through a 116.0 x 44.0 cm plexiglass window. Twenty-four hr lighting is provided by full-spectrum fluorescent light bulbs. Light intensity measures approximately 400 ft-c at the carboy and zooplankton tank surfaces.

Each system shares a common upper and lower air manifold with an adjacent back-to-back mirror image system. Air is received from the main laboratory air system.

Sea water is supplied from Bayboro Harbor. It is filtered through a diatomaceous earth filter and chlorinated to greater than 3.0 ppm in a reservoir. Chlorine is removed by 24 hr aeration. Water is pumped from the reservoir to the culture room as needed.

MATERIALS AND METHODS

One hundred twenty Centropristis melana brood stock (Table 1) were collected by hook and line in the Gulf of Mexico between January and March, 1975, during their natural spawning season. The fishing area was along St. Martin's Reef from Tarpon Springs to Hudson, Florida; the sea bass were usually caught along the base of underwater limestone outcroppings. Males were running ripe and females were in the vitellogenic stage of oocyte development. Despite the stress of capture, no females were found to regress to a resting stage of development.

The fish were placed in 120-liter plastic disposal containers and one live-well box 1.2 x 0.6 x 0.6 m on the boat. The sea bass were placed in an insulated and aerated live-well trailer 2.15 x 1.2 x 1.0 m for transportation from the boat to the laboratory. Upon arrival at the laboratory, the fish were placed in an environment of 10 hr light:14 hr dark, 34 ppt salinity, and 18 $\pm$ 1 C temperature. This regime approximated the natural environment at time of capture. Hoff et al. (in press) give a complete

description of the environmentally-controlled conditioning systems. The brood stock was tagged with Floy fingerling tags in tissues surrounding the second dorsal fin and anal fin spine. Fish were allowed to acclimate for two weeks prior to experimentation.

Fish were fed daily a diet of chopped squid and/or shrimp. The feed was fortified with a proprietary vitamin additive (Table 2). This supplement was added due to a chronic anemia previously found in sea bass maintained with no vitamin supplement.

All fish were anesthetized with 10.0 ppm Quinaldine before handling. Selection of potential female spawners was based on stage and size of ovarian oocytes as described by Hoff et al. (in press). Gonadal sampling of conditioned females was monitored using in vivo catheterization by a 3.0 ml syringe with a dull 10.0 cm catheter needle (Stevens, 1966). The needle was covered with 1.0 mm I.D. polyethylene tubing to protect the ovary and oviduct from excessive trauma. In order to prevent increased suction within the body cavity which could cause ovary damage, a small hole was drilled into the needle end of the syringe so that suction could be released as the catheter was being removed. Oocyte samples were placed on standard microscope slides. In order to reduce egg distortion, three microscope cover slips were placed on each side of the sample, followed by one cover slip placed over the sample. Oocyte stage determinations and measurements were done by using a binocular compound microscope with ocular micrometer. Diameters were measured on viable eggs only. Mean oocyte diameters for the entire sample (Stages III, IV, V, and VI only) were calculated the first day prior to injection and approximately 24 hr after the first injection. Mean diameters for the third day (time of spawning) represent the mean diameters of spawned eggs and not mean diamters of eggs in the overy itself. Photographs were taken using a Wild MK-II camera with either Polaroid or 135 mm film.

Females with predominately Stage IV oocytes were selected for hormone treatment for subsequent spawning. This method was similar to that used by Hoff et al. (in press). Veterinary grade Human Chorionic Gonadotropin (HCG, 2,500 IU amp) from Nutritional Biochemicals Corporation and sterile Sodium Chloride Injection from Cutter Laboratories were used for hormone treatments. Reconstitution was to 250 IU ml^{-1} and injection dosage was based on female body weight. The initial dosage was 0.55 IU g^{-1} body weight, and subsequent doses were 0.275 IU g^{-1} body weight. Injections were made intramuscularly into the anterior epaxial musculature with the needle positioned lengthwise beneath the scales. Following the first injection, treated females were placed into isolated holding tanks with ripe, non-injected males. These tanks were in the same room as the brood stock environmentally-controlled conditioning systems and connected to the same filtration system, therefore, allowing all water parameters to remain the same. Attempts were made to semi-naturally spawn (see Hoff et al., in press) the females. These were unsuccessful and strip-spawning was employed.

All males and females were anesthetized for strip-spawning. Females were manually stripped into large finger bowls containing sea water of the same temperature (17-19 C) and salinity (34 ppt) of the holding and conditioning systems. Eggs would sometimes be stripped into 23 C sea water to eliminate the acclimation period at transfer to the larval rearing system. This did not seem to adversely affect fertilization and subsequent development. Males were also manually milked for sperm. However, sperm was added immediately after stripping, at which time eggs and sperm were gently mixed. Time allotted for fertilization to occur was 20-25 min. At the conclusion of fertilization time, eggs were placed into 80-liter aquaria modified as continuous flow-through incubation chambers (Figure 8). These units allowed fecundity and fertilization determinations and provided excellent water quality during incubation. The chambers consisted of a 0.96 cm PVC drainage-standpipe system below the light louver material grid covered with 800 µm nylon mesh screen to prevent egg loss. The screen was sealed to the aquarium walls with marine silicone sealer. The chambers were placed directly into the filtration units of the larval rearing system; flow into these chambers was through 1.27 cm I.D. plastic tubing and regulated by means of a PVC ball valve connected to the filtration pump.

The rearing silos were filled to capacity (2,000 liters). Since filtration into the silos was not started until day 3 of larval rearing, 32-48 liters of Dunaliella or Nannochloris algae were added to the silos 24 hr before transferring the embryos to maintain water quality. At approximately 32-36 hr after fertilization, embryos were transferred to rearing silos. Silo stocking density varied, ranging from 14,700 to 51,000 embryos stocked in any one silo.

Temperature in the larval rearing laboratory was maintained at 21-23 C. Fluorescent full-spectrum lighting was continuous for the first 5 days of rearing. One incandescent 250 W flood lamp was used over each silo. All lighting was adjusted to 14 hr light:10 hr dark after day 5. Filtration rate was adjusted depending on water quality after day 3. Salinity was adjusted 34 ppt with Instant Ocean Synthetic Sea Salts. Nitrate levels were determined after the method of Kahn and Brezenski, 1967. Nitrite levels were determined by a modification of Strickland and Parsons (1968). Ammonia levels were determined after the methods of Solorzano (1969). Salinity was measured with a refractometer (American Optical). Water quality data are provided in Figures 14, 15 and 16.

Sampling for larval morphology studies was done three times per day for the first seven days, and once every other day from day 8 until day 24. Larvae were removed from the silo by beaker and preserved in Davidson's fixative. Larvae were periodically removed from the silo for feeding behavior determinations and live observations. Larvae were placed in 1.0 ml depression microscope slides, anesthetized with strands of menthol, and then observed under a dissecting scope. Many times it was possible to replace the anesthetized larvae without death occurring.

Food was offered on day 3. Laboratory-reared rotifers (Brachionus plicatilis) were fed from day 3 through day 6, with B. plicatilis, and wild plankton being fed from day 7 through day 13. Artemia nauplii, wild plankton, and TetraMin prepared flake were fed from day 14 until day 24. Food item concentration of at least 10.0 ml^{-1} was maintained. Filtration of rearing silos was stopped for the first 1-2 hr after addition of food.

Algal cultures were grown on an enriched seawater medium (Guilliard f/2 modified; McLachlan, 1973, Table 2-5). Pure cultures were obtained from agar plates. These were cultured in 250 ml Erlenmeyer flasks for approximately one week, then transferred to 2,800 ml Fernbach flasks for approximately another week. From these flasks, two 20-liter carboy cultures per flask were started. Algal density was initially calculated by hemacytometer. These counts were plotted against simultaneous optical density measurements (% transmission). All future measurements of algal density were interpolated from the resulting curve (Figure 9a). A typical growth curve for Dunaliella is given in Figure 9b. Rotifer cultures were started and maintained using a variation of the technique described by Theilacker and McMaster (1971). Carboy cultures (Figure 9b) were inoculated at 3.4×10^3 cells ml^{-1}. The culture was used for food when it reached 1.5×10^6 cells ml^{-1}. Rotifer aquaria was inoculated with algae at 6.0×10^5 cells ml^{-1}. Rotifers (initial inoculation density = 2.0×10^4 per tank) were added when the algal bloom approached 1.0×10^6 cells ml^{-1}. Rotifers would usually reach maximum density after 8-9 days. Stock cultures of rotifers and algae were maintained in a separate temperature and photoperiod control room.

Wild plankton was caught from Fort DeSoto Park, Pinellas County, Florida, by setting a 0.5 m diam plankton net with 64 µm mesh from a bridge during an incoming tide. Wild plankton was returned to the laboratory in aerated carboys and sieved to the appropriate size fraction. Metaframe San Francisco Bay Brand brine shrimp eggs were used. Frozen plankton and prepared flake were obtained from commercial pet supply dealers.

RESULTS AND DISCUSSION

Survival of collected brood stock was 100% from the dock to the laboratory. Some fish died shortly after capture on the boat. These mortalities were usually due to embolism or internal injury caused by the fish hook. Oocyte regression past the vitellogenic stage was never observed despite severe embolism. All the fish were small (Table 1). Size ranged from a female of 13.1 cm standard length (SL) to a male of 21.9 cm SL. The temperature and light regimen used to maintain these fish in a pre-spawning condition worked satisfactorily. Males were flowing milt and females were in an active reproductive state from January until September 1975. However, fish sampled in August and September began to show more variation in initial oocyte diameter and mean diameters were lower.

Shehadeh et al.(1973b) found that female grey mullet, Mugil cephalus, in the absence of continuous exogenous hormone treatment, would become atretic in approximately four weeks during the natural spawning season. Furthermore, they reported that mammalian gonadotropins successfully induced oocyte maturation in females over a long period of time (6 weeks) until mean oocyte diameter reached approximately 750 µm, after which time oocytes would become atretic. Temperature and photoperiod control in duplicating the natural spawning season prolonged vitellogenesis in the present study. No oocyte regression was noticed until late August, five months after the end of the natural spawning season of C. melana. Shehadeh et al. (1973b) also found that under exogenous hormone treatment vitellogenesis would proceed until a certain mean oocyte diameter was achieved. At this time, if there was no spawning stimulus, regression would begin in spite of hormone influence. Also in the same report, groups of fish under hormone treatment exhibited a continual decrease in standard deviations of mean oocyte diameters (s=30 µm) thus exhibiting uniformity in oocyte size as treatment progressed. The control group in that study exhibited large standard deviations (s=150 µm) of mean oocyte diameters thus indicating large variation in size. The latter case was similar to the situation observed in the present study whereby standard deviations were always large.

Spawning

A detailed analysis of spawning is beyond the scope of this report. No statistical analysis of variance has been used. However, the authors suggest that initial mean oocyte diameters may be significantly different in some cases.

Spawning data is summarized in Table 3. Oocyte diameters for days 1 and 2 represent means of approximately 100 measurements. These are random measurements of the entire sample and not measurements of any specific stage or group. Resting Stage II was never measured. Measurements for the third day following injection represent a mean spawned egg diameter of 1,053.66 µm. This number was derived by averaging mean spawned egg diameters from seven C. melana spawns in a previous study (Hoff et al., in press), and three spawns of the present study. By eliminating the need for measuring oocytes the morning of the spawn, personnel were free to devote full attention to the spawning process (care of eggs, system preparation, etc.). This method implies some error. However, the error may not be much greater than the error in a measurement of a catheter sample of eggs just prior to and after ovulation. Thirty hr after initial injection mature Stage VI eggs have worked their way toward the lumen and oviduct, thus displacing immature eggs. Therefore, an unbiased sample cannot be obtained by catheter. This has been observed previously (F. Hoff, personal communication) and in the present study. If only the most advanced group of oocytes is to be measured, then this condition poses no problem. Secondly, unspawned Stage VI mean oocyte diameters do not differ more than 10% from water hardened spawned egg diameters. Some of this difference is a result of the measurement technique. Spawned eggs are measured suspended in seawater.

Their shape is nearly spherical. Eggs obtained by catheter are placed on a slide where much of their mass is supported by one side of the egg. This tends to compress the egg on the axis perpendicular to the plane of the slide. The result is an exaggerated diameter along the plane of measurement.

In every spawn attempt, an effort was made to achieve a semi-natural spawn. Only twice did females spawn eggs into the tank without being stripped. Both times fertilization was not achieved.

Fecundity of strip-spawned females ranged from 23,600 to 80,260 (combined fecundity of spawns 4 and 5), and fertilization ranged from 33-85%.

Hatching ratio for Spawn 1 was 71%. This was the best hatching ratio achieved. Spawn 3 represents the poorest hatching ratio. Spawns 4, 5, and 6 ended in complete mortality during the incubation period due to deterioration of water quality in the stagnant incubation system. After this group was lost, flow-through incubators were designed and connected to the filter system. Hatching ratio for Spawns 7 and 8 combined was 20%. Spawns 9, 10, and 11 were also combined and the hatching ratio was 25%. Similarly, hatching ratio in Spawn 12 was 33%.

As already mentioned, standard deviations were always large in measurements of initial mean oocyte diameters. After hormone treatment deviations usually doubled and in one case (Spawn 9) almost tripled.

Available data suggest that fertilization success is dependent on spawning at a definite time interval after initial injection. This interval probably varies as a function of hormone dosage and initial mean oocyte diameter (Shehadeh et al., 1973a; Hoff et al., in press). The present study indicates that injection of HCG at the specified dosage in females with initial mean oocyte diameters between 425 and 675 µm will result in successful spawning between 41 and 48 hr. The data imply that the interval from initial injection to spawning is inversely related to initial mean oocyte diameter. This is consistent with the observations of Shehedah et al. (1973a). Spawn 11 had a fertilization of 70% at 48 hrs; initial mean oocyte diameter was 429 µm. Spawns 4 and 5 yielded 72% and 50% fertilization at 44 hrs and 46 hrs, respectively; initial mean oocyte diameter was 672 µm.

Semi-Natural Spawns

Two semi-natural spawns occurred, the first was in March, 1975, in a preliminary spawn not included in Table 6. The fish was injected with 0.55 IU HCG g^{-1} body weight, and approximately 24 hr later with another dose at 0.275 IU g^{-1} body weight. At 40 hr after initial injection, the expected semi-natural spawn had not occurred, and at 42 hr the fish was strip spawned and fertilized with milt from two males.

Fertilization was 64% and fecundity was 25,000 eggs. At this time, the fish was given another 0.275 IU g^{-1} body weight injection and placed in a spawning tank with three males. Twenty-two hr later approximately 5,000 eggs were found in the tank. None of the eggs observed were fertilized, and approximately 50% had multiple oil globules.

In Spawn 7 the female was injected with standard dosages and placed in a 1,000-liter tank with three males. At 32 hr (4:00 AM), 3 hr before the lights came on in the spawning room, the lights were manually turned on for about 3 min. This was followed by 1 min of flickering light. All fish responded with increased activity. No spawning behavior was observed, and lights were turned off. At 8:00 AM, approximately 2,000 eggs were found in the tank. Some eggs had multiple oil globules and none were fertilized. The fish was stripped at 9:00 AM yielding eggs with 64% fertilization.

A discussion of the poor success with semi-natural spawning in this study would be speculation. However, in future attempts, lighting cues as a spawning stimulus will be investigated. Hoff et al. (in press) discovered in one series of semi-natural spawns that fish had been spawning unexpectedly at approximately 1:00 AM. After one overnight observation it was found that a faulty timer was turning on the lights at 1:00 AM; and successive semi-natural spawns coincided with simulated sunrise and sunset.

Multiple Oil Globules

Strip-spawning produced many eggs with multiple oil globules. The proportion with multiple oil globules varied from 10-50%. The number of globules per egg also varied. In Spawn 9 the fish was stripped after 29 hr, and some eggs had as many as 30-35 oil globules. The fish was obviously spawned too early (see Frakes et al., in press). Usually, if the number of globules was not over 3 or 4, fertilization and subsequent development were not obviously impaired. In some observations of cleavage, multiple oil globules were seen coalescing into one.

Larval Rearing

The results of three rearing attempts are found in Table 4. By the end of day 24, most larvae had passed all apparent critical stages. At this time their epidermis was heavily pigmented, scales were prominent, and they began a sedentary habit. Survival curves for the three attempts are found in Figures 11, 12, and 13. Water quality data are found in Figures 11, 12, 13, 14, 15, and 16. Yolk sac depletion and growth information is exemplified by Trial 1 (Figures 17, 18).

Hatching

Hatching was marked by the separation of the caudal region from the yolk sac. The process proceeded anteriorly and usually took about 8-12 hr. Some embryos were observed to become separated anterior to the caudal region. These embryos appeared weak and probably never completed hatching.

For the first two days, larval movement consisted of short, rapid undulations followed by periods of drifting. Usually the body was oriented vertically in the water column with the head down. As feeding began, larvae exhibited a more active swimming behavior.

Distribution

Larvae in Trial 1 demonstrated negative phototaxis to high intensity light supplied from day 4. During Trials 2 and 3, the flood light was supplied from hatch and negative phototaxis was not observed. Larvae in all trials descended on the evening of day 3. This descent was not as pronounced as that described by Frakes et al. (in press). The ascent to the surface was similarly undramatic. Larvae were never concentrated in any one section of the silo. Throughout all trials larvae were grouped into two schools. The reasons for this are not clear. After 24 days, larvae maintained this segregated distribution, even when transferred to other systems.

Feeding

Feeding activity began on day 3 concomitant with eye pigmentation, absorption of yolk sac, and development of a functional buccal cavity. Within 1 hr after the addition of food to the silo, larvae exhibited the typical "S" flexure prelude to striking activity. However, analysis of stomach contents revealed that apparently no successful feeding occurred on day 3. During day 4, stomach content analysis of individual fish revealed from 1 to 4 rotifer cuticles and/or undigested algae. Feeding success was also observed macroscopically. From day 7, wild plankton was fed in addition to rotifers. Dominant organisms in wild plankton catches included nauplii and copepodites of calanoid, cyclopoid and harpacticoid copepods. Incidental organisms included cirriped nauplii, polychaete larvae, gastropod and bivalve veligers. After days 8-9, copepod nauplii were also found in stomach contents.

Larvae were maintained on rotifers and wild plankton from day 7 through day 13. On day 11 of Trial 1, Artemia nauplii were offered but not accepted until day 14; then they were eaten almost immediately. Larvae were still transluscent and gut contents, especially Artemia, could be readily seen macroscopically. Larvae in Trials 2 and 3 preyed

upon Artemia nauplii from day 11. Wild plankton was fed approximately every other day from day 14. On day 21, a commercial flake food, frozen plankton, and frozen adult Artemia were accepted. These foods were sieved through a 500 µm sieve.

Feeding activity was continuous during the day, but night observations with subdued lighting revealed no such activity.

Mortality

All trials had mortalities between days 1-6. Frakes et al. (in press) stated that a high incidence of early larval mortality is due to inferior quality of strip-spawned eggs. Results of Trial 1 (Figure 11) presents contrary evidence. Total mortality in Trial 1 was 90.4%. Trial 2 yielded best overall survival (90.0% mortality) despite substantial early mortality (Figure 12). Mortality in Trial 3 was 97.1% (Figure 13). Trials 1 and 3 had substantial mortalities due to physiological edema. Edema was scarcely observed in Trial 2.

Edema has been related to pseudomonad bacteria (E. Houde, University of Miami, personal communication) and to water quality problems (Frakes et al., in press). Comparison of survival with nitrite (NO_2) and ammonia (NH_3) levels showed no definite correlation with the appearance of edema (Figures 11, 12, 13). However, data for Trials 1 and 3 do not exclude the possibility of interaction between these parameters and the occurrence of edema. Pathological analysis in the present study revealed no pathogen in or near the abdominal cavity. Between 30-50% of the larvae in Trials 1 and 3 had edema. Mortality was heavy from day 15 to day 20. During Trial 1, fish appeared abnormal on day 8, and on day 11 in Trial 3. Affected larvae were quite swollen, first laterally, and later dorsally. At first, two symmetrical swellings appeared just posterior to the pectoral fins, and extended toward the anal fin. These enlarged and another swelling formed just posterior to the head. Larvae became positively buoyant, disoriented, stopped feeding, and died. Non-edemic larvae maintained typical body morphology.

Mortality after day 24 was high and due mostly to territorial competition and cannibalism; facilities probably did not provide sufficient habitat and bottom area.

Growth

Larval growth data are presented for Trial 1 only (Figure 17). There was no length increase the first 3 days, although organogenic activity was noted. After larvae began to feed, total length gradually increased from approximately 2.8 mm to 3.7 mm at day 14. On day 14 Artemia nauplii were accepted by this group. By day 16 larvae had grown from 2.7 mm to almost 6.0 mm. This growth indicates that Artemia is an excellent food source for this age larvae. Total length averaged 9.0 mm at day 24.

CONCLUSIONS

Larvae of the southern sea bass (Centropristis melana) can be reared in a silo-type tank from strip-spawned eggs. Survival rate was as high as 10.0%. Rotifers supplemented with wild plankton appear to be a satisfactory food item during the initial fortnight of larval development; Artemia nauplii were suitable thereafter. Physiological edema is a problem in rearing sea bass larvae. It does not usually cause total mortality, but can affect a rearing attempt so as to make the effort unfeasible. Research is needed to outline the source and prevention of edema. The larvae of the southern sea bass are stenohaline organisms. The results of our experiments indicate success in spite of the limited tolerance of this species.

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Table 1. Weights and measurements of Centropristis melana brood stock, collected January through March 1975.

	Males	Females
Average total length (cm)	24.70	19.82
Average standard length (cm)	18.40	15.71
Average weight (g)	184.20	118.50

Table 2. Vitamin fed to brood stock C. melana (Blood Aid, Geisler Pet Products).

Vitamin	Concentration mg ml^{-1}
Thiamine HCL (B_1)	0.1660
Riboflavin (B_2)	0.0830
Pyridoxine HCL (B_6)	0.0083
Cyanocobalamin (B_{12})	0.1660
d-Panthenol	0.0333
Choline Bitartrate	0.8333
Ferric Ammonium Citrate	0.1433
Niacinamide	0.8333
Daily Dosage	2.0 ml kg^{-1} of food

Table 3. Spawning data for C. melana

Spawn No.	Date	Hours from initial injection to spawn	Mean oocyte diameters (µm) Day 1	Day 2	Day 3	Fecundity	% Fertilization	% Hatch	St. dev. Day 1	St. dev. Day 2	Sample Number Day 1	Day 2
1.	41575*	48	542.03	645.32	1053.66	23,600	84	70.7	102.24	174.57	118	141
2.	5675		533.03	745.14	Death	Prior	To Spawning	-	82.90	139.10	76	106
3.	51275	32	625.33	709.05	1053.66	64,350	58	2.6	122.40	243.92	135	77
4.	61675A	44	671.56	851.03	1053.66	51,840	72	-	73.76	201.58	109	102
5.	61675B	46	671.56	851.03	1053.66	28,420	50	-	73.76	201.58	109	102
6.	61675C	43	529.18	719.13	1053.66	35,260	64	-	93.49	267.90	110	104
7.	82175A	46	464.43	574.38	1053.66		64	19.5	110.04	210.65	115	96
8.	82175B	38	394.71	674.88	1053.66	30,400	64	19.5	113.73	155.13	119	82
9.	92775B*	29	447.00	846.15	1053.66	25,000**	33	25.0	91.22	288.70	53	13
10.	92775B*	43	447.00	846.15	1053.66	25,000**	45	25.0	91.22	288.70	53	13
11.	92775C*	48	429.3	568.86	1053.66	30,000**	70	25.0	122.75	209.28	58	70
12.	10175A*	41	501.3	--	1053.66	60,000**	85	33.0	97.41		47	

* Eggs from these spawns were successfully hatched and reared through metamorphosis
** This figure represents estimates and not aliquot measurements

Table 4. Stocking densities, original hatches, and survival of three C. melana larval rearing trials.

	Stocking density (embryos)	Number hatched	Survival through day 24
Trial 1	14,700	10,290	1000 (9.61%)
Trial 2	39,464	10,000	1000 (10.0%)
Trial 3	51,000	17,000	500 (2,94%)

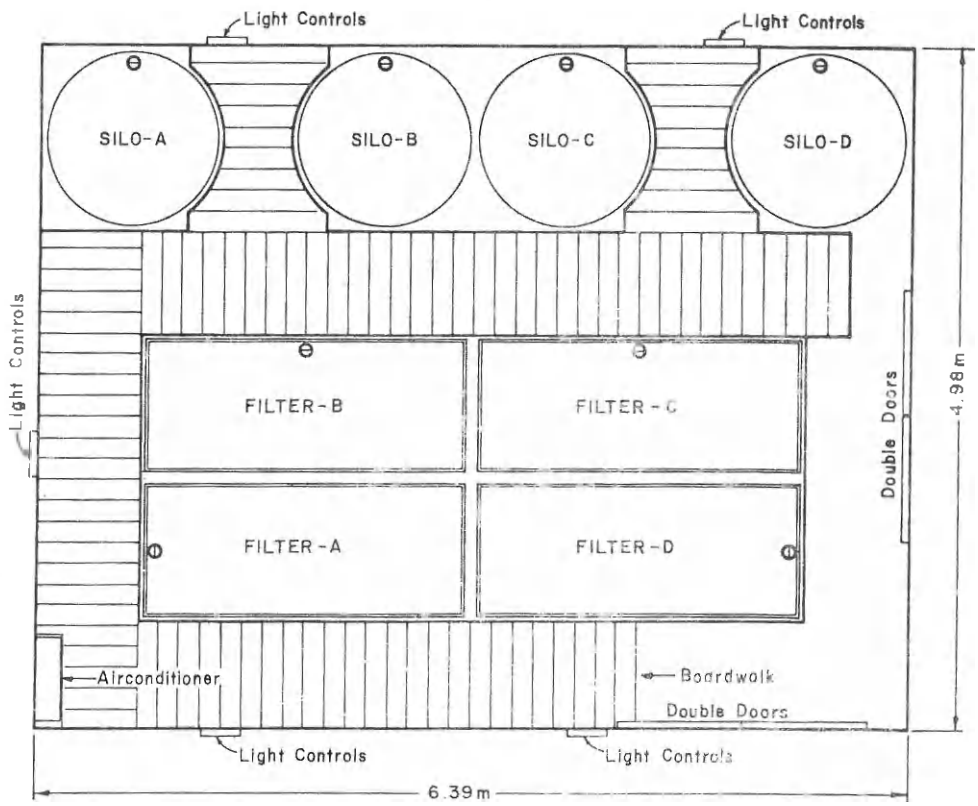

FIGURE 1. LARVAL REARING LABORATORY FLOOR PLAN SCHEMATIC.

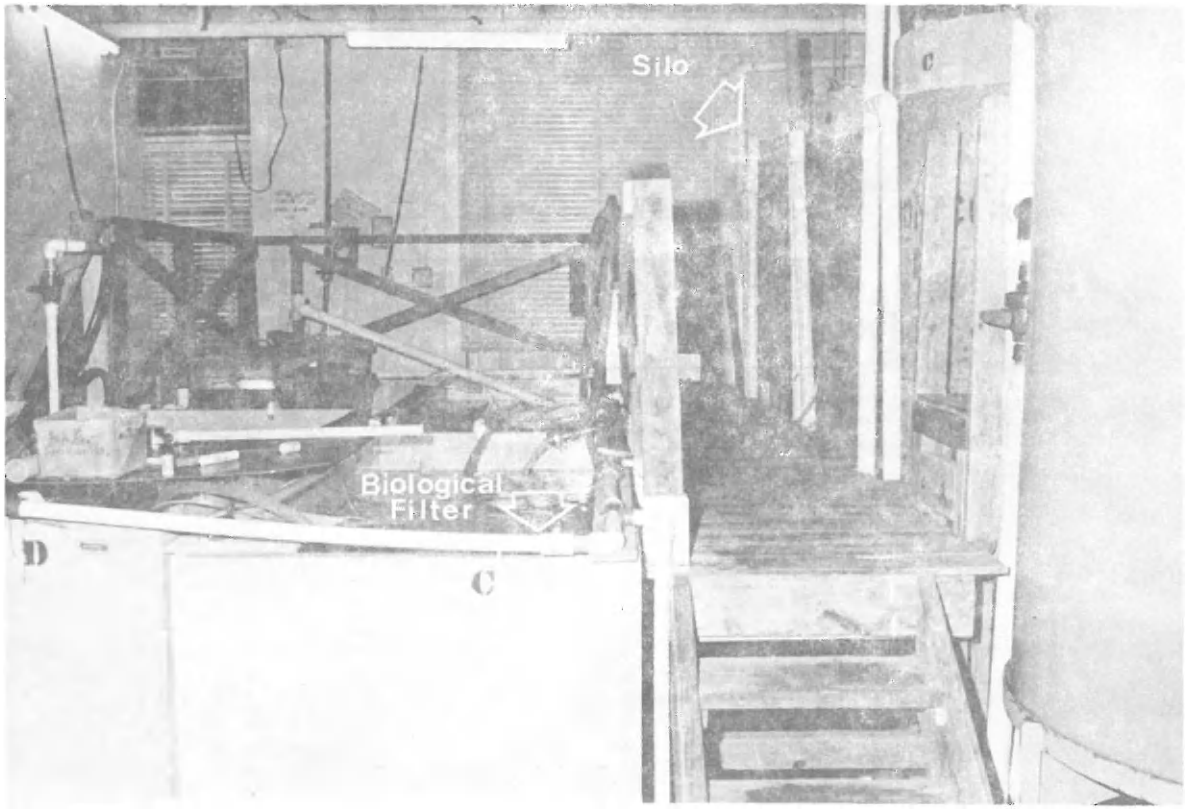

FIGURE 2. LARVAL REARING LABORATORY (PHOTOGRAPH) SHOWING FILTERS AND REARING TANKS.

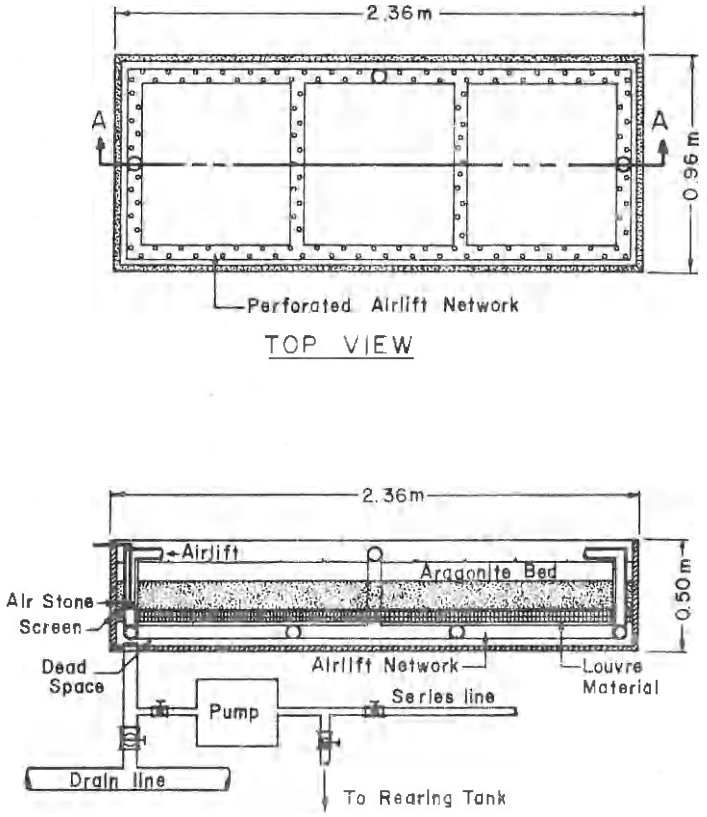

FIGURE 3. FILTER VAULT SCHEMATIC. TOP VIEW AND LONGITUDINAL SECTION.

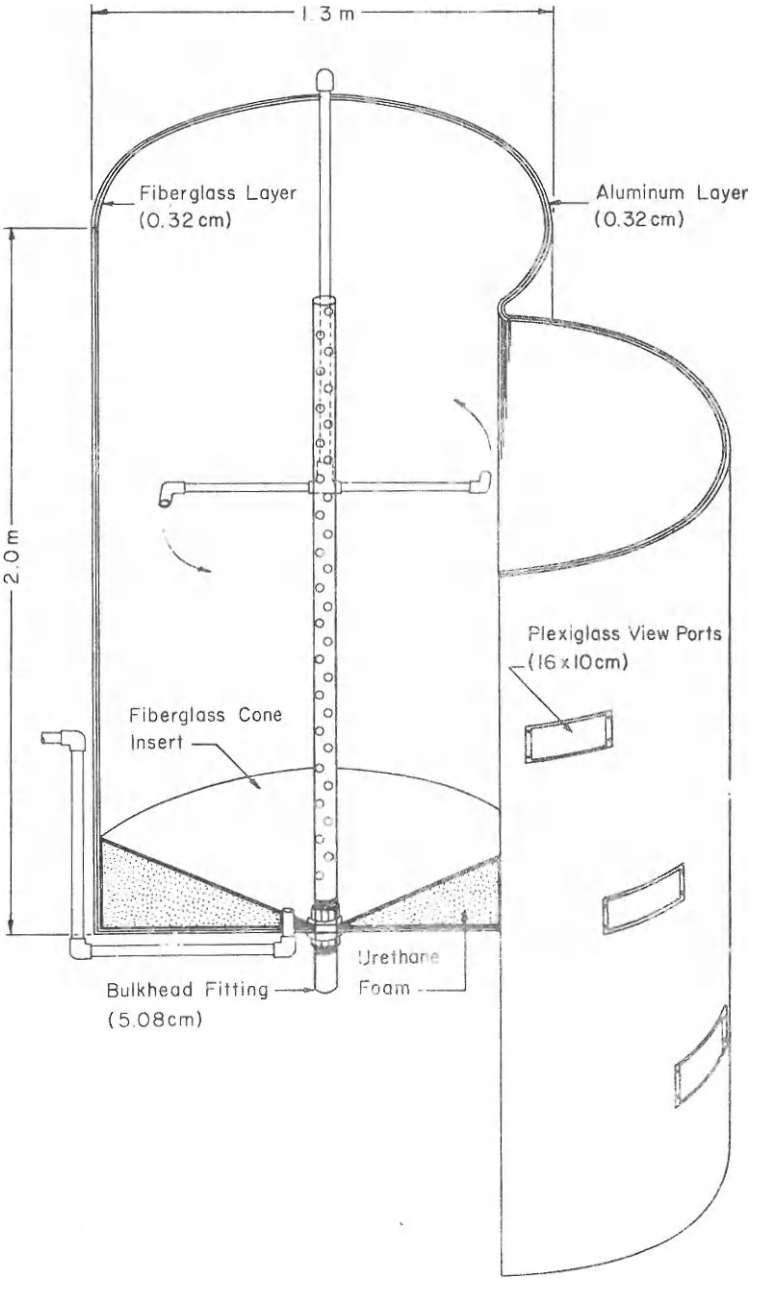

FIGURE 4. LARVAL REARING SILO SCHEMATIC.

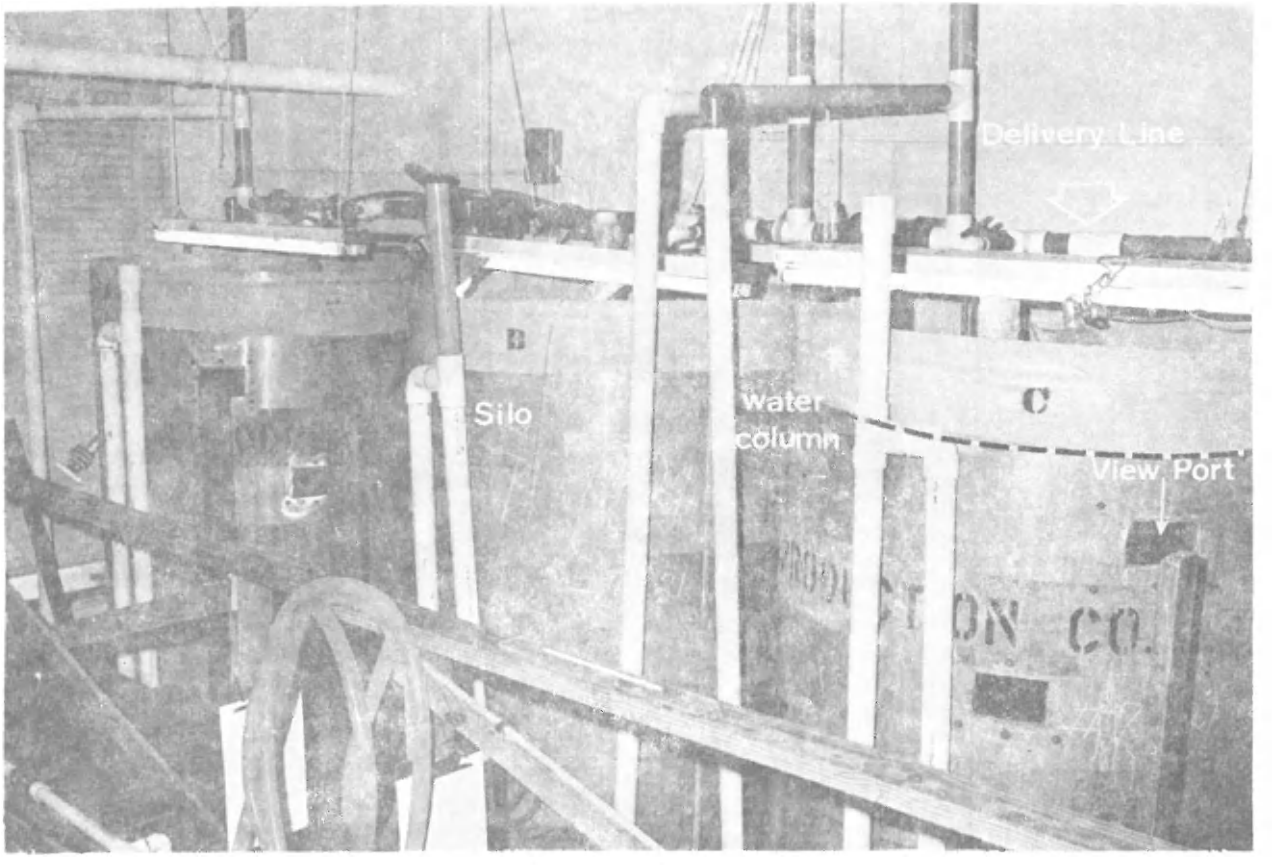

FIGURE 5. LARVAL REARING SILOS.

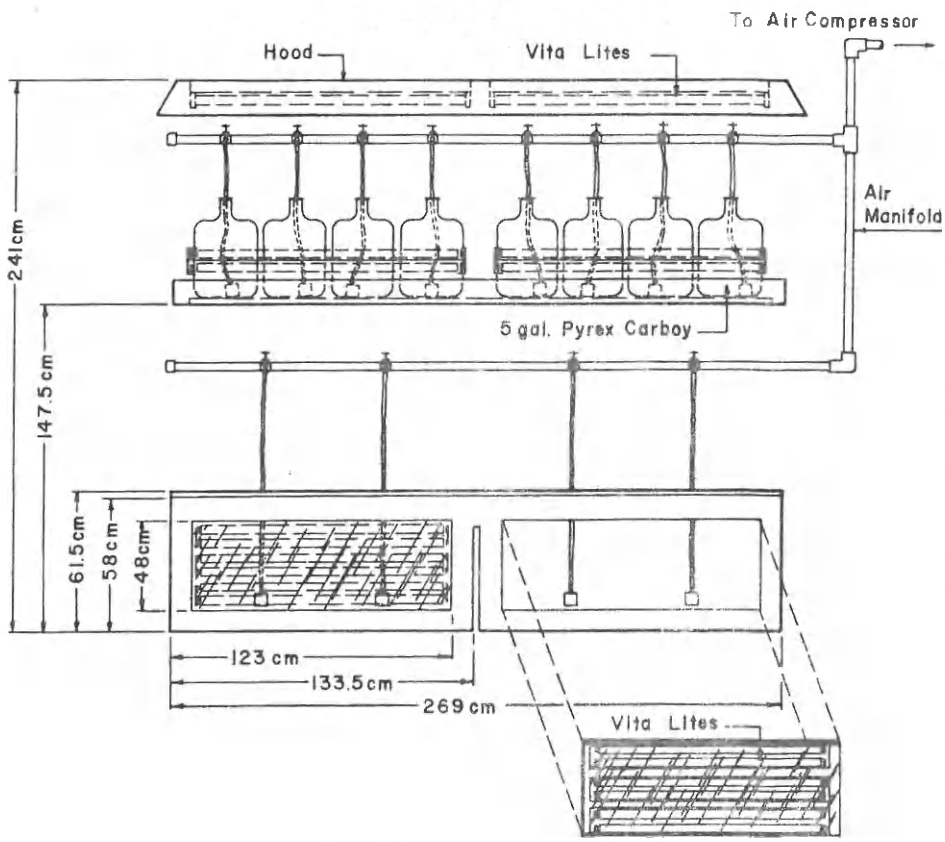

FIGURE 6. MASS FOOD CULTURE SYSTEM SCHEMATIC (1 UNIT).

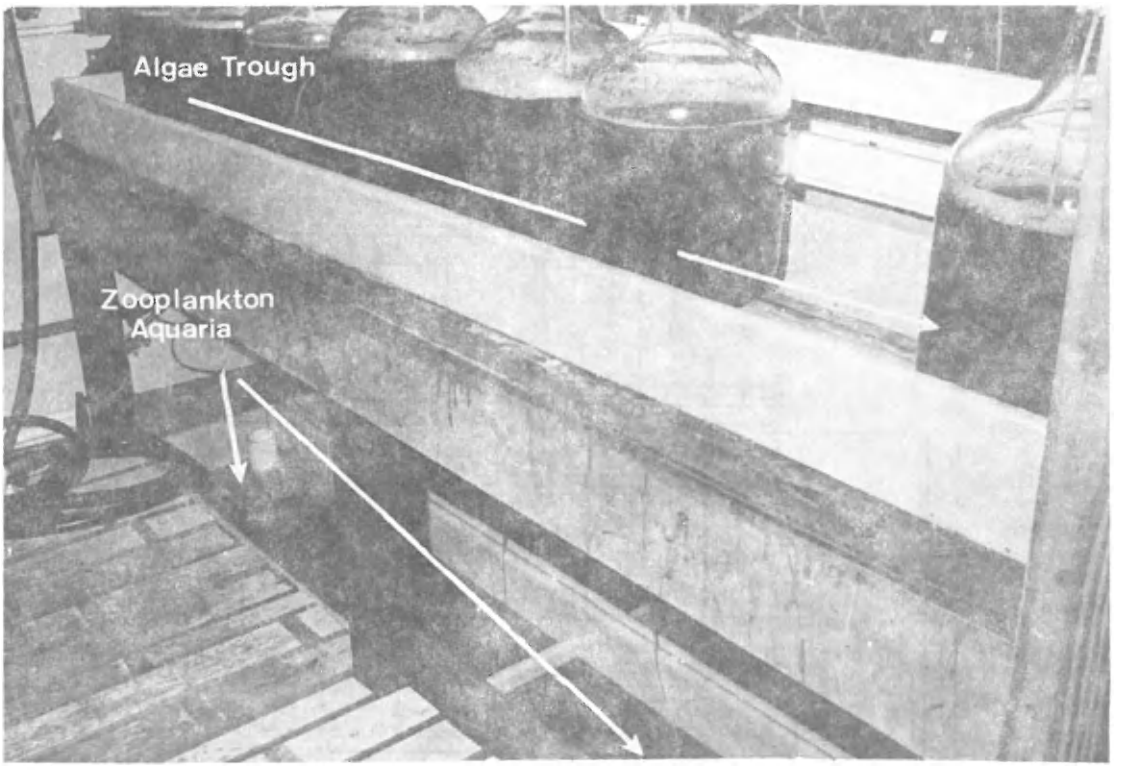

FIGURE 7. MASS FOOD CULTURE SYSTEM (1 UNIT) SHOWING THE UPPER ALGAE CULTURE TROUGH AND THE LOWER ZOOPLANKTON CULTURE AQUARIA.

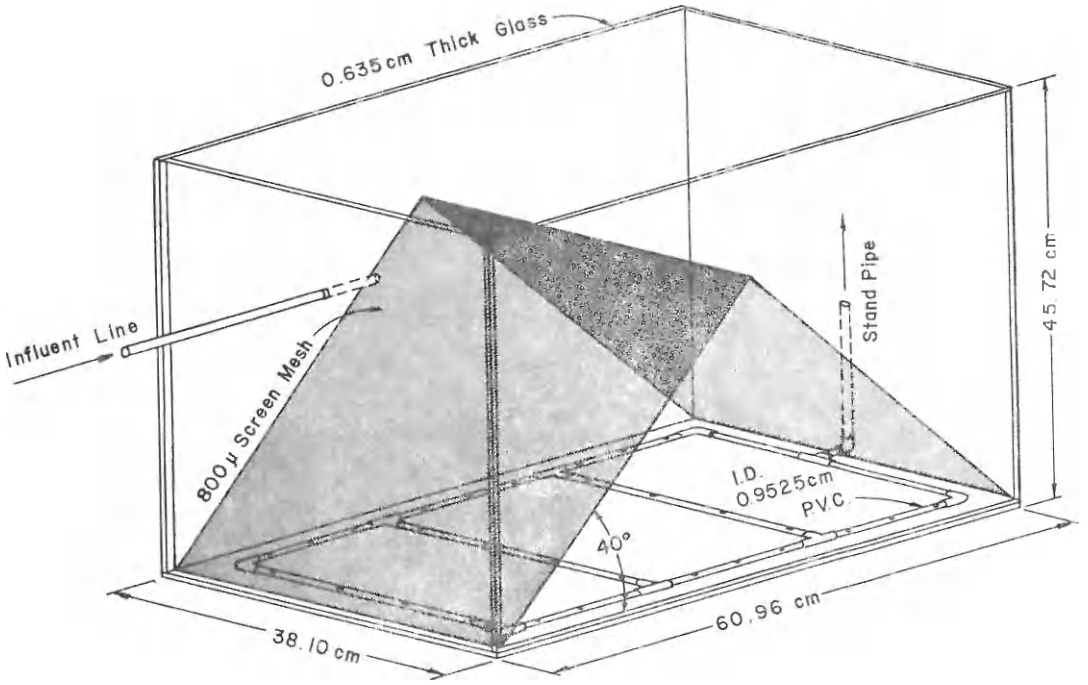

FIGURE 8. INCUBATION CHAMBER. SLOPED SCREENING ALLOWS DEAD EGGS TO SINK TO EXTREME ENDS WHERE THEY CAN BE SIPHONED. LIVE BUOYANT EGGS FLOAT NEAR THE SURFACE.

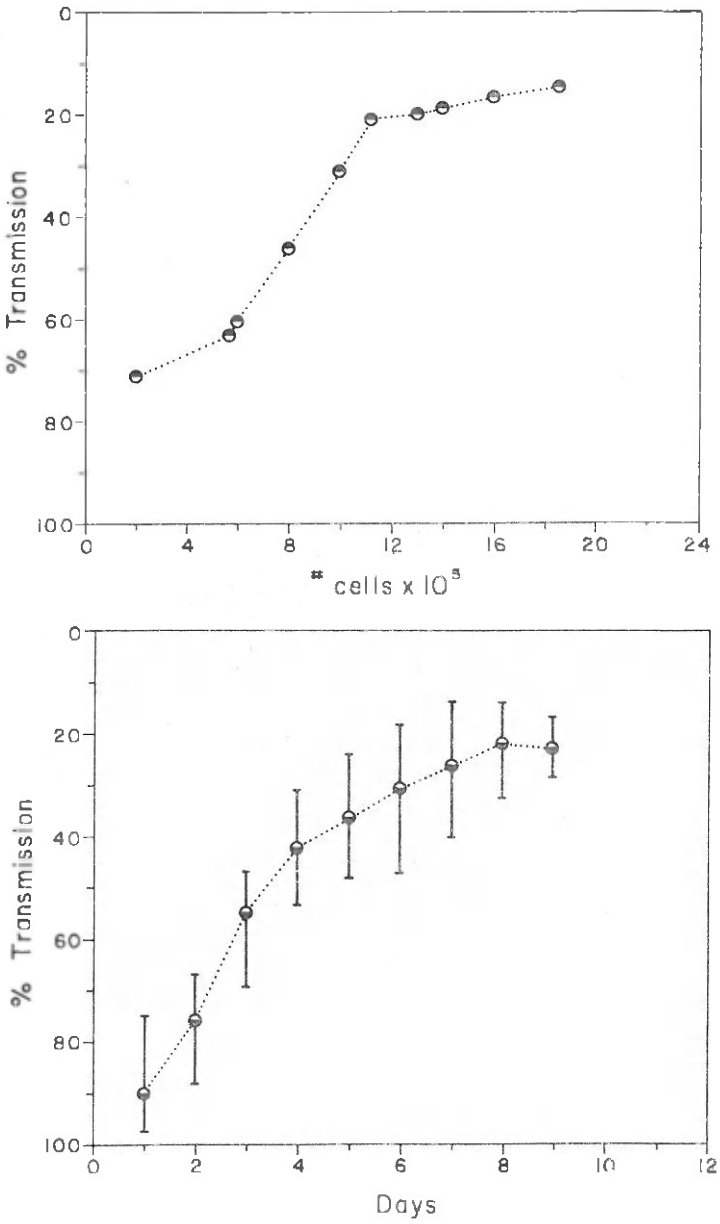

FIGURE 9. A) INTERPOLATION GROWTH CURVE FOR DUNALIELLA SP. OPTICAL DENSITY OF DAILY SAMPLES WAS PLOTTED AGAINST HEMACYTOMETER (AO BRIGHT LINE) COUNTS. B) TYPICAL GROWTH CURVE FOR DUNALIELLA SP. GROWN IN 20 L CARBOYS.

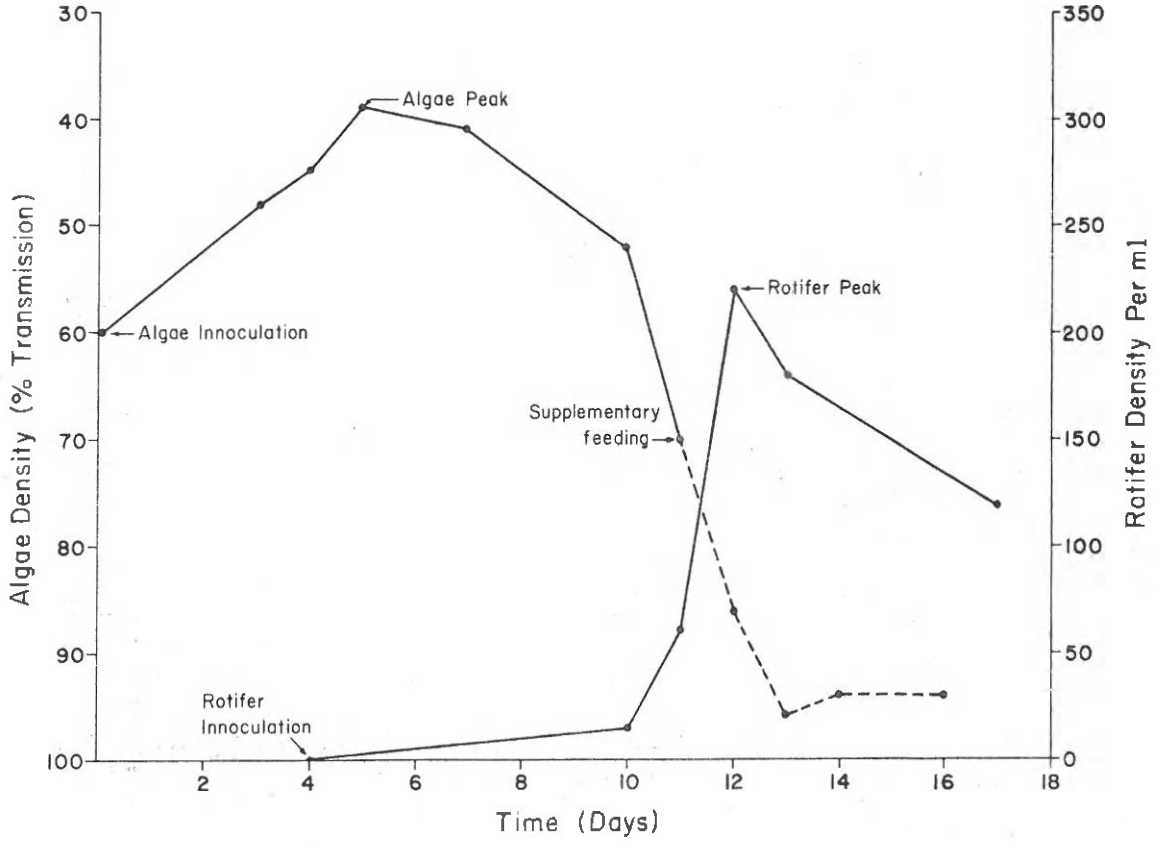

FIGURE 10. ROTIFER CULTURE CHRONOLOGY SHOWING ALGAE INOCULATION, ALGAE PEAK, ROTIFER INOCULATION, AND ROTIFER PEAK. SUPPLEMENTARY ALGAE WAS REQUIRED AFTER DAY 11.

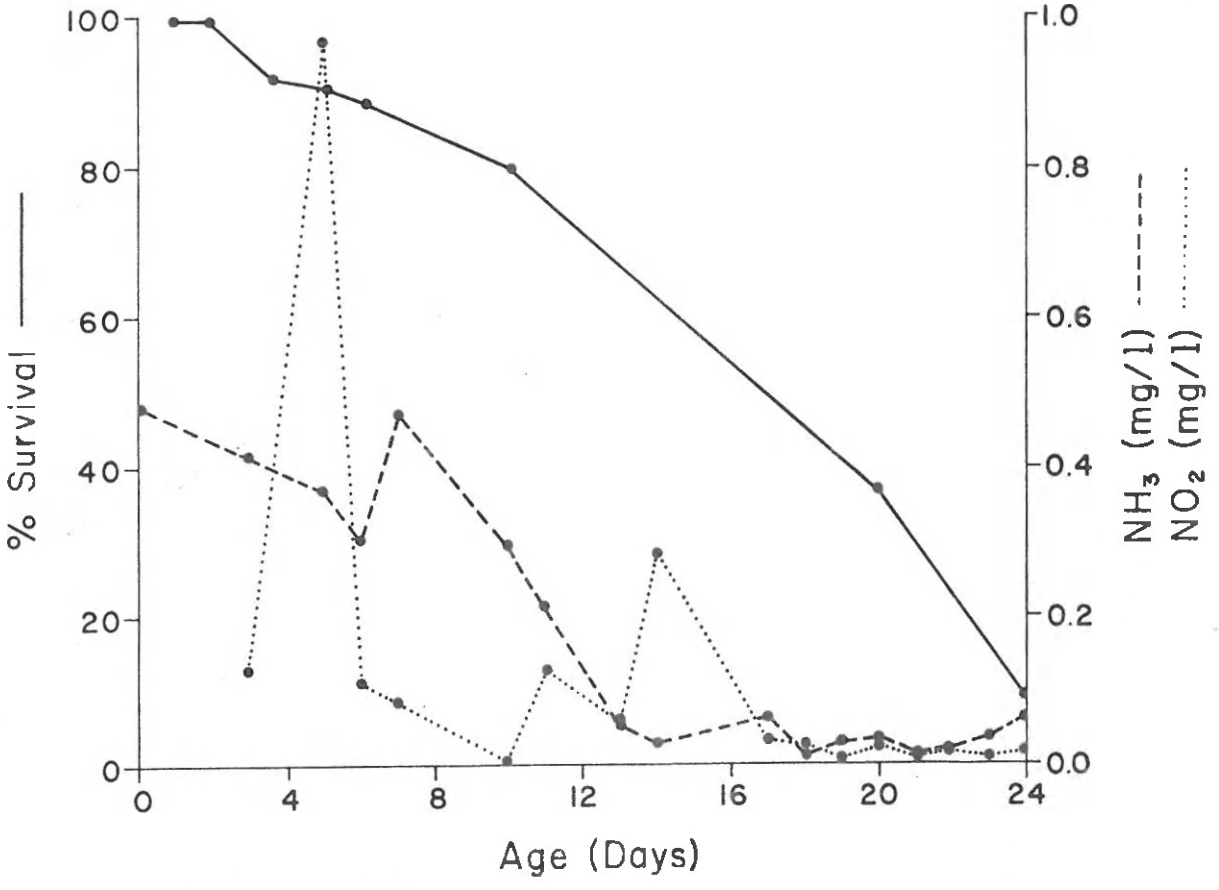

FIGURE 11. LARVAL SURVIVAL, TRIAL 1.

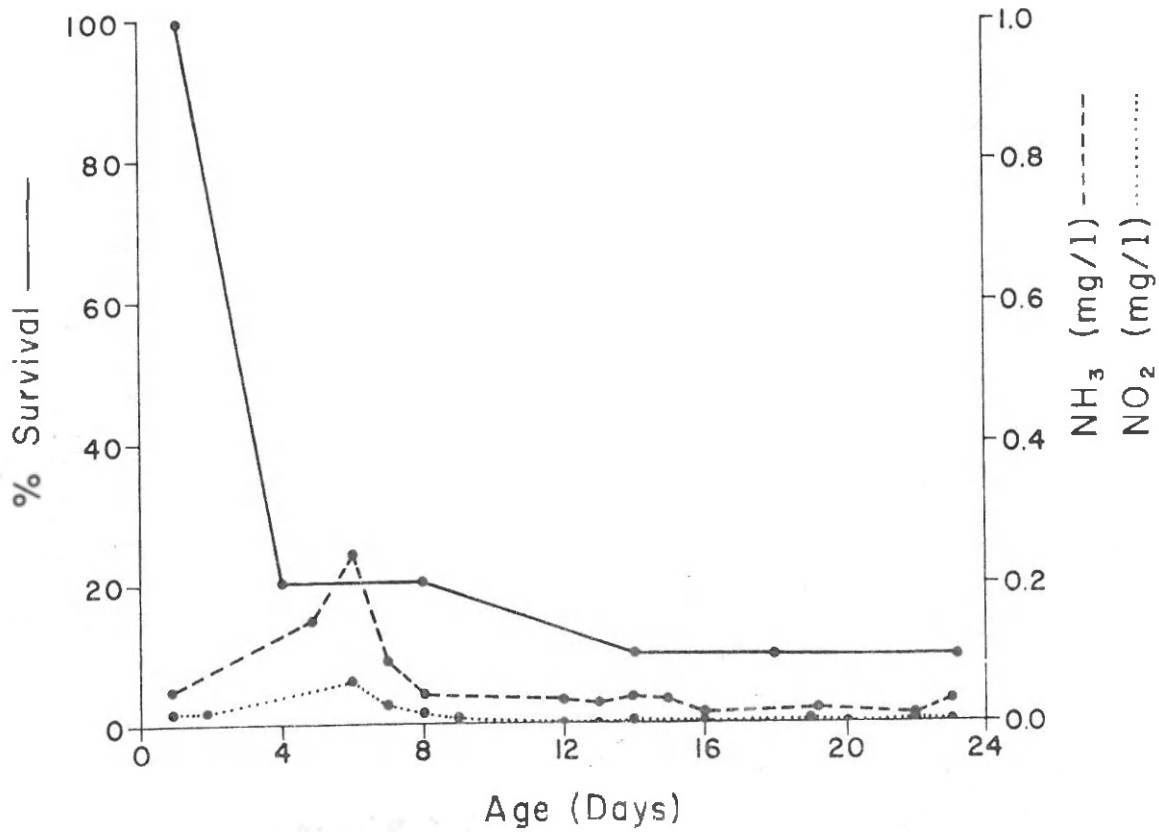

FIGURE 12. LARVAL SURVIVAL, TRIAL 2.

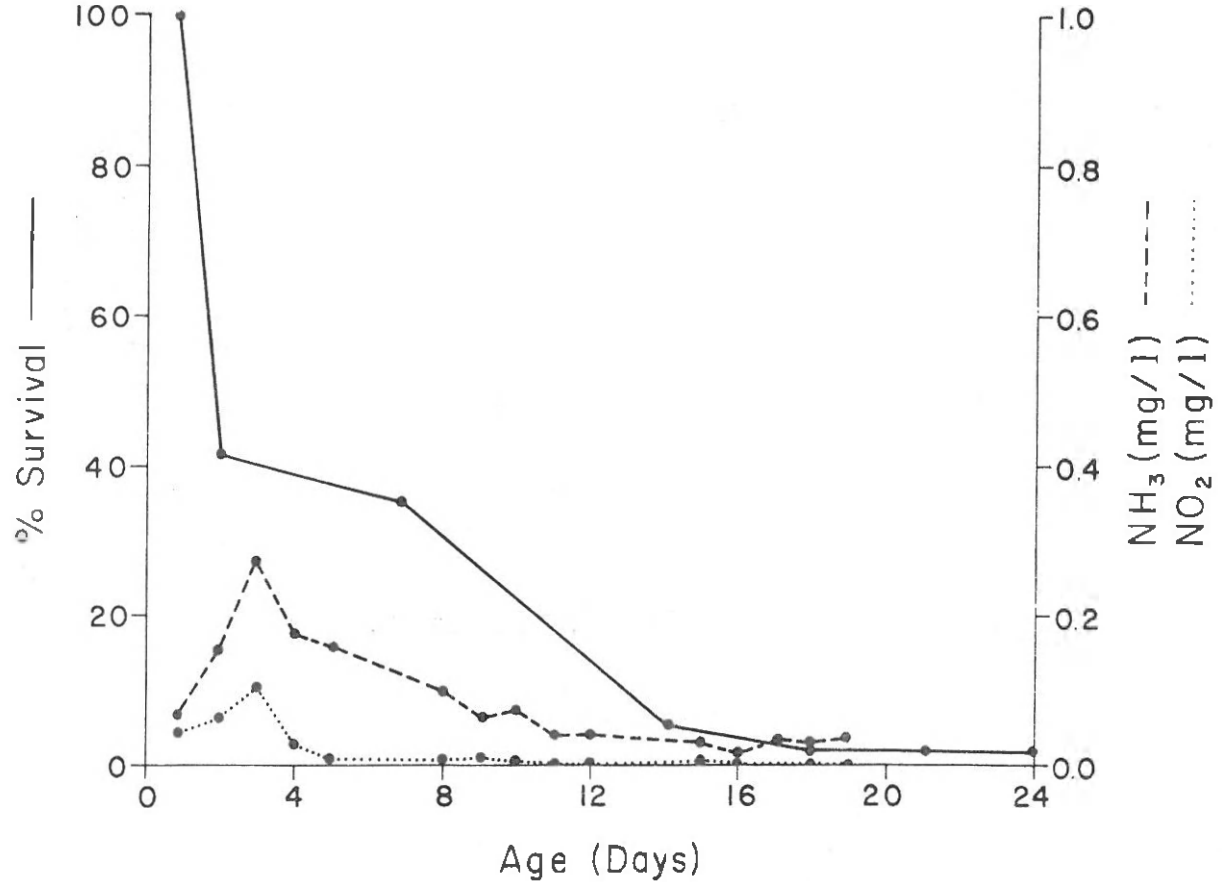

FIGURE 13. LARVAL SURVIVAL, TRIAL 3.

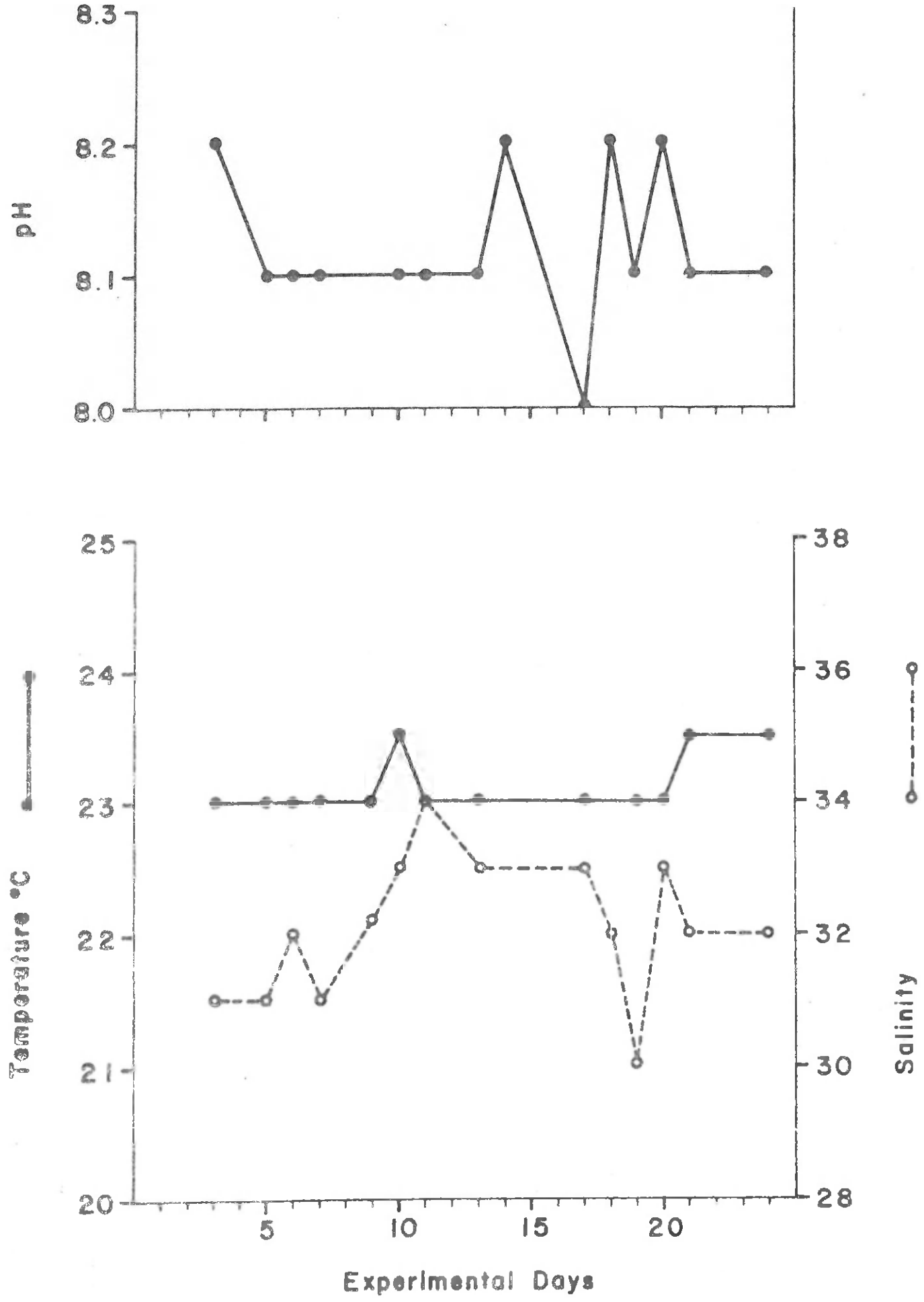

FIGURE 14. WATER QUALITY DATA FOR FILTER A, TRIAL 1.

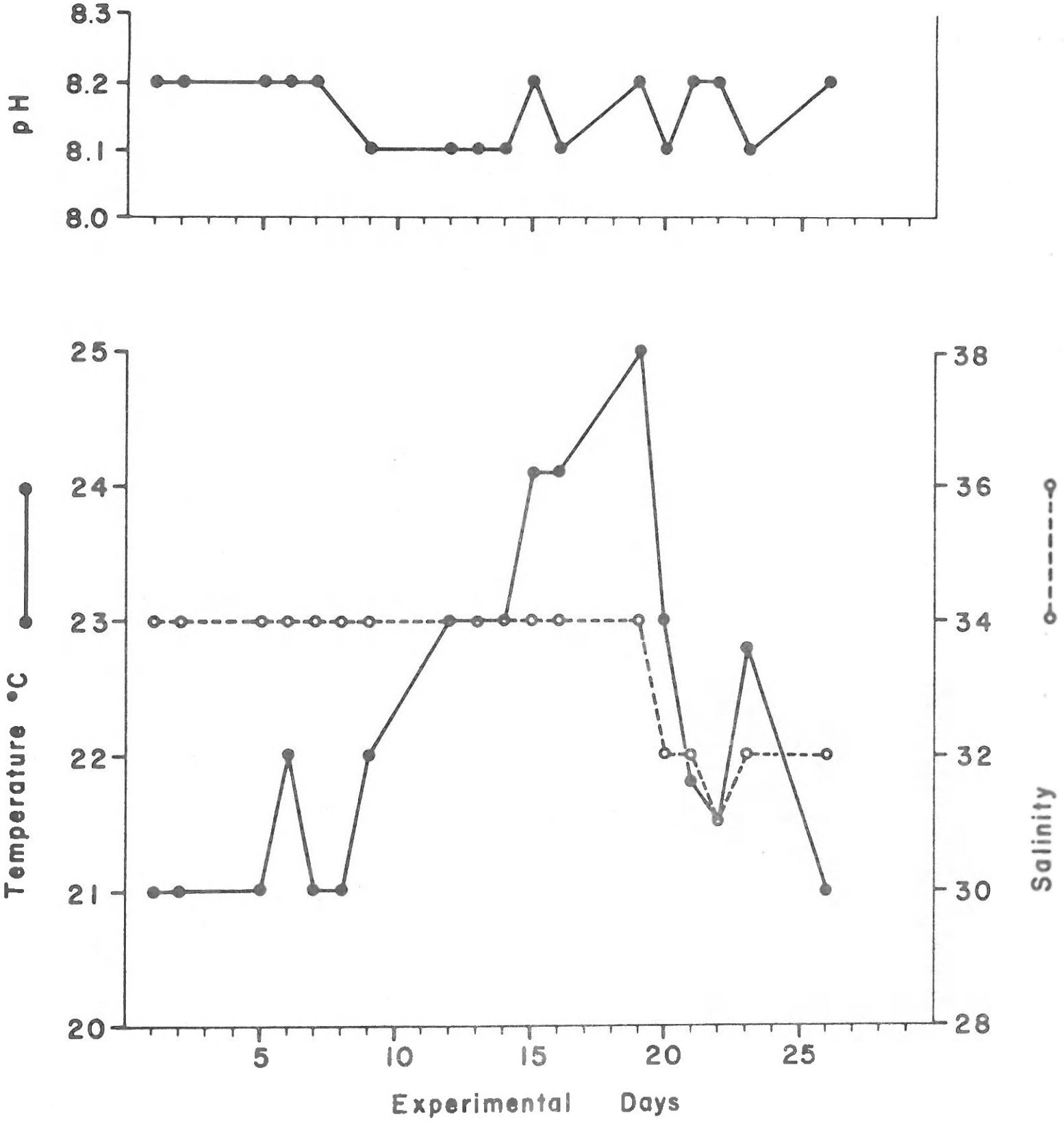

FIGURE 15. WATER QUALITY DATA FOR FILTER C, TRIAL 2.

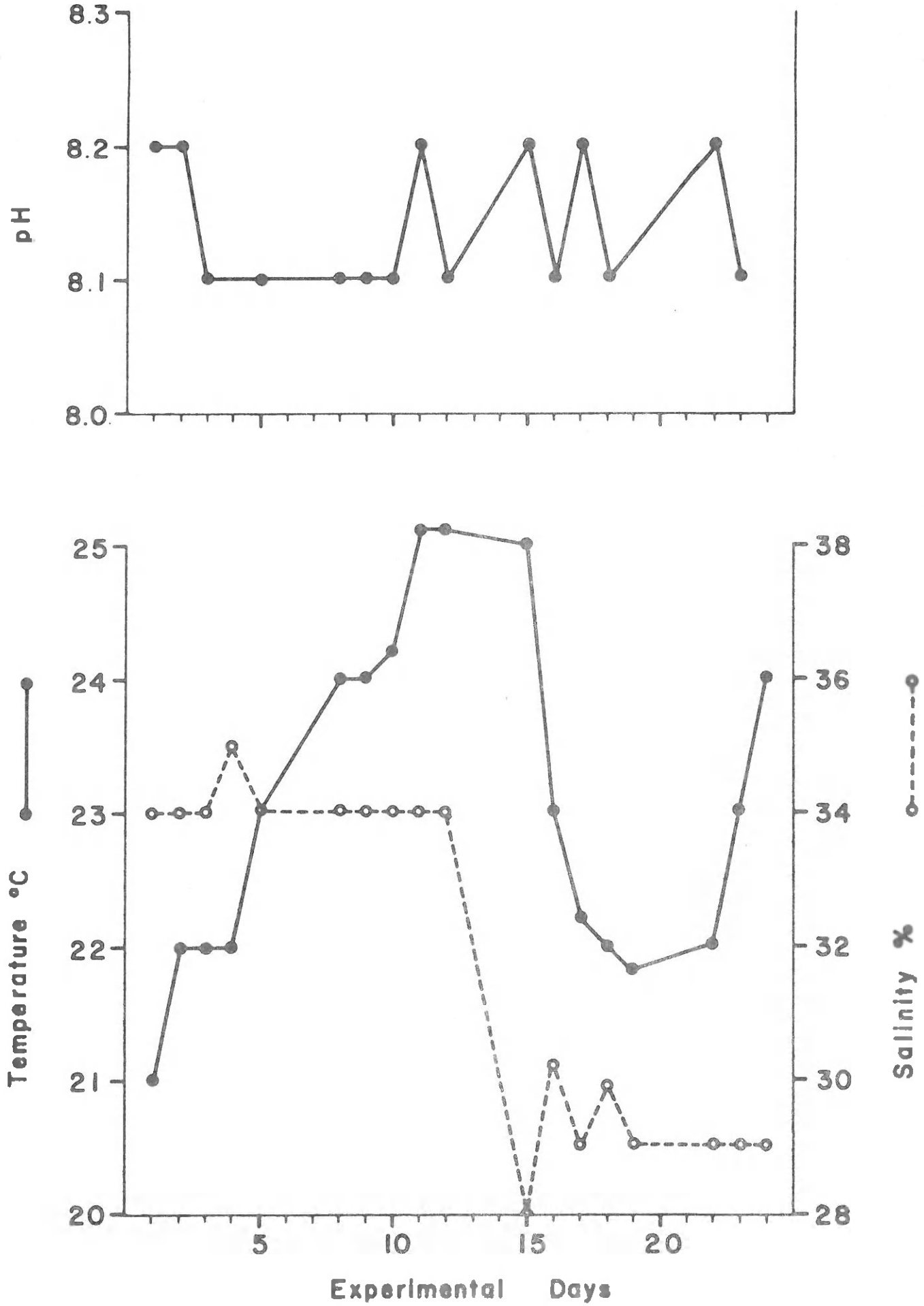

FIGURE 16. WATER QUALITY DATA FOR FILTER C, TRIAL 3.

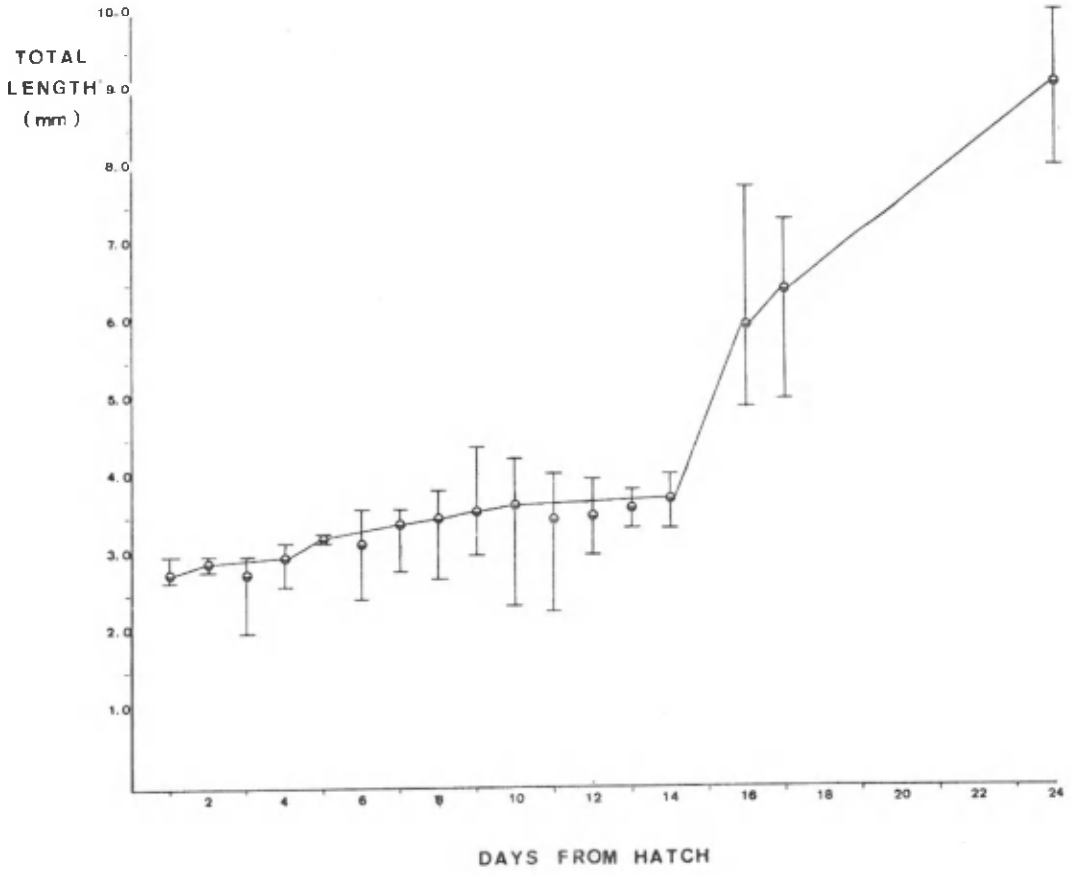

FIGURE 17. LARVAL GROWTH CURVE FOR TRIAL 1.

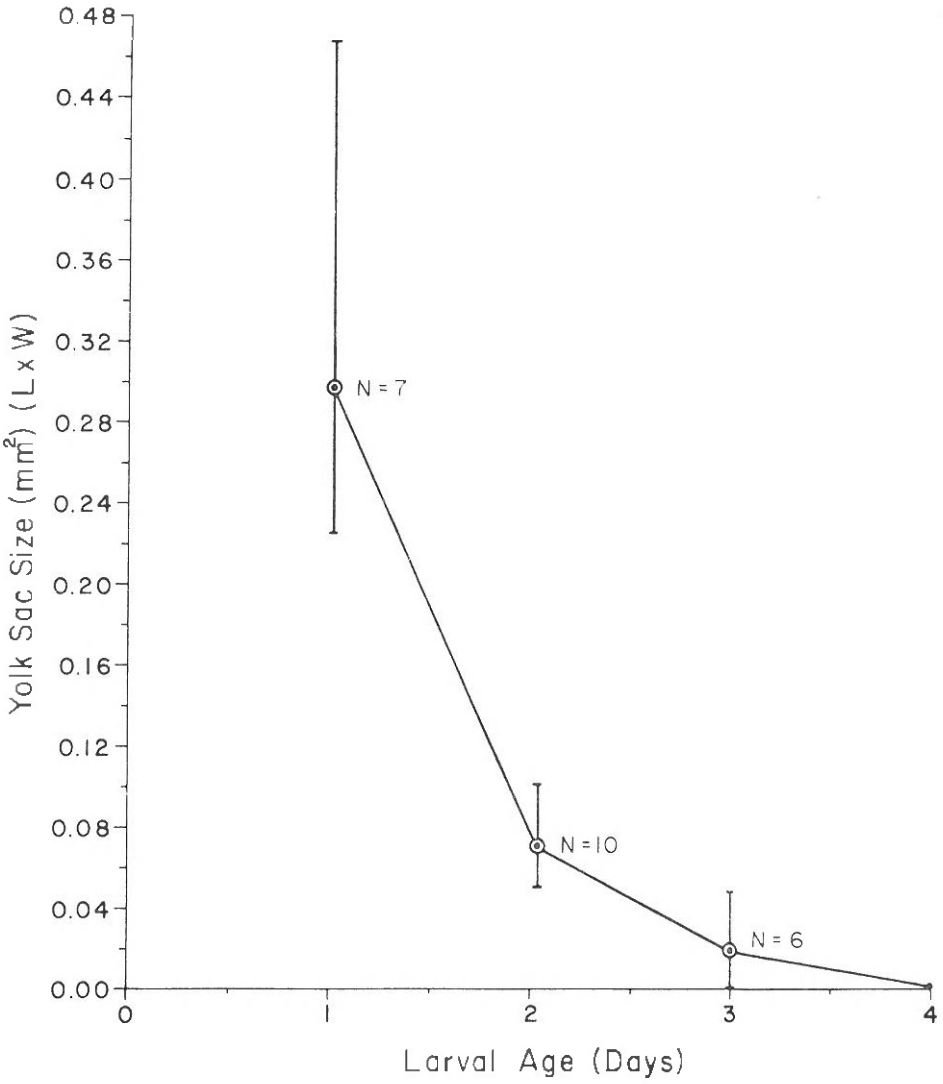

FIGURE 18. YOLK SAC DEPLETION CURVE FOR TRIAL 1.

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