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CONTROLLED REARING OF DUNGENESS CRAB LARVAE AND THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THEIR SURVIVAL

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CLOSING REPORT

November 16, 1965 to June 30, 1971

By

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CRAB LARVAL REARING STUDY

ABSTRACT

It is possible that the large fluctuations in annual Dungeness crab landings are caused by displacement of crab larvae by currents. We studied the effects of various environmental factors on crab larvae survival and behavior. Tests were conducted on cultured zoeae and megalopae and on wild megalopae.

Our studies showed that the highest survival (93.3%) of crab larvae through the fifth zoeai stage occurred when they were reared in a closed environment. Newly hatched San Francisco brine shrimp, when fed at concentrations of five per ml of rearing water, were the most satisfactory food source. Optimum larvae densities appeared to be five zoeae per 200 ml of rearing water. All cultured crab megalopae were significantly smaller than those larvae collected from Yaquina Bay. Diet concentration appeared to affect size.

Temperature-salinity tests showed that optimum ranges to rear crab larvae are 10.0 to 13.9 C and 25 to 30 $^{\circ}/\infty$, respectively.

We were unsuccessful in rearing Tanner crab larvae through the first zoeal stage using handling and rearing techniques developed for Dungeness crab larvae.

Postlarval Dungeness crabs exhibited a preference for a sand substrate over a gravel substrate and rejected plexiglass and mud bottoms.

Crab larvae displayed variable responses to different light intensities. Generally, the cultured larvae displayed a positive phototactic behavior to low light intensities and a negative response to high light intensities.

Cultured Dungeness crab larvae displayed a passive response to current; whereas wild megalopae swam actively in the current chamber. A swimming capability of 62.7 mm per second was observed for the wild megalopae. Although the wild megalopae exhibited a slight positive rheotaxis, there is no evidence that the larvae at sea would display this response. In considering the speed and direction of the ocean currents adjacent to the Pacific coast, it is reasonable to expect larvae to be transported at the mercy of the currents.

Controlled Rearing of Dungeness Crab Larvae and the Influence of Environmental Conditions on Their Survival

1971 ANNUAL PROGRESS REPORT (Part 1)

INTRODUCTION

Commercial landings of Dungeness crab (*Cancer magister*) in Oregon since 1948 have ranged from 3.5 to 15.0 million pounds and averaged 8.9 million pounds. Changes in the ocean environment at the time larvae are present may be responsible for these drastic fluctuations in the adult crab population. Concern over these fluctuations prompted our laboratory studies to determine what effects selected environmental factors have on survival, growth, and behavior of larvae since this is presumed to be the period of highest mortality. After factors associated with laboratory survival were determined, we intended to monitor ocean environmental conditions to see if these specific factors are related to eventual adult abundance.

As reported in the 1971 preliminary report, Oregon State University is studying the distribution of Dungeness crab larvae off the Oregon coast, one of our anticipated objectives during FY 1972. To avoid duplication of effort, we are concluding our crab larvae studies with this report which is presented in two parts. The first section reports progress made during FY 1971. The second part reviews accomplishments during the entire project period (1966-71).

Objectives during the July I, 1970, through June 30, 1971, study period were to determine the following: (1) the effects of light on growth and survival of Dungeness crab larvae; (2) the effects of aeration on growth and survival of Dungeness crab larvae; (3) the effects of diet concentration and crab larvae density on growth and survival of Dungeness crab larvae; (4) the effects of light intensity on the swimming behavior of Dungeness crab larvae; (5) the effects of water current on the swimming behavior of Dungeness crab larvae; and (6) develop techniques for rearing Tanner crab larvae (*Chionoecetes tanneri*) in the laboratory since determining distribution of crab larvae by species off the Oregon coast can only be accomplished if those larvae collected can be identified.

METHODS AND MATERIALS

Developing Culture Techniques

Dungeness crab larvae were subjected to similar rearing conditions as previously reported (Gaumer, 1969). The only significant change was that I increased the rearing water temperature from II C to 14 C to shorten the rearing period for the larvae. Previous work (Reed, 1967) showed that rearing time could be halved by an increase of 3 C without sacrificing survival.

Survival and growth of crab larvae was monitored through the first five zoeal stages to the first appearance of the megalopa. To reduce the problem of cannibalism, each newly hatched megalopa was removed from the rearing containers and placed into individual 250 ml flasks. Megalopae continued to be fed at the same diet concentrations. Rearing water was changed and larvae fed twice a week. Dead larvae and exuviae were removed with each change of water. All crab larval rearing studies were discontinued after most of the fifth stage zoeae had molted to the megalops stage. The megalopa were then sacrificed and measured from tip of rostral spine to tip of telson, when fully extended, to determine size.

Effects of Light on Growth and Survival. The effects of light on growth and survival of crab larvae was tested by placing 100 newly hatched zoeae (10 per flask) into black painted 250 ml flasks containing 200 ml of rearing water. Ten clear flasks containing 10 zoeae each were used as a control. Larvae were fed a diet of brine shrimp at a concentration of 10 shrimp per ml of rearing water. Caps were placed on all flasks to eliminate dust particles.

Effects of Aeration on Growth and Survival. Tests were made to determine the effects of aeration on growth and survival of crab larvae. Newly hatched zoeae were placed into 250 ml flasks (10 per flask) containing 200 ml of sea water. Larvae were fed a diet of brine shrimp at a concentration of 10 shrimp per ml.

Flasks were aerated by inserting an air line through the top of the plastic covers. Air flow to each flask was regulated by individual valves. The amount of air released was not measured but was regulated so that a continuous series of air bubbles was discharged. Crab larvae reared at 10 per flask in nonaerated flasks were used as a control.

<u>Effects of Diet Concentration and Crab Larvae Density on Growth</u> <u>and Survival</u>. Crab larvae were reared at three diet and larvae concentrations to study combined effects on survival. Newly hatched zoeae were placed in 250 ml flasks at larvae concentrations of 5, 10, and 20 per flask and fed at diet concentrations of 5, 10, and 20 brine shrimp (*Artemia salina*) per ml of rearing water. A control group of unfed crab larvae were reared at concentrations of 5, 10, and 20 per flask.

Rearing Techniques for Tanner Crab Larvae (Chionoecetes tanneri). Berried female Tanner crabs were collected at 300 fathoms in December 1970 by commercial fishermen fishing in the Pacific Ocean adjacent to Coos Bay. These crabs were brought to Newport and held in 7 C water in a closed aquaria. Zoeae were collected as they hatched and placed in 250 ml flasks at larvae concentrations of 5 and 10 per container. These larvae were divided into two groups and reared at 7 and 14 C. Larvae were fed a diet of newly hatched brine shrimp at a concentration of 10 shrimp per ml of rearing water. Rearing water was changed and larvae fed twice a week.

Physical Parameters and Their Effects on Dungeness Crab Larvae Behavior

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Effects of Light Intensity on Swimming Behavior. Work continued to improve our techniques for measuring the effects of light intensity on the swimming behavior of crab larvae. The plexiglass chamber was moved from a horizontal to a vertical position and an adjustable incandescent microscope light, capable of producing 8,700 foot-candles (ft-c) of light, was placed at the top of the chamber. The plexiglass top was removed to eliminate light reflection. A scale marked in mm was placed behind the chamber to measure crab larva movement. The chamber was placed in a darkened temperature controlled room to eliminate incidental light and heat and was filled with filtered and ultraviolet treated sea water at 14 C ±i C. The water was changed after approximately I hour to minimize temperature increase. Crab larva of the 5 zoeal stages (reared at a density of 10 larvae per flask and fed a diet concentration of five brine shrimp per ml) and laboratory megalopa were introduced individually in the top of the chamber and subjected to one of four light intensities (0, 2,000, 4,000 and 8,000 ft-c). The position of each crab larva at the end of 1, 2, 3, 4, and 5 minutes was recorded. Position of the larva in the unlighted chamber was observed at the end of each 1-minute interval by using a flashlight covered with a red cellophane cover.

Effects of Water Current on Swimming Behavior. The cylindrical plexiglass chamber, used in 1970 to measure the effects of water current on Swimming behavior of crab larva, was modified by placing the chamber in a vertical position. The old painted chamber was replaced with a clear one. Water was pumped up through the chamber. Flows were measured by a calibrated flow meter and the pump was regulated by a rheostat. A metric scale was placed behind the chamber to measure crab larva movement. Water

temperatures were maintained at 14 C \pm 1 C. Crab larvae were introduced individually at the center of the chamber through a 15 mm diameter hole. The hole was then plugged and chamber slowly filled. Water velocities were then adjusted to one of three velocities, (0, 33.8, and 55.9 mm per second). Position and movement of the larva at the end of 1, 2, 3, 4, and 5 minutes was recorded at each velocity.

RESULTS

Developing Culture Techniques

Effects of Light on Growth and Survival. Survival of Dungeness crab larvae, reared in dark and clear flasks after 44 days, was 50 and 76%, respectively (Figure 1). This observation was made immediately prior to the molt of the fifth stage zoeae thereby reducing the effects of cannibalism by the megalopae. Difficulties fifth stage zoeae have in shedding their exuvia also contributes to an accelerated mortality during this molt. This accelerated mortality is illustrated in Figure 1 where after 54 days, survival was only 21 and 50% for larvae reared in dark and clear flasks, respectively. Mortalities under each condition were highest between day 47 and 51 and were generally constant throughout the test period although the rate of mortality was higher for those larvae reared in the dark containers.

An analysis of variance showed that survival of zoeae reared in the clear flasks was significantly better than for those reared in dark containers $(F_{.95} = 25.73)$. Differences in survival for the two groups of larvae can probably be attributed to difficulties zoeae had in capturing food in the dark containers. This assumption is partially substantiated by data in Figure 1. Survival of zoeae through day 20 was slightly better for larvae reared in dark flasks. Previous experiments have shown that Dungeness crab

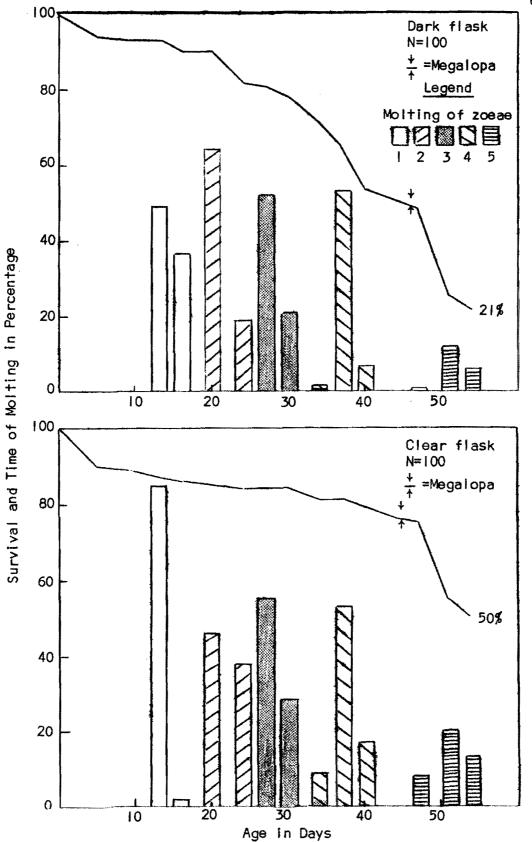


Figure 1. Survival of Dungeness Crab Zoeae (Line) and Molt of Survivors (Histogram) Reared in Dark and Clear Flasks, 1971

larvae can survive 12 days without any food (Gaumer, 1969). Increased nutritional needs as the larvae grow older are probably reflected in the increased mortality for dark reared zoeae after day 20.

Growth as measured by the percentage of the larvae molted per observation period was generally similar for each group of zoeae. The most significant difference in time of molting was observed for the first zoeal stage where a preponderance of the larvae reared in clear flask molted on a single day. The first observed molting of zoeae from the first, second, third, fourth, and fifth zoeal stages occurred on the 13th, 20th, 27th, 34th, and 47th day, respectively, for each group (Figure 1).

The average length of the megalopae reared in clear and dark flasks and wild megalopae collected from Yaquina Bay were 8.6, 8.7, and 11.7 mm, respectively (Figure 2). Megalopae reared last year in clear flasks at 11 C were 8.1 mm long (Gaumer, 1970).

Effects of Aeration on Growth and Survival. Survival of fifth stage crab zoeae reared in aerated and nonaerated flasks after 45 days and just prior to their moit to the megalops stage, was 54.0 and 63.5%, respectively. Survival through the megalops stage was 24.0 and 41.5%, respectively (Figure 3). An analysis of variance showed that survival of fifth stage crab zoeae was significantly higher for those larvae reared in nonaorated flasks (F $_{95}$ = 5.96).

The characteristic increase in mortality during the molt of the fifth zoeal stage was not observed for those larvae reared in nonaerated flasks. These larvae did experience an unexplained increase in mortality during their first molt. Lower survival in the aerated flasks can probably be attributed to the difficulty zoeae had in capturing their food in the moving water.

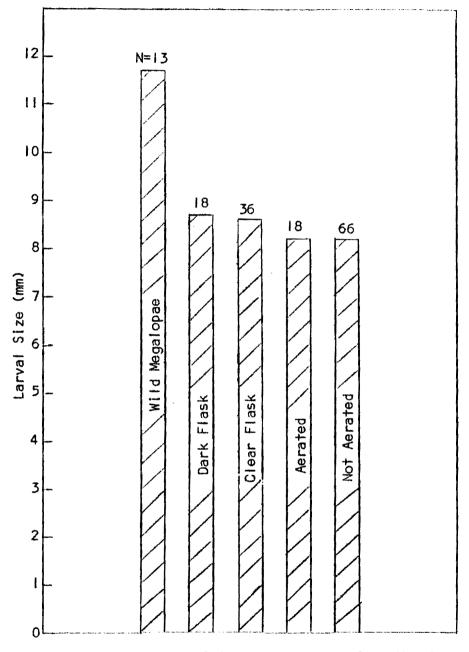


Figure 2. Size Composition of Dungeness Crab Megalopae, by Rearing Condition, 1971

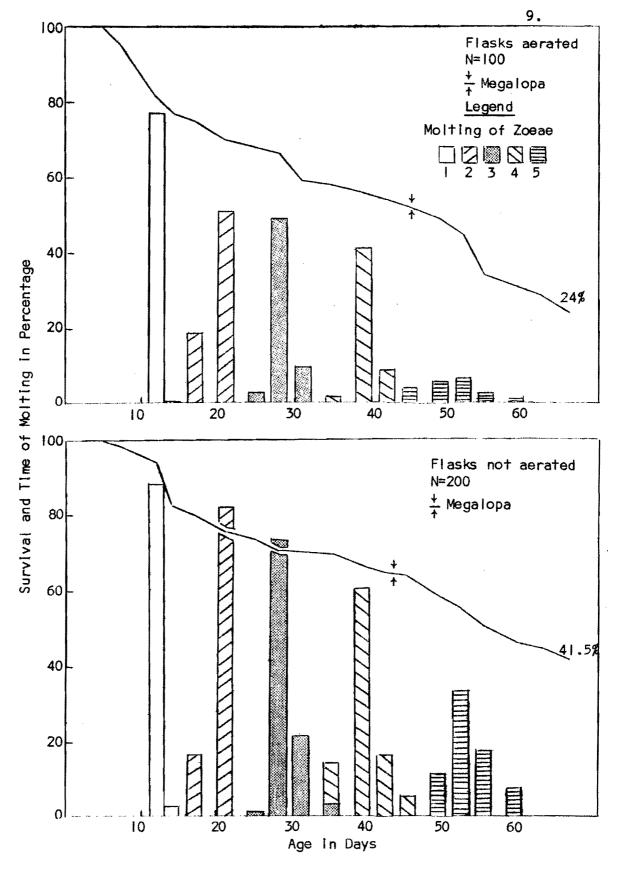


Figure 3. Survival of Dungeness Crab Zoeae (Line) and Molt of Survivors (Histogram) Reared in Aerated and Nonaerated Flasks, 1971

Growth and time of molting were generally similar for the two groups of larvae. The preponderance of molting of each zoeal stage occurred on a single day most frequently for those larvae reared in nonaerated flasks. The first observed molting of the first, second, third, fourth, and fifth zoeal stages occurred on the 12th, 17th, 25th, 35th, and 49th day, respectively for each group (Figure 3).

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Average size of the megalopae reared in aerated and nonaerated flasks was 8.2 mm (Figure 2).

Effects of Diet Concentration and Crab Larval Density on Growth and Survival. Survival of crab larvae through the fifth zoeal stage was highest (88%) for those reared at a larvae density of five zoeae per flask and fed at a diet concentration of five brine shrimp per ml (Figure 4). Survival was poorest (0%) for crab larvae reared at a larvae density of five and 10 zoeae per flask and fed at a diet concentration of 20 brine shrimp per ml (Figure 6). Generally, as larvae or diet concentrations were increased, survival of crab larvae decreased (Figures 4, 5, and 6).

Crab larvae, reared at densities of 5, 10, and 20 zoeae per flask, experienced a high mortality at each density during the first 10 days of rearing when fed at a diet concentration of 20 brine shrimp per ml (Figure 6). Following this rapid die-off, mortality rates leveled off indicating a change in relationship between predator and food organisms. Mortalities at other larvae and diet concentrations were generally consistent throughout the rearing period. Mortalities of larvae fed a diet concentration of 20 brine shrimp per ml at each larvae density were also consistent following the rapid die-off during the first zoeal stage.

All crab larvae in the unfed group were dead by the 14th day. None molted into the second zoeal stage.

Survival of laboratory-reared megalopae was highest (44%) for larvae reared at a density of five per flask and fed at diet concentrations of five and 10 brine shrimp per ml (Figures 4 and 5). All megalopae were sacrificed and measured before molting into the postlarval stage. Generally, the more advanced the zoeal stage, the greater the range in molting time (Figures 4, 5, and 6). No differences in first observed molting were recorded for the first and second stage zoeae. The greatest delay in time of molting was recorded for the fifth zoeal stage. This delayed development is clearly shown in Figure 4 where zoeae reared at 20 larvae per flask and fed five brine shrimp per ml molted on day 59. This delay in molting might be attributed to insufficient diet. Fifth stage zoeae fed at diet concentrations of five and 10 brine shrimp per ml exhibited delays in time of molting with increased larvae density. No fifth stage zoeae reared at five and 10 zoeae per flask and fed at a diet concentration of 20 brine shrimp per ml molted into the megalops stage.

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Size composition of crab megalopae, by larvae and diet concentration, and wild megalopae collected from Yaquina Bay is shown in Figure 7. Due to the small numbers of crab megalopae collected from each group, this measure of larvae or diet concentration on size is questionable. Generally, at each larvae density, size of megalopae was larger at the higher diet concentrations. All crab megalopae reared in the laboratory were smaller than wild megalopae collected from Yaquina Bay. Growth is apparently restricted by the environment that crab larvae encounter when reared in the laboratory.

Rearing Techniques for Tanner Crab Larvae (Chionoecetes tanneri). I was unsuccessful in rearing Tanner crab larvae through the first zoeal stage. Crab larvae reared at a density of five zoeae per flask and 7 C were dead by the tenth day. All crab larvae reared at five zoeae per

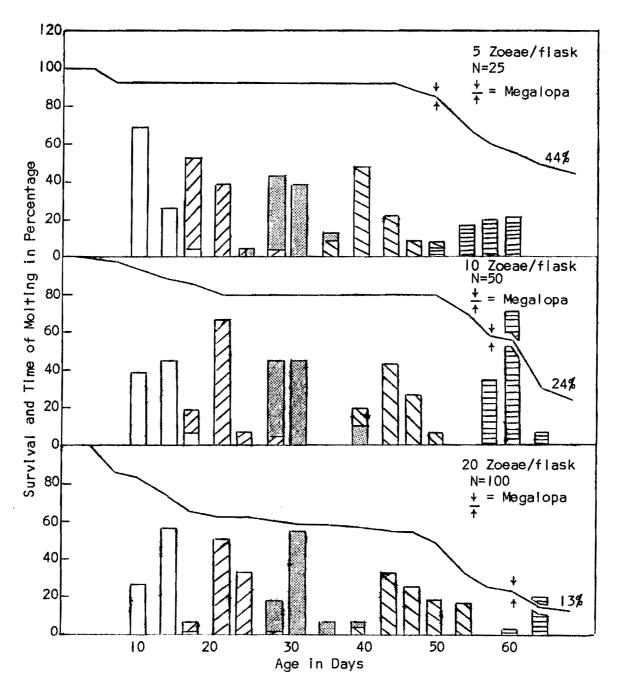


Figure 4. Survival of Dungeness Crab Zoeae (Line) and Molt of Survivors (Histogram) at Three Larvae Densities and Fed Five Brine Shrimp per ml of Rearing Water, 1971 (Legend same as Figure 3)

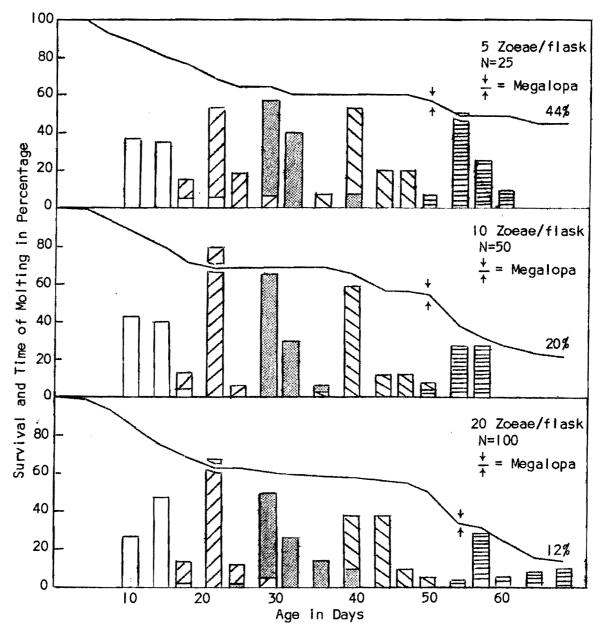
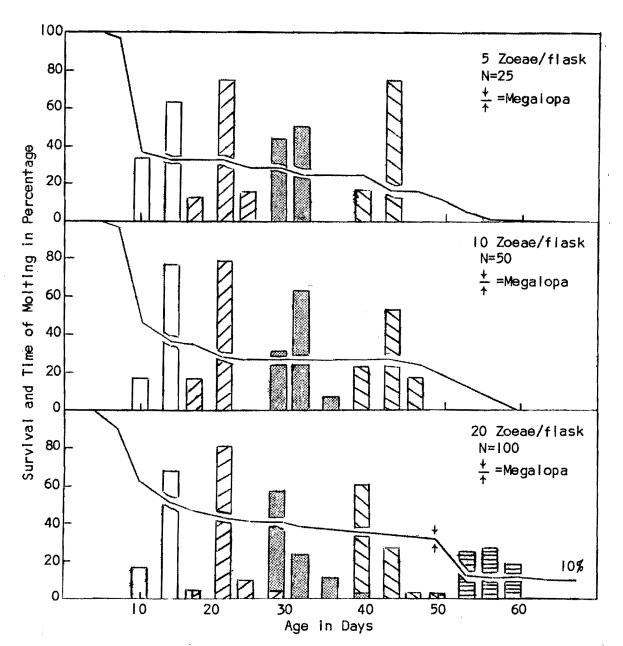


Figure 5. Survival of Dungeness Crab Zoeae (Line) and Molt of Survivors (Histogram) at Three Larvae Densities and Fed Ten Brine Shrimp per ml of Rearing Water, 1971 (Legend same as Figure 3)



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Figure 6. Survival of Dungeness Crab Zoeae (Line) and Molt of Survivors (Histogram) at Three Larvae Densities and Fed Twenty Brine Shrimp per ml of Rearing Water, 1971 (Legend same as Figure 3)

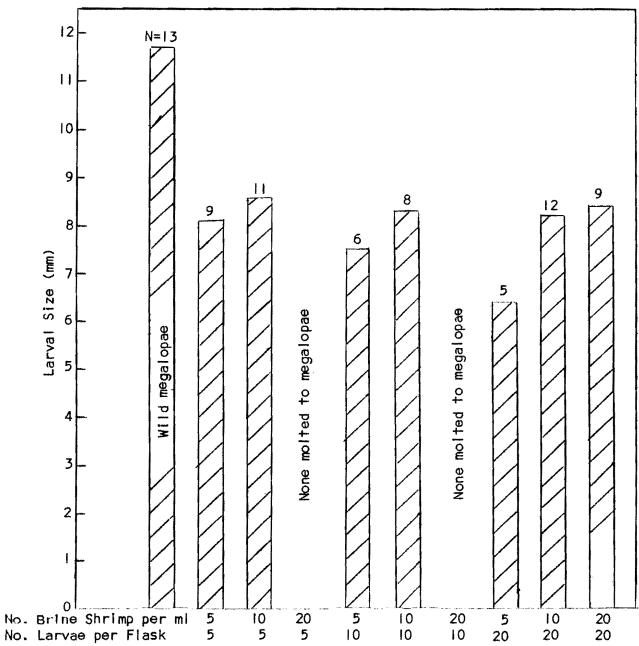


Figure 7. Size Composition of Dungeness Crab Megalopae, by Larvae and Diet Concentrations, 1971

flask and 14 C were dead by the fourth day and at 10 zoeae per flask at 7 and 14 C by the second day. Poor survival of the newly hatched zoeae can probably be attributed to stresses encountered by the gravid female Tanner crabs and/or eggs during capture and shipment to the laboratory.

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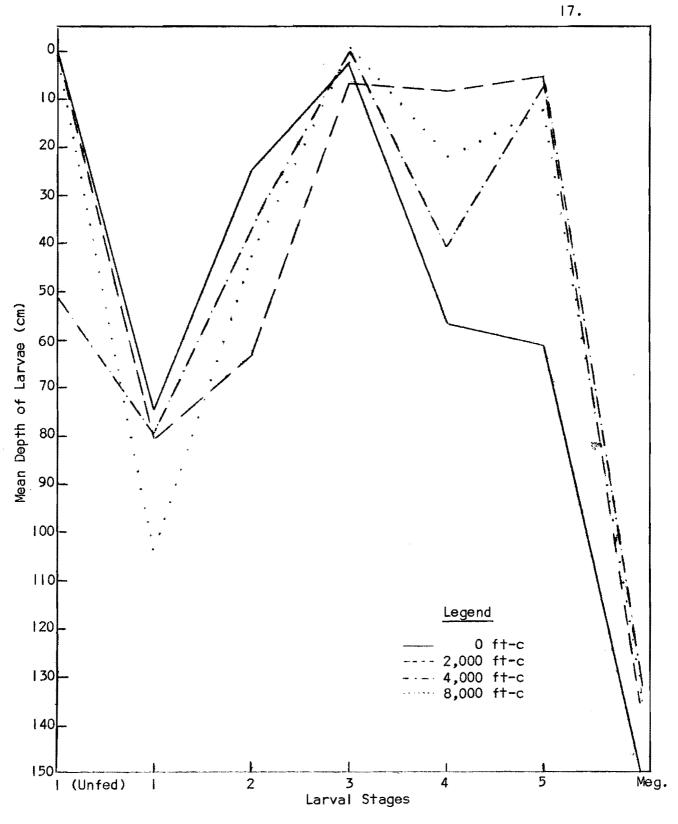
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Physical Parameters and Their Effects on Dungeness Crab Larvae Behavior

Effects of Light Intensity on Swimming Behavior. Results of analysis of variance showed that crab larvae of all stages generally made similar responses to each of the three light intensities (2,000, 4,000, and 8,000 ft-c), but made a significantly different one to a dark environment. Consequently, the experimental lighting conditions were categorized as "dark" (0 ft-c) and "light" (2,000 to 8,000 ft-c).

Figure 8 shows the mean depth preference of crab larvae by stage and light intensity. Unfed first stage zoeae were significantly more surface oriented at each light intensity than the fed first stage zoeae. The data indicate that the fed larvae become strongly surface oriented as they progress in development from stage 1 through stage 3. This trend then reversed so that by stage 5 larvae in the dark chamber behaved in about the same way as they did during stage 1. Fourth stage zoeae, in the lighted chambers, also exhibited this reversed trend but then became strongly surface oriented during the fifth zoeal stage. Differences in depth preference of crab larvae at each light condition was significant only for the fifth stage zoeae.

Response of laboratory-reared crab megalopae to each of the four light intensities was generally similar. Of the larval stages, they exhibited the least positive response to light. A slight but insignificant difference in mean depth preference was noted for those subjected to the three light intensities and those placed in the dark chamber ($F_{.95} = 3.2I$).



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Figure 8. Relationship of Surface Orientation of Dungeness Crab Larvae, by Larval Stage and Light Intensity, 1971

All but one of the megalopae placed in the dark chamber had reached the bottom of the chamber by the end of 2 minutes. At the end of 3 minutes all megalopae rested on the bottom of the light chamber and remained there for the duration of the 5-minute test period.

Effects of Water Current on Swimming Behavior. Tests were completed on only the first stage zoeae and laboratory-reared megalopae due to operational problems encountered with the current-chamber pump. These tests showed that first stage zoeae either swam or settled to the bottom of the chamber at a speed of 2.6 mm per second in 0 water velocity. At water velocities of 33.8 and 55.9 mm per second, first stage zoeae were swept from the current chamber at a rate of 28.0 and 61.1 mm per second, respectively. This indicates a slight positive rheotaxis of 5.8 mm per second for larvae in a water velocity of 33.8 mm per second and a negative rheotaxis of 5.2 mm per second for larvae when placed in a water current of 55.9 mm per second. Last year, first stage zoeae when subjected to a water current of 33.8 mm per second in the horizontal swimming chamber exhibited a positive rheotaxis of 9.4 mm per second (Gaumer, 1970). Part of this difference could be attributed to the change in position of the chamber.

Reaction of laboratory-reared crab megalopae to static water in the current chamber was comparable to that of first stage zoeae. Megalopae settled to the bottom at 2.1 mm per second. At a water velocity of 33.8 mm per second, megalopae generally maintained their position in the chamber. In 1970, crab megalopae exhibited a slightly stronger positive rheotaxis (6.4 mm per second vs. 0.3 mm per second). At a water velocity of 55.9 mm per second the megalopae exited the current chamber at 38.8 mm per second indicating they were actively swimming against the current at a speed of 17.1 mm per second.

SUMMARY OF ACCOMPLISHMENTS, 1966-71 (Part 2)

Successful rearing of Dungeness crab larvae through the megalops stage is a recent accomplishment. Mir (1961) described the first zoeal stage of *(Cancer magister)* Dana. Poole (1966), although experiencing high mortalities in his crab larvae studies, successfully reared and described the 5 zoeal stages and megalops instar of the Dungeness crab. A major portion of our study was determining the parameters that give optimum growth and survival of crab larvae. Laboratory-reared crab larvae were then subjected to various environmental conditions, normally encountered in nature, and their swimming behavior recorded. These studies were considered a prerequisite to a planned field study to determine the factors causing the large fluctuations in annual crab landings along the Oregon coast.

Objectives of the Crab Larvae Rearing Study in chronological order of study are as follows:

- 1. Develop a crab larvae rearing system.
- 2. Develop basic crab larvae rearing techniques.
- Define the effects of wide ranges of temperature and salinity on survival and growth of crab larvae.
- 4. Determine the effects of various laboratory diets and diet concentrations on survival and growth of crab larvae.
- Determine the effects of crab larvae density on survival and growth of crab larvae.
- 6. Determine the effects of diet concentration on cannibalism.
- 7. Determine the effects of light intensity on the swimming behavior of crab larvae.
- 8. Determine the effects of water current on the swimming behavior of crab larvae.

- 9. Determine the preference of postlarval crab for different bottom types.
- 10. Determine the effects of light on growth and survival of crab larvae.
- 11. Determine the effects of aeration on growth and survival of crab larvae.
- 12. Develop techniques for rearing Tanner crab larvae in the laboratory.

Developing Culture Methods

<u>Development of Rearing Equipment</u>. Initially, a semi-closed aquaria system, patterned after one used by Modin and Cox (1967) for ocean shrimp larvae (*Pandalus jordani*) was designed for our Dungeness crab larvae rearing studies (Reed 1966). Crab larvae were reared in compartmented plastic boxes with screened bottoms (202μ Nitex) and suspended in a recirculating sea water system. Sea water for the system was pumped directly from Yaquina Bay, Oregon. Long spines of the Dungeness crab zoeae were caught in the screens necessitating other means of culture. Subsequent studies were conducted in 250 and 500 ml Erlenmeyer flasks containing 200 and 400 ml of rearing water, respectively. Caps on the flasks kept out dust particles.

Two types of closed systems were tried. The first employed the use of a room with temperature control which maintained air temperatures at 36 F (2.2 C) (Reed, 1967). Required water temperatures in the experimental water baths were maintained with thermostatically controlled heating units. Poor working conditions, a result of long exposures of project personnel to these cold air temperatures, led to the purchase of a temperature controlled water bath in 1968. All subsequent crab larvae rearing experiments were conducted in this water bath. Sea water used to rear crab larvae in the closed systems was drawn from Yaquina Bay and was filtered through a Microfloc-polyvinyl chloride filter to eliminate particles larger than 5µ. Ultraviolet treatment was used to suppress bacterial growth. Required salinities for each water change were achieved by dilution with distilled water from a glass-lined still.

Effects of Diet Types on Growth and Survival. Criteria for selecting a suitable food organism for Dungeness crab larvae are: high zoeae survival potential, uniform zoeal growth potential, ease of handling, and availability of the food organism.

Five single organism diets and six combination diets were studied (Reed, 1968) (Figure 9). Size of food organisms are shown in Table I. Small newly hatched Utah brine shrimp were fed immediately to the crab larvae. Large Utah brine shrimp (second stage nauplii) were attained by rearing them for an additional 18 hours. Barnacle nauplii were collected from gravid adults and held for 24 hours at 11 C and 30 °/oo before they were used for food. Mussel larvae were obtained for food by using 0.03 M KCL to Induce adults to spawn. Gametes were than fertilized and embryos were cultured to the shell stage. Basket cockles were ground up with a blender. Particles that passed through a 354 μ screen and were retained by a 155 μ screen were fed to crab larvae immediately. Crab larvae were fed more food than they could consume between feedings. Food concentrations were uniform on any one day but varied from one feeding to the next. A control group of unfed zoeae was maintained to provide a reference point for the different diets.

All zoeae in the control group died by the 14th day (Figure 9). The best survival (100%) occurred when zoeae were fed small Utah brine shrimp. Furthermore, any combination diet containing small Utah brine shrimp resulted

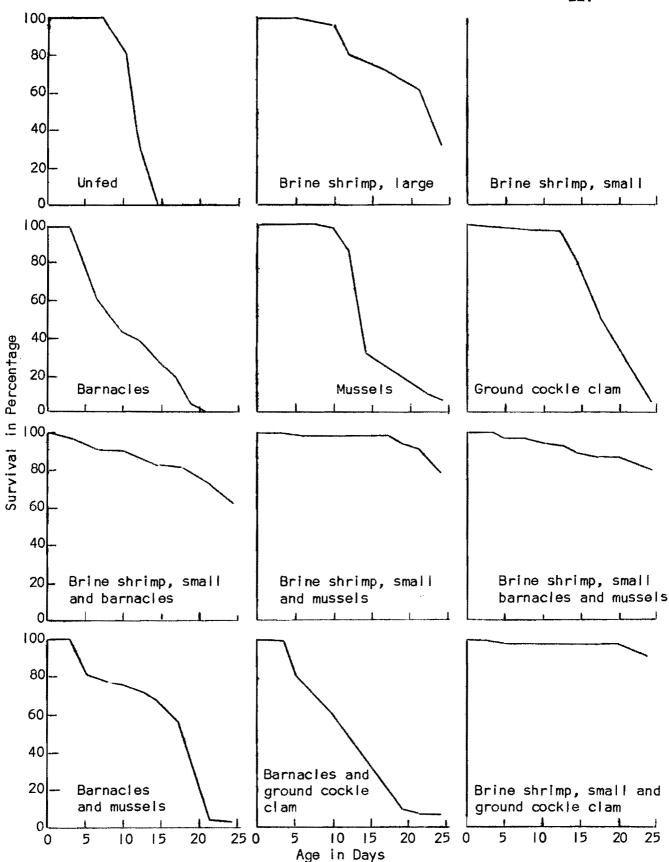


Figure 9. Survival of Dungeness Crab Zoeae Fed Different Diets, 1968

Table I.	Size of	Dungeness	Crab	Larvae	and	Food	ltems	Used	in	Diet	Studies,	1968
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Species	Size Range	Average Size	Remarks			
Dungeness crab ((<i>ancer magister)</i> larvae	2.0-2.3 mm	2.1 mm	First zoea, tip or dorsal to tip of rostral spine			
Utah brine shrimp (Artemia salina) nauplii-large	466-891 µ	761 µ	Body length			
Utah brinə shrimp (Artemia salina) nauplli-small	421-524 μ	479 µ	Body length			
San Francisco brine shrimp (Artemia salina) nauplii-small	365-454 μ	405 µ	Body length			
Barnacle (Balanus glandula) nauplii	354 - 445 μ	417 µ	Body length			
Bay mussel (<i>Mytilus edulis</i>) larvae	86-106 µ	95 µ	Greatest diameter			
Prepared diet, ground basket cockles (Clinocardium nuttalli)	155-354 μ		Screened for size			

in good survival (64 to 91%). No other single or combination diet compared favorably with any of the above shrimp groups. This study was terminated after 24 days since it was apparent that small brine shrimp were well suited as a food organism for Dungeness crab larvae.

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Tests were conducted to determine differences in survival of crab larvae when fed brine shrimp from Salt Lake, Utah, and San Francisco Bay. Shelbourne (1968) reported that survival of plaice (*Pleuronectes platessa*) and sole (*Solea solea*) larvae, when fed Utah brine shrimp, was lower than for those fed San Francisco brine shrimp.

Crab larvae were fed at a diet concentration of one brine shrimp per ml of rearing water. After 38 days of rearing, survival of zoeae, fed San Francisco brine shrimp, was better (46%) than survival of those fed Utah brine shrimp (7%). Growth was also better for larvae fed San Francisco brine shrimp. Moiting of second stage zoeae was earlier for those fed San Francisco brine shrimp. There was no molting of third stage zoeae fed Utah brine shrimp. This experiment was interrupted prematurely when the ecto-commensal protozoan *Vorticella* was found to be infecting the crab larvae. In 6 years of crab larvae studies, this was the only occurrence of this protozoan.

Effects of Diet Concentration and Larvae Density on Growth and Survival. Diet concentration and larvae density studies, using San Francisco brine shrimp for food, were conducted to determine optimum concentrations of food organisms to feed Dungeness crab larvae. Preliminary studies were conducted throughout the 1966-70 study period and culminated with studies reported in the i97i annual progress report.

Survival of each group of crab larvae was compared prior to the molt of the fifth stage zoeae to the megalopa instar. The effects of cannibalism by the megalopae on the fifth zoeai stage was thereby eliminated. Megalopae were killed and preserved for growth studies.

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Survival of Dungeness crab larvae was generally highest when reared at larvae densities of five zoeae per flask and fed at a diet concentration of five or 10 brine shrimp per ml of rearing water (Table 2). Results of one experiment in 1969 showed that 93.3% of the zoeae survived through the fifth zoeal stage when reared at five larvae per flask and fed 10 brine shrimp per ml. In four experiments at this diet and larvae concentration, survival through the fifth zoeal stage ranged from 60.0 to 93.3%. Survival of crab larvae in 1971 was highest (88.0%) for those reared at a density of five zoeae per flask and fed five brine shrimp per ml. No zoeae survived when reared at five per flask and fed 20 brine shrimp per ml. This is perplexing since results of the 1969 study showed an 80.0% survival. Rearing techniques other than an increase of 3 C in water temperature were similar.

No unfed crab larvae molted into the second zoeal stage. Generally, as larvae or diet concentrations were increased above five larvae per flask and 10 brine shrimp per mi, survival decreased. This is possibly due to overcrowding of the larvae in the 200 mi of rearing water.

Variability in time of molting was apparent for crab larvae reared at different larvae and diet concentrations. As larvae densities increased at each diet concentration, time required to molt increased. As diet concentrations increased at each larval density, time required to molt decreased. Generally the more advanced the zoeal stage, the greater the range in time of molting.

	Water Temp.						Larvae -	- Brine	Shrimp	Combinat	ions					
Year	ic;	5/01/	5/1	5/5	5/10	5/15	5/20	10/0	10/5	10/10	10/20	20/0	20/5	20/10	20/20	40/10
1969	11	0.0	63.3	80.0	93 .3	76.6	80.0	10 00 0000								
1970	11	**			70.7					70.0				65.0		11.0
1970	11	-			60.0					33.3						
1971	14	0.0		88.0	60.0		0.0	0.0	80.0	56.0	0.0	0.0	41.0	49.0	33.0	

Table 2. Summary of Survival of Dungeness Crab Larvae by Larvae and Diet Concentration, 1969-71

 $\frac{1}{2}$ First number represents number of larvae per flask; second number represents number of brine shrimp per ml of water.

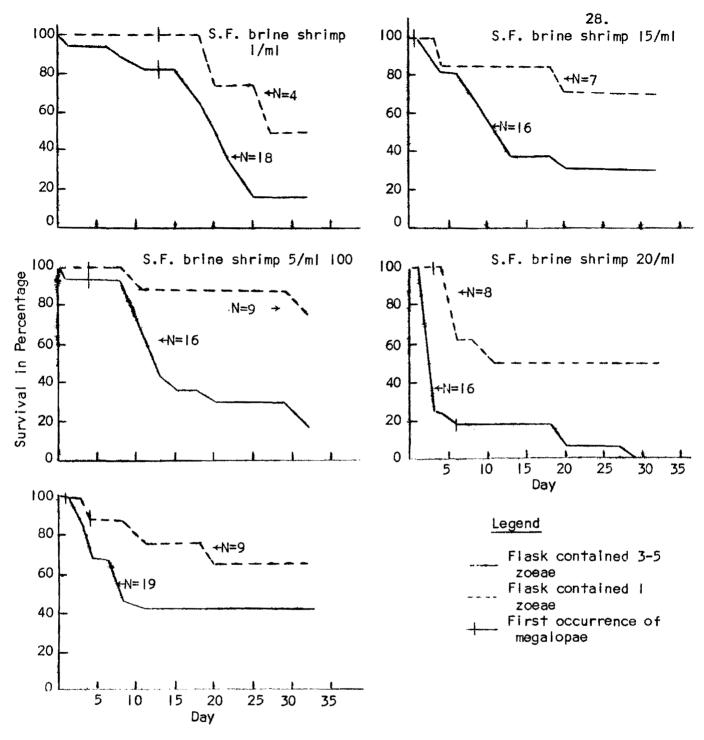
Size of Dungeness crab megalopae reared in the laboratory were significantly smaller than those caught in Yaquina Bay. Mean lengths of megalopae reared in 1971 ranged from 6.4 to 8.7 mm. The smaller megalopae were those reared at higher larvae densities and lower diet concentrations. Mean lengths of the megalopae generally increased as diet concentration increased. Mean length of megalopae caught in Yaquina Bay was 11.7 mm. Growth of the laboratory-reared crab larvae is apparently inhibited by the artificial conditions encountered in the controlled system.

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<u>Effects of Diet Concentration on Cannibalism</u>. Reed (1967) reported that newly metamorphosed megalopae were cannibalistic towards those zoeae remaining in the flasks. To determine the significance of this cannibalism, survival was compared for crab megalopae reared individually and at densities of three to five per flask and fed at diet concentrations of 1, 5, 10, 15, or 20 brine shrimp per ml. All mortalities observed were classified as results of cannibalism although natural mortality and cannibalism could not always be differentiated.

Survival of crab megalopae, after 32 days, was higher at all diet concentrations for larvae reared individually than for those reared together (Figure 10). Highest and lowest survival recorded for megalopa held individually was 78% (fed five bring shrimp per ml) and 50% (fed one and 20 brine shrimp per ml). Survivals of 42 and 0% (fed 10 and 20 brine shrimp per ml, respectively) were demonstrated for megalopae reared in a group. The lower survivals recorded for each group of megalopae at the higher diet concentrations indicate a possible overcrowding.

Effects of Light on Growth and Survival. Studies done in 1971 showed survival of crab larvae reared through the fifth zoeal stage in dark and clear flasks to be 70 and 76%, respectively. Analysis of variance showed



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Figure 10. Effects of Diet Concentration on Survival of Dungeness Crab Zoeae from Cannibalism by Megalopae, 1969

this difference in survival to be significant. Differences in survival can probably be attributed to difficulties zoeae in the dark flasks had in capturing their food. Time of observed molting was the same for the two groups of larvae. Average size of the megalopae reared in the clear and dark flasks was 8.6 and 8.7 mm, respectively.

Effects of Aeration on Growth and Survival. Studies made in 1971 showed survival of crab larvae reared through the fifth zoeal stage in aerated and nonaerated flasks was 54.0 and 63.5%, respectively. Lower survival in the aerated flasks is probably due to the difficulty zoeae had in capturing food in the moving water. Time of molting was generally similar for the two groups of larvae. Crab larvae reared in nonaerated flasks more frequently molted on a single day than those in aerated flasks. Mean size of the megalopae reared in aerated and nonaerated flasks was 8.2 mm.

Effects of Temperature and Salinity on Growth and Survival. The effects of wide ranges of temperature and salinity on survival and growth of Dungeness crab larvae have been published (Reed, 1967). Survival was monitored through the first 5 zoeal stages to the appearance of megalopa. Zoeae were subjected to 25 different combinations of temperatures and salinities. Temperatures of 6.1, 10.0, 13.9, 17.8, and 21.7 C (43, 50, 57, 64, and 71 F) and salinities of 10, 15, 20, 25, and 30 $^{\circ}/\infty$ were chosen.

Survival of crab larvae was not significantly affected by temperatures and salinities approximating those conditions found along the Oregon coast during the zoeal period (9 to 15 C and greater than 30 $^{\circ}/_{\circ\circ}$). No zoeae reared at 6.1 or 21.7 C, regardless of salinity, and at 10.0, 13.9, and

17.8 C at salinities of 10 and 15 $^{\circ}/\infty$ molted into the megalops stage (Figure II). Survival of crab zoeae ranged from 60 to 88% when reared at temperatures of 10.0, 13.9, and 17.8 C and at salinities of 25 and 30 $^{\circ}/\infty$. Survival was highest (88%) when the larvae were reared at 13.9 C and 30 $^{\circ}/\infty$.

An analysis of variance showed that the effects of temperature, salinity, and their interaction on survival of crab larvae were all significant with salinity buffering temperature. At favorable temperatures, unfavorable salinities resulted in complete mortality whereas favorable salinities at unfavorable temperatures allowed some survival.

Growth of the zoeae was directly related to temperature. As temperature increased, the growth rate increased. Larvae reared at 6.1 C and 30 $^{\circ/\infty}$ salinity survived for 125 days but did not complete zoeal development. Time required for complete zoeal development ranged from 105 days at 10.0 C and 20 $^{\circ/\infty}$ to 45 days at 17.8 C and 25 $^{\circ/\infty}$. Salinities between 20 and 30 $^{\circ/\infty}$ varied little in their effect on the growth rate of zoeae.

<u>Rearing Techniques for Tanner Crab Larvae</u>. We were unsuccessful in rearing Tanner crab larvae through the first zoeal stage. Stresses encountered by the berried female Tanner crabs and/or eggs during capture at sea and shipment to Newport probably contributed to the poor viability of the newly hatched zoeae.

Physical Parameters and Their Effects on Dungeness Crab Larvae Behavior

There have been few studies on the behavorial development of Dungeness crab larvae. Consequently little is known of the behavior and ecology of the larvae. One of the reasons for the lack of these studies is the difficulty of rearing large numbers of larvae in the laboratory. In the course of rearing crab larvae for our diet studies responses of larvae when subjected to different bottom types, light intensities, and water currents were also recorded.

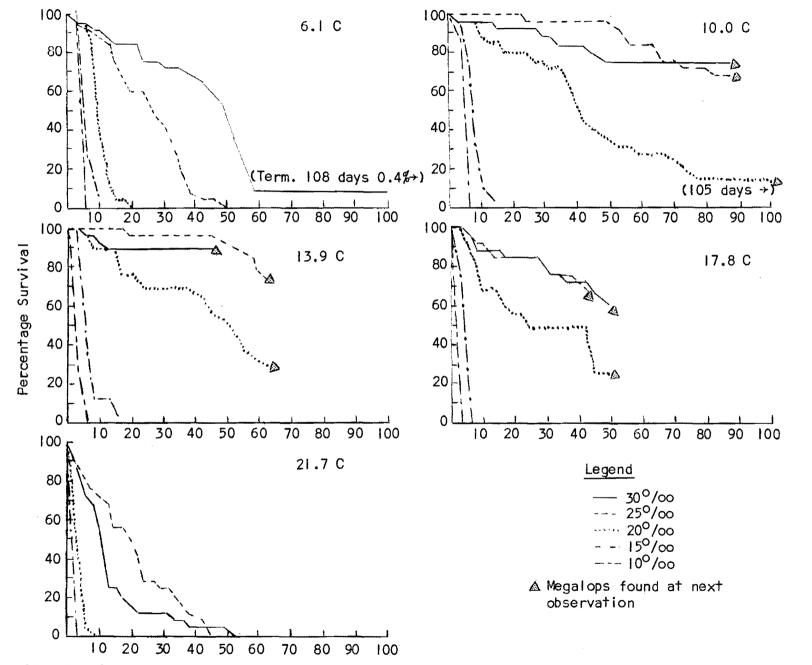


Figure 11. Survival in Number of Days of Dungeness Crab Zoeae Cultured at Different Temperatures and Salinities, 1967

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Preference for Different Bottom Types. Tests were conducted to determine the preference of postlarval crabs for different bottom types. Megalopae were collected from Yaquina Bay and placed in an aquarium with a choice of four substrates (plexiglass, mud, sand, and gravel 26 to 52 mm in diameter). Results of these tests showed that 4 days after release 0.0, 4.0, 32.0, and 64.0% of the newly molted crabs had established positions on mud, plexiglass, sand, and gravel bottoms, respectively. By the lith day, all crabs were located on the sand (60.9%) and gravel (39.9%) substrates. Additional tests were conducted using only sand and gravel as substrate materials. Results of these tests showed that by the third day, 80.8% of the crabs had established a position on the sand bottom.

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> Effects of Light Intensity on Swimming Behavior. Laboratory experiments were designed to test the effects of light intensity on the swimming behavior of Dungeness crab larvae. It was anticipated that the results of these tests would give us insight into the swimming behavior and vertical distribution of wild crab larvae. If behavioral patterns were conclusive then a sampling program could be designed which would eventually assist us in determining distribution of crab larvae at sea.

Results of preliminary studies, using a horizontal plexiglass light chamber, showed that crab zoeae displayed a positive response to light intensities of 25 and 340 ft-c and a negative response to 990 ft-c. Laboratory megalopae exhibited a similar response to these light intensities. Maximum observed swimming speeds of cultured megalopae exposed to 25, 340, and 990 ft-c were 1.9, 0.6, and 0.2 mm per second, respectively. Maximum speeds of wild megalopae at the same light intensities were 19.3, 11.0, and 15.4 mm per second, respectively.

In 1971, the light chamber was moved from a horizontal to a vertical position. This change more closely simulated a water column under natural conditions. In another effort to simulate natural conditions, an incandescent light capable of producing 8,700 ft-c of light was placed at the top of the chamber.

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Crab larvae were subjected to two light conditions: "dark" (0 ft-c) and "light" (2,000 to 8,000 ft-c). Results of these tests showed that unfed first stage zoeae, under both light conditions, were more strongly surface oriented than fed first stage zoeae. Behavior of the fed crab larvae through the third zoeal stage was generally the same at the two light conditions. As the larvae developed, each zoeal stage became more surface oriented. Zoeae placed in the dark chamber were slightly more surface oriented than those exposed to light. This trend then reversed with the fourth and fifth stage zoeae exhibiting a positive phototactic behavior. Differences in mean depth preference of crab larvae in the light chamber, at each light condition, were significant only for the fifth stage zoeae.

Laboratory-reared crab megalopae were exposed to the "dark" and "light" conditions. They exhibited the least positive response of all the larval stages.

Effects of Water Current on Swimming Behavior. A horizontal open trough was originally built to test the effects of water current on crab larvae. Problems with controlling water velocity and air bubbles necessitated changing the apparatus to a horizontal cylindrical swimming chamber. Additional problems of crab larvae settling to the bottom of this chamber and remaining there necessitated changing the position of the tube to a vertical one.

Results of the effects of water current on the swimming behavior of crab larvae were inconclusive. Problems encountered in designing and building a suitable swimming chamber precluded adequate testing of zoeae of each of the larval stages. Response of those larvae tested is presented in the following text.

Swimming behavior of crab larvae in the horizontal chamber was variable. When subjected to a water current of 33.8 mm per second (the slowest speed obtainable with the existing apparatus), crab larvae usually drifted with the current and settled to the bottom. Zoeae once on the bottom generally remained there for the duration of the 5-minute observation period. Megalopae reared in the laboratory generally were able to maintain their position in the water column whereas wild megalopae actively swam back and forth in the swimming chamber.

Swimming speed, measured before the larvae touched the bottom, for the first, second, third, fourth, and fifth stage zoeae averaged 9.4, 7.8, 1.3, 13.1, and 0.54 mm per second, respectively. Laboratory and wild megalopae before touching the bottom exhibited swimming capabilities of 27.4 and 62.7 mm per second, respectively. This difference in swimming behavior precludes equating the swimming capabilities of crab larvae reared in the laboratory with wild larvae in the sea.

Due to operational problems encountered with the swimming chamber pump, swimming tests in the vertical tube were completed only on the first stage zoeae and laboratory-reared megalopae. Larvae were subjected to one of three water velocities (0, 33.8, and 55.9 mm per second).

First stage zoeae settled to the bottom of the chamber at a rate of 2.6 mm per second in 0 velocity. Larvae were swept from the chamber at a rate of 28.0 and 61.1 mm per second when subjected to a current of 33.8 and

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55.9 mm per second, respectively. This is a slight positive rheotaxis to a current of 33.8 mm per second, but a negative rheotaxis at a current of 55.9 mm per second.

Crab megalopae, in static water, settled to the bottom of the swimming chamber at a speed of 2.1 mm per second. In a water velocity of 33.8 mm per second the megalopae generally maintained their position in the chamber. The megalopae, when subjected to a water velocity of 55.9 mm per second, were swept from the swimming chamber at a speed of 38.8 mm per second. This indicates a positive rheotaxis of 17.1 mm per second.

DISCUSSION

Rearing Technique Study

Although our early rearing attempts were marked by only limited success, recent studies resulted in good survival of crab larvae through the zoeal stages. Water quality, temperature, salinity, diet types, and diet concentrations were all determined to be important in determining zoeal survival. Survival as high as 93.3% was achieved with the development of a closed rearing system. Highest survival of crab larvae was realized when they were reared in clear Erlenmeyer flasks containing filtered, unaerated, and ultraviolet treated sea water.

Food Study

Results of this study showed that San Francisco brine shrimp were an excellent food organism for Dungeness crab larvae. The brine shrimp satisfied all the basic criteria desired of a food organism: (1) high zoeal survival potential; (2) uniform zoeal growth potential; (3) ease in handling; and, (4) ready availability.

For optimum survival, zoeae should be reared at a larvae density of five per 200 ml of rearing water and fed a diet of brine shrimp at a

concentration of five newly hatched brine shrimp per ml of water. Rearing water was changed and larvae fed twice a week. Volume of rearing water appeared to restrict the number of larvae that can be reared. Although sample sizes were small, the size of megalopae increased slightly as diet concentrations increased.

Cultured megalopae were approximately 3 mm smaller than wild megalopae collected from Yaquina Bay. Poole (1969) also reported a pronounced difference in size of cultured and wild megalopae. These differences in size concern us since one of the objectives of this study was to rear crab larvae for testing against environmental factors. Due to the size differences, response of cultured crab larvae to different environmental parameters could be significantly different than the reaction of wild megalopae to the same conditions.

Molting time varied for crab larvae reared at different larvae and diet concentrations. As larvae densities increased, or diet concentrations decreased time required to molt increased. This suggests that the planktonic period for wild crab larvae could be extended because of insufficient diet. If this should occur, exposure of crab larvae to predator animals or unfavorable ocean currents could influence survival.

Temperature-Salinity Study

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Optimum ranges of temperature and salinity for cultured Dungeness crab larvae are 10.0 to 13.9 C and 25 to 30 $^{\circ}$ /oo, respectively. Zoeal growth was directly related to water temperatures. Salinities alone had little effect on zoeal growth. Interaction between temperature and salinity was significant. Caution should be used in making statements about either variable independent of the other.

Survival of crab larvae in the laboratory was good at temperatures and salinities approximating ocean ranges of these variables as found along the Oregon coast during the time zoeae are present.

This suggests that temperature and salinity changes alone are not the cause of large fluctuations in zoeal survival. Reduced temperatures and the resulting prolonged zoeal development combined with unfavorable current transport or exposure to predator animals may be determining factors in survival of Dungeness crab larvae.

Tanner Crab Study

I suspect that stresses encountered by the gravid female Tanner crabs in their capture and shipment to our laboratory resulted in low viability of eggs. These Tanner crab were captured by bottom fishermen fishing with trawl nets in approximately 300 fathoms (550 meters) of water off the Oregon coast. After being separated out of the bottomfish catch, the Tanner crab were held on board ship for up to several days before delivery to the laboratory. It appears that special handling procedures are needed to insure delivery of high quality gravid female crabs.

Substrate Preference Study

Given a choice of a sand or gravel substrate, 80.8% of the juvenile crabs selected the sand. Bottom substrates of plexigiass and mud were rejected by the crabs. This behavior was anticipated since Dungeness crabs are typically found on sandy bottom areas of the Pacific Ocean. The crabs seek protection by burrowing down into the sand leaving only their eyestalks exposed. The other substrate materials precluded the crabs from burrowing.

Light Intensity Study

Results of tests showed that Dungeness crab zoeae and megalopae generally displayed a positive phototaxis to light intensities of 25 and 340 ft-c and a

negative response to light intensities of 990 ft-c. Cook (1968) reported a similar response for brown shrimp larvae (*Penaeus astecus*) when subjected to light intensities ranging from 3 to 960 ft-c. Studies by Hardy and Bainbridge (1954) showed that the copepod (*Calanus finmarchicus*) displayed a positive and negative response to weak and strong light, respectively.

Mean depth preference of cultured Dungeness crab larvae by stage and light condition was variable. Unfed first stage zoeae and third stage zoeae were more strongly surface oriented than the other stages. This was not considered a phototactic response since larvae of the same stages placed in a dark chamber were also attracted to the surface.

Of the larval stages, the megalopae exhibited the strongest negative response to the water surface or light. Irving and Coffin (1960) reported that of the larval stages of the pea crab (*Fabia subquadrata*), the megalopae exhibited the strongest negative phototactic behavior. Knudsen (1960) stated that megalopae of four species of newly metamorphosed pebble crabs (*Xanthidae* sp.) exhibited a positive phototactic behavior while older megalopae displayed a negative phototactic response.

Herrnkind (1966) described the swimming behavior of the fiddler crab (Uca pugilator) megalopae as being active during the first week following their molt. Then prior to their molt into the crab stage, the megalopae would settle to the bottom and remain there unless mechanically disturbed or exposed to a strong light beam. This response might be a trait of cultured megalopae. I have observed evidence of contrasting behavior in wild Dungeness crab megalopae. On several occasions, I caught megalopae actively swimming on the surface of Yaquina Bay. I suggest that this could be a premolting activity because, within 24 hours after being placed in running sea water in the laboratory, most had molted into juvenile crabs.

The variability in response of cultured Dungeness crab larvae to different light intensities and depths, limits the use of the results of this study in predicting vertical distribution of wild crab larvae in the ocean. It is reasonable to expect larvae of all the zoeal stages and megalopae to range in depth distribution dependent on light intensity. Their positive phototactic response at low light intensities might suggest that the larvae would approach the water surface at night. The opposite response should probably be expected during the daylight hours.

Current Study

It has been suggested that displacement of Dungeness crab larvae by unfavorable ocean currents could be the cause of annual fluctuations in crab landings. If this displacement can be shown to occur, then careful monitoring of oceanic currents along the Oregon coast could lead to year class predictions.

Results of tests on the swimming behavior of Dungeness crab larvae showed that wild megalopae had a swimming capability of 62.7 mm per second. Duration of this swimming capability was not determined. Equating the swimming behavior of cultured Dungeness crab larvae and wild larvae was not practical because of the extreme variability between the two groups of animals.

Steuer (1910) measured the swimming speed of five species of Decapoda larvae (Galathea strigosa, Galathea dispersa, Eupagurus sp., Pandalus sp., and Porcellana sp.). Swimming speeds ranged from 10.9 mm per second for (Porcellana sp.) larvae to 22.2 mm per second for (Galathea strigosa) larvae.

It has long been recognized that near shore ocean currents along the Pacific coast flow in the direction of the wind. Studies by Wyatt, Burt, and Pattullo (1972) showed that the mean speed of the northward flowing

Davidson current (October through February) off the Oregon coast averages 0.2 knots (103 mm per second). During the period of their study (1961-71), this surface current extended westward 100 miles and occasionally was detected 165 miles offshore to the edge of their study area. These surface currents were reported as far north as 50 and 60° N (southeastern Ålaska). Collins (1968) reported winter currents at 20 and 120 meters depth flowing northward and extending westward 30 miles off the Oregon coast.

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Wyatt, et al., (1972) also reported that the northward flowing surface currents along the Oregon coast shift direction in March and April and flow southward from May through August at a mean speed of 0.2 knots.

Studies by Stevenson, Pattullo, and Wyatt (1969) found that the depth of the southward current off Newport was a minimum of 10 meters deep and averaged 0.18 knots at that depth.

Upwelling caused by strong northwest winds, is a common phenomenon along the Oregon coast during the spring and summer. When this occurs, a southward moving offshore current will establish near the coast (Collins, 1968).

It is apparent from the results of these studies that ocean transport can be extremely important in influencing the distribution of planktonic organisms. Cultured crab larvae, reared at II C, require approximtely 109 days to reach the crab stage (Gaumer, 1969). This should be considered the minimum time required for complete larval development since mean water temperature off of Oregon is slightly lower than II C. We must assume the developmental time for wild megalopae is generally the same as for cultured larvae. From these observations it is apparent that time of hatching, current velocity, and direction govern larvae distribution. Larvae hatching in December would be carried by a northward current during their entire

planktonic period. Since the mean velocity of this current is 0.2 knots (241 km or 150 miles per month), larvae hatched off northern California would settle out of the water column off British Columbia. If these larvae hatched in January, they would be carried to a position off Washington. One month of their larval period could be spent at the mercy of the period of transitional currents. The later the hatching, the stronger the influence the southern summer currents would have on their distribution. It is reasonable to expect that some crab larvae drift northward for a period of time and then southward to their original point of origin.

The early establishment of spring upwelling and offshore southern currents, and a corresponding late crab larvae hatch conceivably has the greatest impact on crab larvae survival. These currents could transport great numbers of crab larvae to a hostile environment of deep, unproductive waters and at the same time subject the larvae to vast numbers of predators.

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