CULTURE METHODS AND RESPONSE OF LABORATORY-REARED PINK SHRIMP *(PANDALUS JORDANI)* LARVAE TO SELECTED SIMULATED ENVIRONMENTAL FACTORS

INFORMATION REPORT

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CULTURE METHODS AND RESPONSE OF LABORATORY-REARED PINK SHRIMP *(PANDALUS JORDANI)* LARVAE TO SELECTED SIMULATED ENVIRONMENTAL FACTORS

Little is known of the larval life of *P. jordani*. Published studies of *P. jordani* larvae have been largely restricted to descriptions of laboratory-reared larval stages (Modin and Cox 1967; Lee 1969). Pearcy (1972) attempted to collect *P. jordani* off the Oregon coast, with little success.

The major objective of this study was to refine culture methods for shrimp larvae. Further objectives were to ascertain influence of water current and light intensity on survival and behavior of initial zoeal stages. The time period of larval emergence was also studied.

METHODS AND MATERIALS

Collecting and Holding Adult Shrimp

In Late March 1971, ovigerous *P. jordani* were caught by shrimp trawl off Coos Bay, Oregon. They were held in 136.5 liter (30 gallon) plastic containers and transported to the Fish Commission of Oregon marine laboratory at Newport, Oregon. These female shrimp were held in a recirculating seawater aquarium designed by Lukas (1973).

Each shrimp used to obtain larvae was removed from the aquarium and placed in a 2000-ml beaker containing 1500 ml of sea water. Adult shrimp were kept near the bottom of the beakers by a large-mesh plastic screen to prevent cannibalization of larvae. Minced fresh cockle clams *(Clinocardium nuttallii)* or gaper clams *(Tresus capex)* were fed to these shrimp.

Larvae Culture Techniques

Shrimp larvae culture methods were patterned after those of Reed (1969) and Gaumer (1969, 1970) for Dungeness crab *(Cancer magister)* larvae.

Only larvae less than one day old and actively swimming near the water surface

were used. When larvae appeared in beakers, they were transferred by large-mouth pipette to either 250-ml Erlenmeyer flasks containing 200 ml of water or 500-ml Erlenmeyer flasks filled with 400 ml of water. Plastic caps were placed loosely over flasks to eliminate air-borne contamination. Flasks were aerated by inserting an air line through tops of the caps. The amount of air introduced was regulated so that a continuous, but slow, series of bubbles was discharged. In one experiment, to determine importance of water aeration of survival, flasks were not aerated.

All rearing containers were placed in a constant temperature water bath initially maintained at 10 c $\pm$ 0.5 c. This temperature closely approximated ocean conditions (9.3 to 10.9 c surface and 9.5 to 10.0 c near bottom) when the majority of larvae hatched naturally on the northern Oregon shrimp grounds in March 1968 (Robinson and Milburn in press). When shrimp reached zoeal stage 9, the water bath temperature was lowered and maintained at 7.2 c ($\pm$ 0.5 c) to simulate known ocean floor temperatures of 6.1 to 7.8 c (Lukas and Hosie 1973) in spring and early summer. It was assumed this was the time of metamorphosis.

Sea water was obtained from Yaquina Bay and filtered through a Microfloc-polyvinyl chloride filter to eliminate particles larger than 5 μ. Bacterial growth was suppressed by ultraviolet treatment. Salinity was maintained at 30 $^{0}/_{00}$, $\pm$ 1 $^{0}/_{00}$, by dilution with distilled water.

Shrimp were exposed to 12 hours of flourescent light of 69 to 73 ft. c from 0600 to 1800 hours, with complete darkness during the rest of a 24 hour day. Light measurements were made with a model 756 Weston illumination meter.

Water in the containers was changed, shrimp fed, development noted, and the presence of mortalities and exuviae recorded three times per week. Larvae were considered dead if they failed to respond to agitation with a small pipette. Flasks were cleaned periodically to prevent algae growth. Larvae were fed newly hatched

nauplii of San Francisco variety brine shrimp *(Artemia salina)* at a concentration of 15 nauplii/ml of rearing water via a calibrated automatic pipette.

Effects of Light on Survival

The effects of light on survival was tested by placing 59 newly-hatched zoeae (about 16/flask) into four black painted 250-ml flasks containing 200 ml of water. Four transparent 250-ml flasks containing 52 newly-hatched zoeae (about 13/flask) were used as a control.

Effects of Aeration and Lack of Food on Survival

Experiments were conducted to ascertain effects of aeration on survival of: (a) unfed newly-hatched larvae and (b) feeding larvae reared through the zoeal stages. In the first experiment, survival comparisons were made between two groups of 60 unfed recently-hatched zoeae. They were reared until death in either aerated or non-aerated 500-ml flasks at 30 zoeae/flask.

In the second experiment, the effect of aeration on feeding larvae was tested by placing 60 newly-hatched zoeae into two non-aerated 500-ml flasks (30 zoeae/flask) containing food. Four aerated 500-ml flasks containing 120 newly-hatched feeding zoeae (30/flask) were used as a control.

The maximum number of days newly-hatched zoeae could live without food was determined in the unfed larvae aeration experiment.

Larvae Emergence

To determine number of days necessary for all eggs to hatch and the specific time of larvae emergence during a day, an experiment was conducted using three ovigerous shrimp. Each shrimp was placed in an individual 1000-ml beaker. Observations for emergent larvae were made each day for 24 days. Each morning at 0800 hours newly-emerged larvae were removed and their numbers recorded. To ascertain

specific emergence time, beakers were checked during four continuous days at eight times per day (0400, 0600, 0800, 1200, 1700, 2000, 2200, and 2400 hours).

Effects of Light Intensity and Water Current on Behavior

Observations were made on response of first and third stage *P. jordani* zoeae to light intensity and water current using a light chamber and a current cylinder (Figure 1) developed by Gaumer (1970). Only early stage shrimp zoeae were utilized because Gaumer (Fish Commission of Oregon, pers. comm.) found late stages of laboratory-reared Dungeness crab larvae displayed different reactions in these containers than ocean-collected larvae.

The light chamber and current cylinder were in a darkened controlled temperature room. A metric scale was attached to the side of each container to measure larval movement. Both containers were filled with filtered and ultraviolet-treated 30 $^{0}/_{00}$ ($\pm$ 1 $^{0}/_{00}$) sea water maintained at 12c ($\pm$ 1c).

The light chamber was vertical, plexiglass, 150 cm in height by 15 cm in diameter and closed at both ends. An adjustable incandescent microscope light was placed at the top of the chamber. Water and zoeae were introduced through a 2.5 cm hole at the top of the chamber. Zoeae were exposed to one of six light intensities (0, 100, 500, 1000, 2000, and 8000 ft. c.). The 8000 ft. c. (576 largleys/day) is about equivalent to the maximum recorded solar energy (622 largleys/day) at the ocean surface at Nanaimo, British Columbia (Takahashi and Parsons 1973). Light intensities were measured with a photometer placed directly above the water surface. In darkness, the location of larvae was determined by using a flashlight covered with red cellophane.

Newly hatched (less-than-one-day-old) first stage zoeae, three-day-old first zoeae, and seventeen-day-old third zoeae were used. Newly hatched zoeae, because of small size and transparent appearance, were introduced one per trial into the

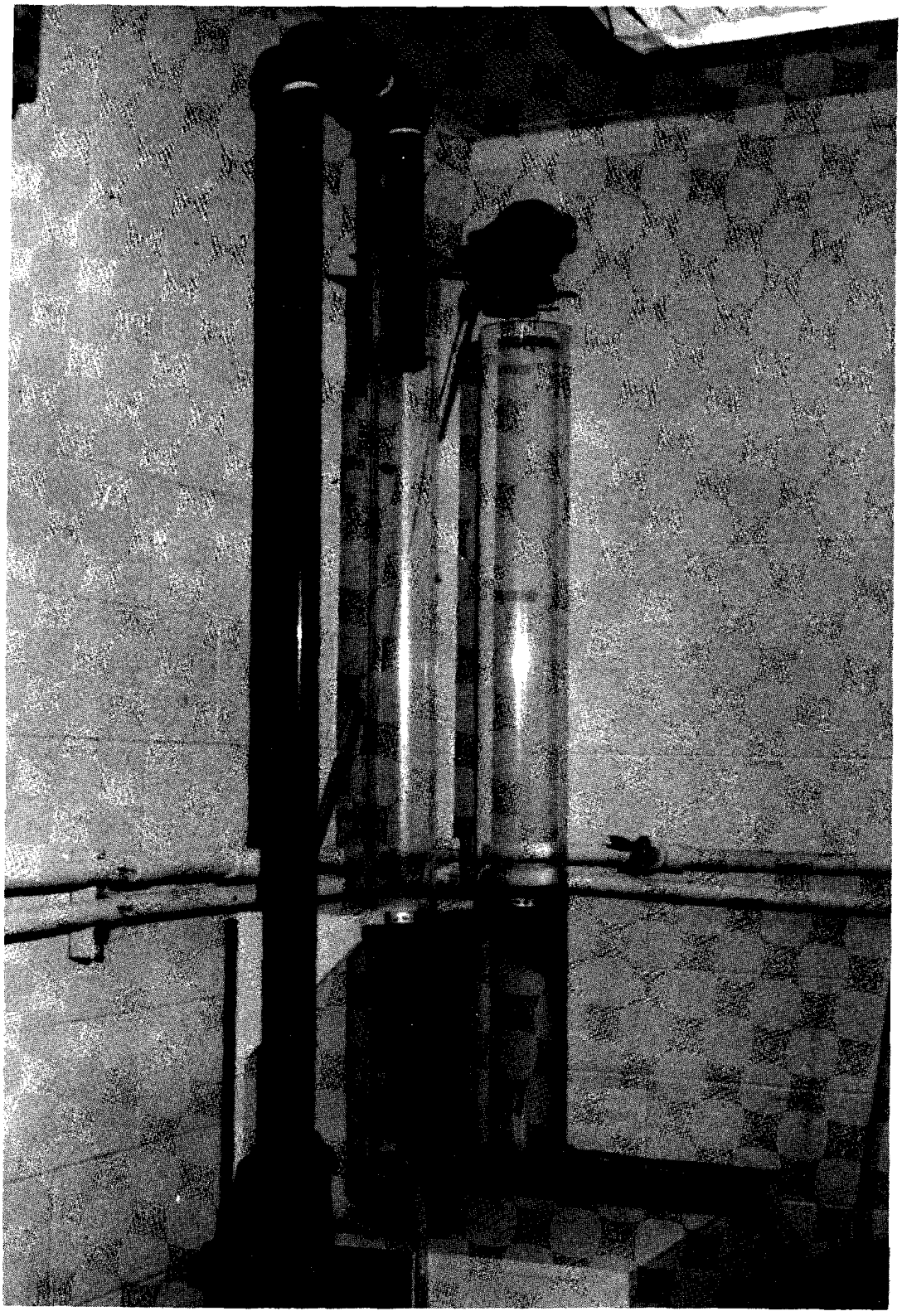

Figure 1. Light Chamber (background) and Current Cylinder (foreground) Used to Measure Swimming Behavior of *P. jordani* Zoeae

light chamber. Four trials were conducted at each light intensity with a total sample size of 24. Older first stage zoeae and third stage zoeae, because of greater visibility, were placed into the chamber at five per trial. One trial was conducted at each light intensity, with a sample size of 30 for each of these latter two zoeal types. Location of larvae in the chamber was recorded at 5, 7, 10, and 12 minutes. Water was changed after each test to avoid a temperature increase.

The cylinder used to ascertain response of zoeae to a water current was vertical, plexiglass, and 110 cm in height by 10 cm in diameter, with a base-mounted pump connected to a seawater source. A vertical upward moving current was used because Gaumer (1969) reported Dungeness crab placed in a horizontal current container did not stay in the current, but instead settled to the bottom and remained there, possibly because of gravity. Flow was regulated by a rheostat. First and third stage zoeae were introduced (one per trial, with five trials at each zoeal stage) through a 1.5 cm hole into the middle of the chamber, half filled with water. The opening was then closed, the cylinder slowly filled with water and velocity adjusted to a flow of 33.8 mm/sec (0.7 knots). About 15 seconds were allowed for acclimation of zoeae to the upward current in the cylinder. Position of the larvae was then recorded at the end of either one and two minutes or when they left the chamber.

RESULTS

Larvae Culture Techniques

Effects of Light on Survival. Zoeae reared to 13 days in light versus dark flasks showed no significant difference in mortality ($X^2_{1d.f.} = 0.19 < 3.84$ n.s.). Survival was 42.3% and 49.2% respectively for light reared and dark reared zoeae (Figure 2). All surviving larvae had reached or passed through the second zoeal stage when the experiment was terminated.

It was more difficult to determine the aeration rate and culture zoeae in the dark flasks.

Effects of Aeration and Lack of Food on Survival. Some aerated unfed zoeae lived to 16 days while non-aerated zoeae were dead by 12 days (Figure 3). However, results were too similar and number of replicates too few to justify conclusions about effects of aeration on unfed larvae survival. Unfed zoeae cannibalized one another. Hence, some unfed larvae reached the second zoeal stage before dying.

Survival of feeding larvae at zoeal stage 6 (maximum age 47 days) was 7.0% and 42.7% respectively in non-aerated and aerated rearing conditions (Figure 4). The experiment on non-aerated feeding zoeae was terminated at stage 6, as only 4 individuals were still alive.

Larvae Emergence

Seven, nine, and ten days were required for complete hatching of eggs from three females (Table 1). Most zoeae emerged within a four day period from each female. Total numbers of zoeae hatched per female were 717, 1083, and 1463 respectively.

Hatching occurred only between 1700 and 0600 hours, with 77.4% of this emergence between 2200 and 2400 (Figure 5).

Hatching was usually accompanied by vigorous beating of the female's pleopods,

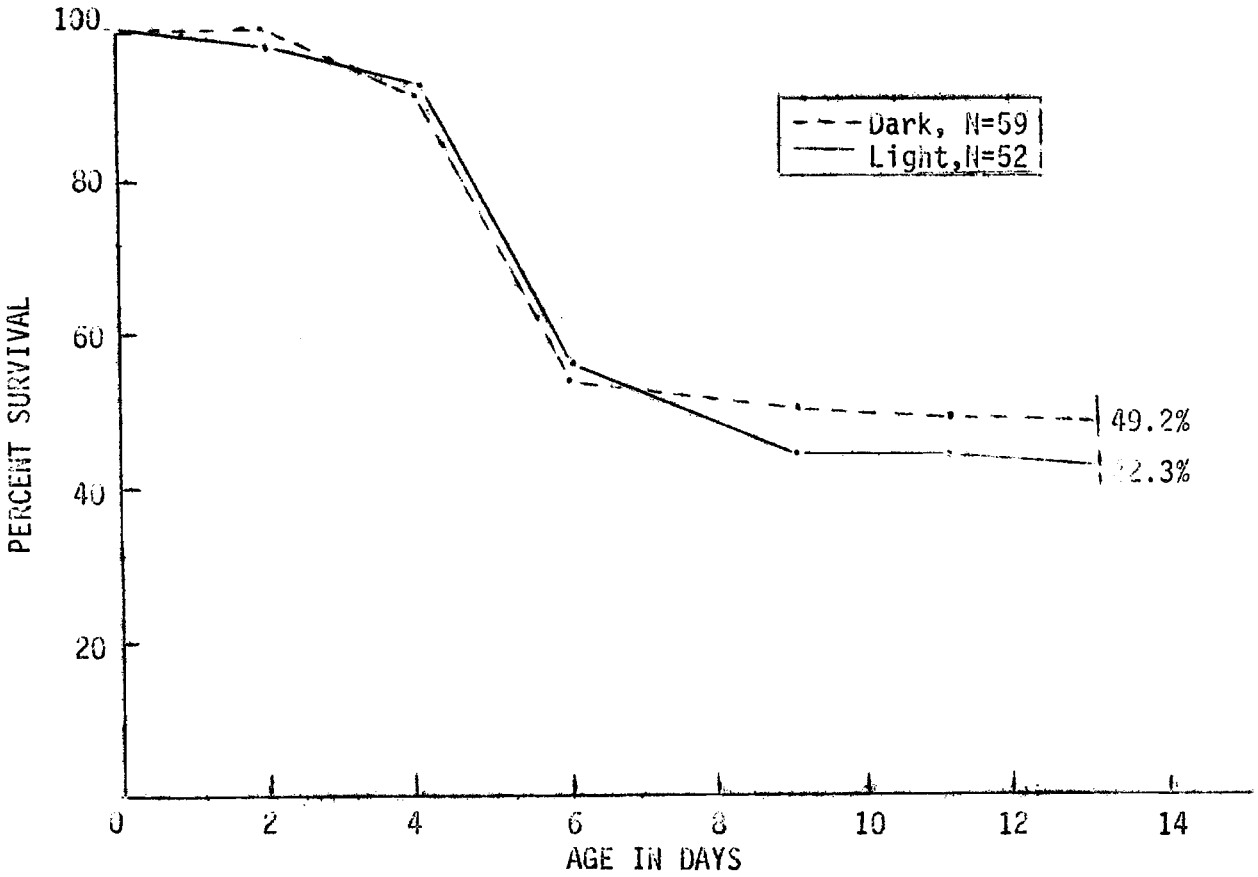

Figure 2. Survival of Newly-Hatched *P. jordani* Fed Larvae Reared for 13 Days In Light Versus Dark Flasks.

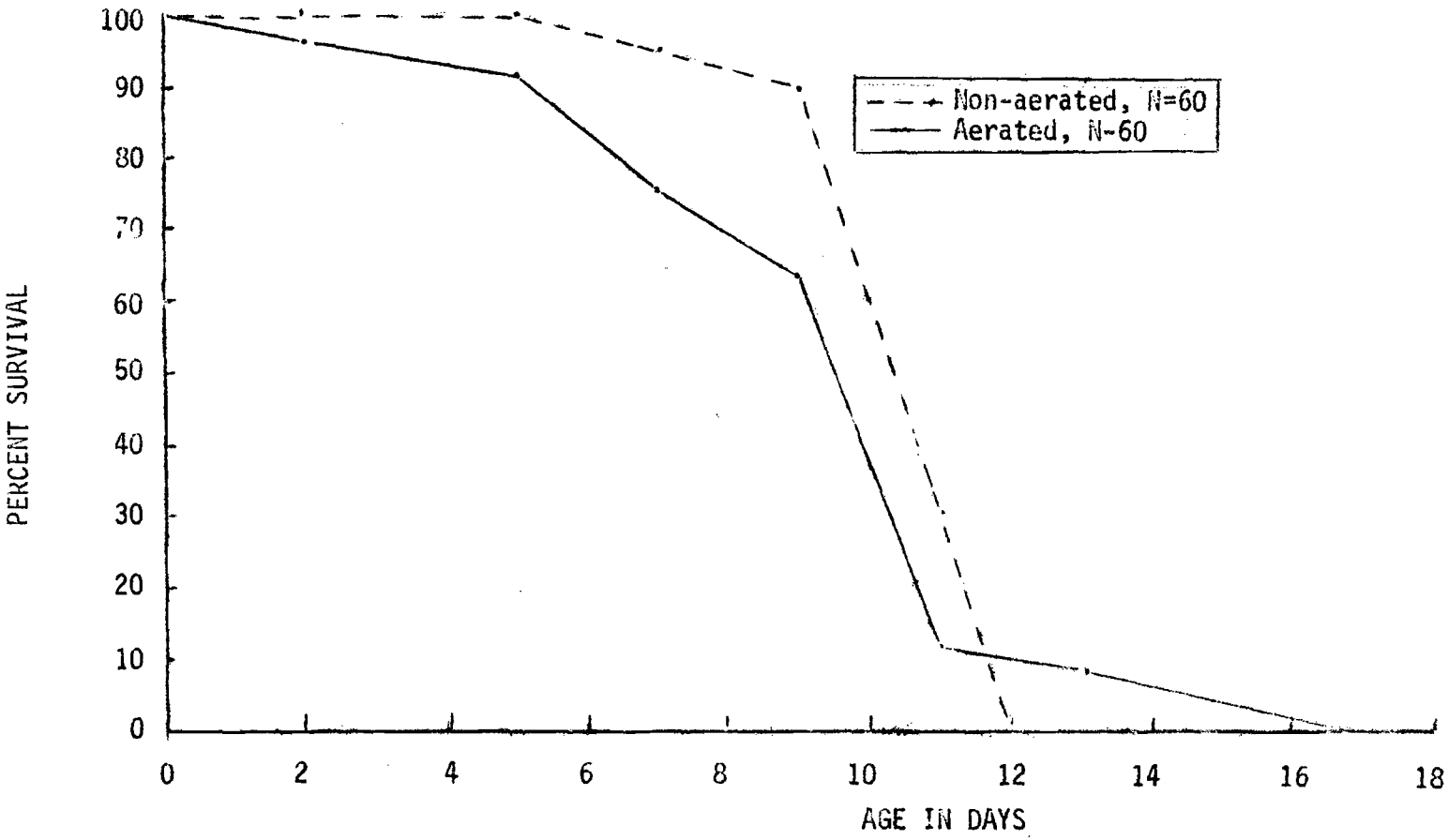

Figure 3. Survival of Newly-Hatched Unfed *P. jordani* Larvae Cultured In Aerated Versus Non-aerated Flasks.

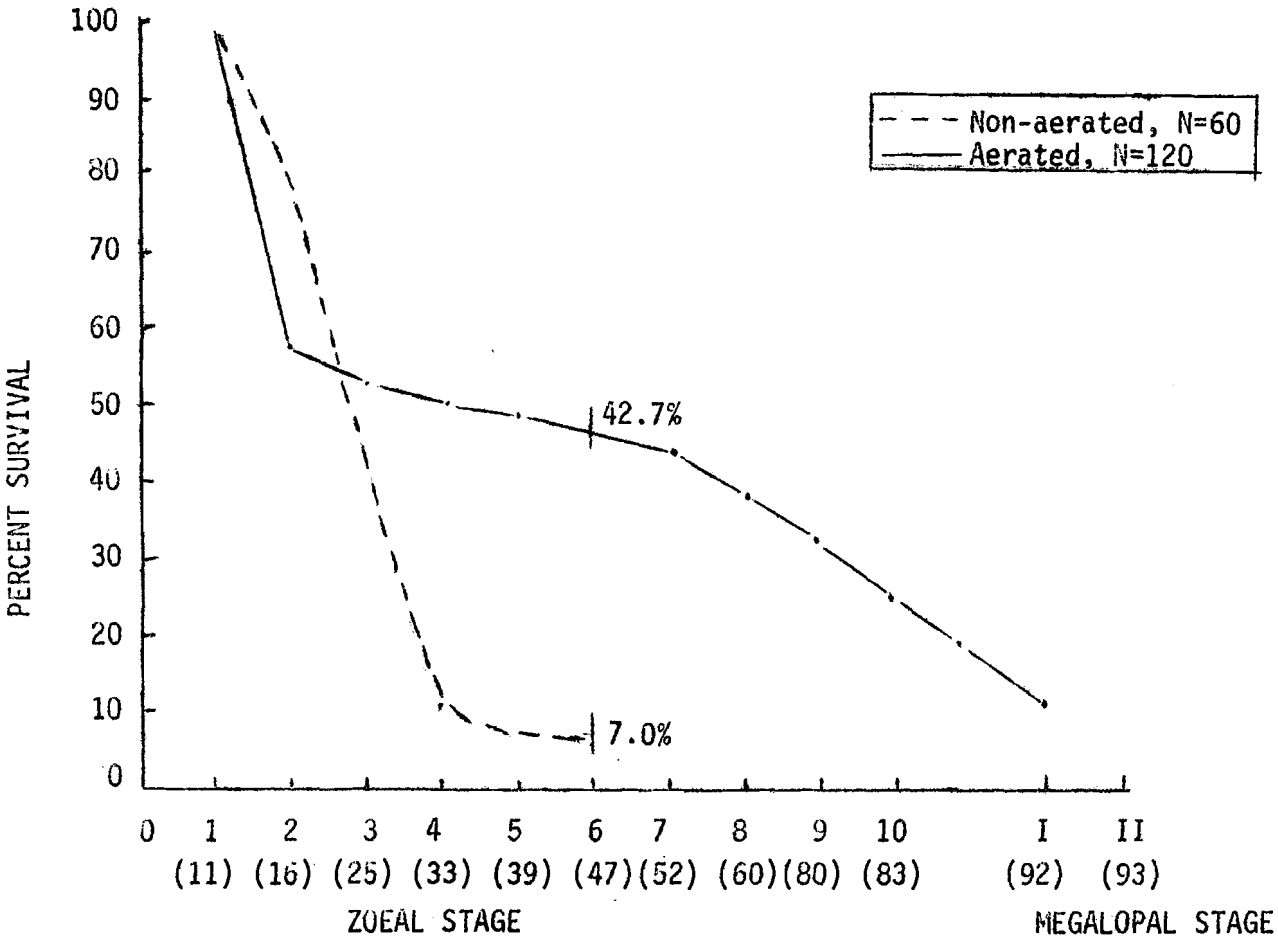

Figure 4. Survival of Feeding *P. jordani* Larvae Cultured From Emergence to a Given Stage in Aerated Versus Non-aerated Flasks, Numbers in parentheses are maximum ages, in days, of each stage.

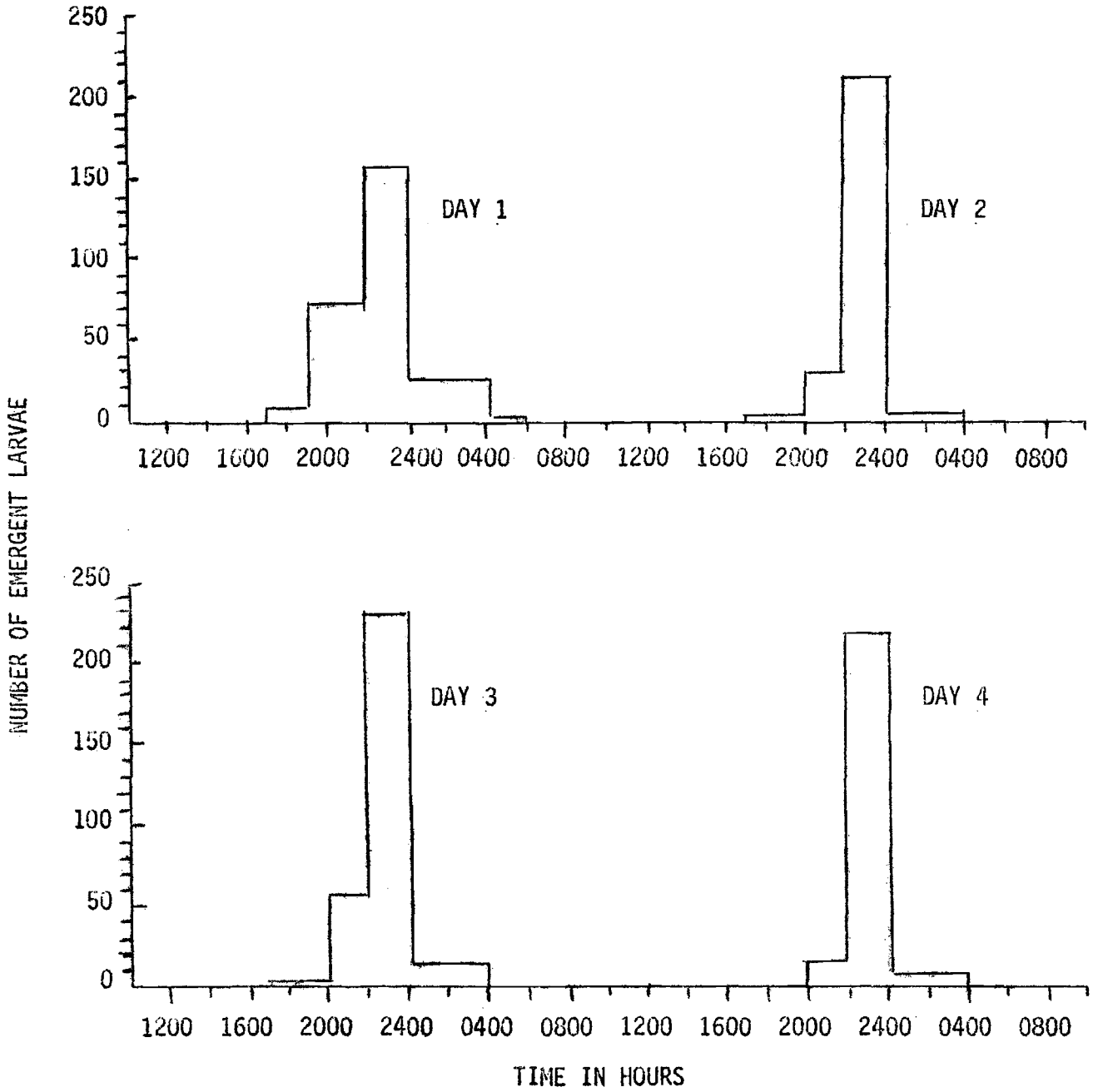

Figure 5. Time of Emergence of *P. jordani* Larvae in the Laboratory Over a Four Day Period

causing large numbers of larvae to be liberated. When each larvae emerged, it swam upward and assumed a vertical position near the water surface with its carapace down and telson up. No pre-zoeae were seen.

Table 1. Number of Days Necessary for Zoeae to Emerge From Eggs of Each of Three Ovigerous Female *P.jordani*.

Day	Number of Emergent Zoeae		
	Female I	Female II	Female III
1	2	14	348
2	83	26	162
3	220	507	150
4	284	693	34
5	238	166	20
6	127	40	0
7	85	6	3
8	40	6	0
9	3	5	0
10	1	0	0
Total zoeae per female	1,083	1,463	717
Female carapace length(mm)	21.5	21.0	22.5

Effects of Light Intensity and Water Current on Behavior

There were no major differential light responses within each of the zoeae types tested, even though responses were statistically different between the three ages of zoeae used (Table 2 and Figure 6). No noticeable variation occurred in the mean reaction to light between the 5 to 12 minute observation period. Individual shrimp however, showed a wide variation in their response to light.

Table 2. Analysis of Variance on Effects of Different Light Intensities on Behavior of Newly Hatched and Third Stage *P. jordani* Zoeae.

Source of Variation	Sum of Squares	df	Mean Square	F
Treatments	2.5104	11		
Light Intensity	0.8758	5		
Zero ft. c. Versus other ft. c.	0.3220	1	(=M.S.)	2.70 n.s.
Zoeal Stages	1.3928	1	(=M.S.)	11.68[1]/
Stages x Lights	0.2418	4		<1 n.s.
Residual	5.0116	42	0.1193	
Total	7.5220	53		

1/ Significant difference between zoeal stages at 1% level.

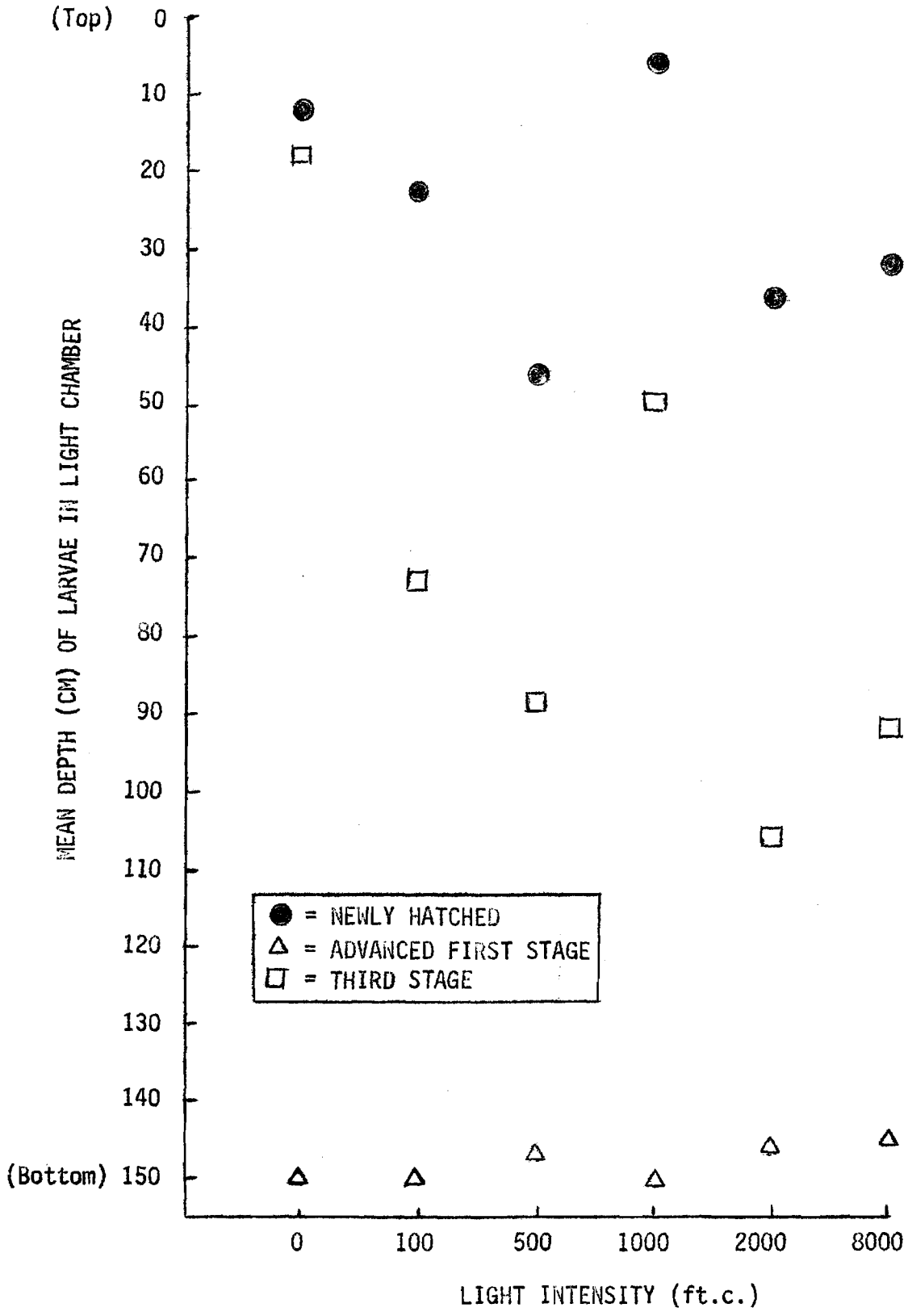

Figure 6. Mean Depth Maintained in a 150 cm Vertical Light Chamber by Three Types of *P. jordani* Larvae Exposed to Six Different Light Intensities. Light Source was at Top of the Chamber.

Newly hatched first stage zoeae primarily stayed in the upper one-fourth of the chamber near the light source at mean depths of 6.1 cm (1000 ft.c.) to 45.5 cm (500 ft.c.). Three-day-old first stage zoeae swam to the bottom of the chamber and remained either on the bottom or near there. Seventeen-day-old third stage zoeae were more scattered in the chamber, swimming at a mean depth of 17.8 cm (0 ft.c.) to 106.8 cm (2000 ft.c.).

The first and third stage zoeae subjected to the upward moving 33.8 mm/sec. current were swept out of the chamber within approximately one minute of release. First stage zoeae had a mean positive movement of 4.2 mm/sec. in the water current used. Third stage zoeae were swimming against the current at a mean velocity of 15.1 mm/sec. All zoeae oriented themselves with carapace down and telson up and actively swam against the current.

DISCUSSION

Results of this study provided no conclusive evidence survival of cultured newly-hatched larvae differed in dark versus clear flasks. This suggests that rearing newly-hatched *P. jordani* in dark containers would not reduce mortality of first stage zoeae cultured in transparent vessels. However, the light-survival experiment was terminated after only 13 days, due to other commitments. Reeve (1969) and Gaumer (1973) found for other decapod larvae a significantly higher mortality in dark containers, attributed to probable increased difficulties of capturing food.

The higher rate of survival of feeding *P. jordani* zoeae in aerated conditions showed future larvae rearing in 500-ml Erlenmeyer flasks should include mild aeration. This agrees with Modin and Cox (1967), who briefly mention no success in culturing *P. jordani* zoeae in non-circulating water.

Results of the aeration experiment on non-fed zoeae suggest laboratory-reared larvae have a maximum of 16 days after emergence to find food before starving to death.

The 7-to-10 day laboratory hatching period of *P. jordani* larvae is identical to what Price and Chew (1972) reported for laboratory held *P. platyceros*. Also, the 4 day peak of larval emergence from each female is the same Kurata (1955) found for *P. kessleri*.

A large number of eggs on female III (Table 1) hatched on the first day of the study, suggesting some of its eggs had hatched previously to collection.

Hatching of *P. jordani* in this study primarily occurred from 2000 to 2400 hours, with 77.4% of this from 2200 to 2400. Peter Rothlisberg (Oregon State University, Department of Zoology, pers. comm.) in uncontrolled lighting conditions, observed *P. jordani* zoeae primarily hatching between 2000 and 0200 hours. Night time preference for emergence has also been reported for other Pacific ocean panalid shrimp (Berkeley 1930, Kurata 1955, Price and Chew 1972).

Results of this larval emergence study suggests that in nature *P. jordani* zoeae are hatched at night. It is probable that night emergence of *P. jordani* zoeae is advantageous to the survival of the species. Newly hatched larvae might have a greater chance of avoiding predation if emergence occurred in darkness.

Lack of larvae reaction to varying light intensities in the light chamber suggest that behaviour patterns were caused by some other stimuli. Gaumer (1973) using Dungeness crab zoeae in the same light chamber, found similar differences between zoeal stages. However, unlike this study, he and Cook (1968) found different responses based on light condition, i.e., a positive phototactic response to low light and a negative response to high light.

The mean swimming velocities found for first stage zoeae (4.2 mm/sec) and third stage zoeae (15.1 mm/sec), though measured in the abnormal condition of an upward moving current, are probably about what would be their maximum velocities in nature, as the larvae actively swam into the current. Gaumer (1973), using the same chamber and current velocity, reported first stage Dungeness crab zoeae swam at 5.8 mm/sec. Steur (1910) found that swimming velocities of five species of European decapod larvae ranged from 10.9 mm/sec. for *Porcellana sp.* to 22.2 mm/sec for *Galathea strigosa*. He reported individuals of a *Pandalus sp.*, which were late larvae, swam at speeds from 14.9 to 20.8 mm/sec.

The laboratory swimming reactions of first stage and third stage *P. jordani* zoeae show them to be weak swimmers, unable to withstand a current of 33.8 mm/sec. This velocity is below or close to the minimum horizontal current reported over the continental shelf in Oregon waters by previous workers, including Wyatt, Burt, and Pattullo (1972) for surface currents; Pillsbury, Smith, and Pattullo (1970) for midwater currents; and, Gross, Morse, and Barnes (1969) as well as Harlett (1972) for bottom currents. The actions of these currents are not fully understood, but it is probable that the initial zoeal stages of *P. jordani*, being feeble swimmers,

are at the mercy of whatever water mass they occupy.

It is hypothesized from the results of this and other studies that oceanic transport can be important in affecting *P. jordani* larval distribution and survival.

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