Culture Methods and Effects of Temperature and Salinity on Survival and Growth of Dungeness Crab (*Cancer magister*) Larvae in the Laboratory

PAUL H. REED

Fish Commission of Oregon
Research Division, Newport, Ore., USA

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Recent interest in causes of Dungeness crab (*Cancer magister*) population fluctuations led to a study of temperature and salinity effects on survival and growth of zoeae.

Preliminary work developed methods for culturing larvae in flasks with good survival. A comparison of survival of larvae fed two different diets showed the nauplii of the barnacle *Balanus glandula* and larvae of the bay mussel *Mytilus edulis* were suitable and unsuitable food organisms, respectively.

The optimum ranges of temperature and salinity for laboratory-cultured *C. magister* zoeae were 10.0–13.9 C and 25–30‰, respectively. Zoeal survival was not significantly affected by temperatures and salinities approximating ocean ranges of these variables off the Oregon coast during the larval period. The growth rate of *C. magister* zoeae was directly related to temperature, but salinities that favored survival did not appear to affect the zoeal growth rate.

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INTRODUCTION

THE DUNGENESS CRAB (*Cancer magister* Dana) is the object of an important commercial fishery in Oregon. From 1957 through 1965, annual landings in Oregon ranged from 3.5 to 12 million lb and averaged 7.5 million lb. These figures reflect the magnitude of the fishery and show the range of fluctuations that occur in annual landings. Substantial fluctuations are believed to result from the influence of environmental factors on crab survival. Concern over poor landings prompted studies to determine what effects selected environmental factors have on larval survival and growth. Studies were confined to the larval period since this is presumed to be the time of highest mortality.

Larvae hatch from the abdominal egg mass of females between December and April in Oregon waters. The pelagic larvae pass through five zoeal instars and a megalops instar during a 3-month planktonic period.

Studies of *C. magister* larvae have been restricted to descriptions of larval stages. The first zoea of three species of *Cancer* including *C. magister* have been described (Mir, 1961). Poole (1966) described all six instars of laboratory-reared *C. magister* larvae. Other reports on the larvae of this species consist of occasional observations.

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The objectives of this study were to develop laboratory culture methods and determine what effects wide ranges of temperature and salinity had on survival and growth of *C. magister* larvae.

MATERIALS AND METHODS

Crab

Gravid females were obtained from local commercial fishermen and held in a laboratory tank with circulating salt water. When zoeae appeared, the tank was drained, cleaned, and refilled and first zoea less than a day old were collected for studies. Only zoeae actively swimming at the surface were used.

Food Organisms

The three larval organisms fed to different groups of zoeae during these studies were Utah brine shrimp nauplii (*Artemia salina*), bay mussel larvae (*Mytilus edulis*), and barnacle nauplii (*Balanus glandula*). *Artemia salina* nauplii were cultured at 21 C and at least 30‰ salinity from commercial eggs. Adult *M. edulis* were induced to spawn with 0.03 M KCl; gametes were then fertilized and embryos were cultured to the shell stage following procedures described by Breese et al. (1963). Adult *B. glandula* were scraped off rocks in Yaquina Bay and the ripe dark-brown larval lamellae were collected. The lamellae were maintained in 16 C aerated salt water of 25–30‰ salinity for 1 day to obtain nauplii. Food organisms were cultured for each feeding.

Basic Culture Methods

Salt water for all studies was drawn from Yaquina Bay, Oregon, and was filtered through a Microfloc-polyvinyl chloride filter to eliminate particles larger than 5 μ. Ultraviolet treatment to suppress bacterial growth was employed for the temperature–salinity study but not for the food study. Required salinities for each water change were achieved by dilution with distilled water from a glass-lined still.

Early *C. magister* culture studies were conducted in a system patterned after Modin and Cox (1967) for ocean shrimp larvae (*Pandalus jordani*). *Cancer magister* zoeae were reared in compartmented plastic boxes with screened bottoms suspended in a recirculating saltwater system. Although this method was adequate for *P. jordani*, the long spines of *C. magister* zoeae were caught in the screens necessitating other means of culture. The food organism *A. salina* was also a suspected factor in initial culture problems.

Subsequent studies were conducted in 250-ml Erlenmeyer flasks with 200 ml of salt water per flask. Zoeae were placed in the flasks and mortalities and the presence of exuviae were recorded at the time salt water was changed. Zoeae were examined individually and considered dead if they failed to respond to agitation within a small transfer pipette. Flasks were cleaned with salt water and a brush periodically to prevent the growth of algae. Food organisms were dispensed in equal volumes to flasks after water changes. Enough food was fed so live food organisms remained in the flasks at the next water change.

Food Study

Initial culture problems ascribed to methods, food, or both, led to a search for a suitable food organism for zoeae. *Balanus glandula* and *M. edulis* larvae were chosen as possible food organisms based on size, reproductive timing, ease of handling, and proximity to the laboratory.

Newly hatched zoeae were divided into two groups of 30 each and set in culture flasks, 6 zoeae per flask, in a 16 C controlled temperature laboratory. Salinities ranged from 31 to 34‰. One group was fed *M. edulis* larvae; the second group was fed *B. glandula* nauplii. Water in the culture flasks was changed three times a week and fluorescent lights were on in the laboratory at irregular intervals during the daytime. Survival of zoeae fed the different diets was studied to determine the suitability of each food organism.

TEMPERATURE–SALINITY STUDY

Five water baths were placed in a constant temperature laboratory (3 C) to study the combined effects of temperature and salinity on survival and growth of zoeae (Fig. 1). The baths were galvanized washtubs covered on the outside with fiberglass wall insulation. Each bath contained 45 liters of water and was equipped with a thermostatically controlled heating unit, a water circulating unit, a suspended grate for culture flasks, and a maximum–minimum thermometer (Fig. 1). The baths were covered with transparent plastic sheets to reduce evaporation.

FIG. 1. Water baths in a constant temperature laboratory showing dispensing of food to a culture flask (*top*), and a detailed view of a water bath (*bottom*).

Zoeae were tested at 25 different combinations of temperature and salinity. To test the widest possible response of zoeal survival and growth to these factors, 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‰ were chosen. Salinities above 30‰ were not used because of water supply limitations. Both factors were controlled with as little variation as possible (Table 1). In two instances equipment failures

TABLE 1. Ranges in temperature and salinity values.

Temp (*C*)	6.1	10.0	13.9	17.8	21.7
Temp variation (*C*)	5.0–6.7	8.3–11.1[a]	11.1–15.6[b]	17.2–18.9	21.1–22.8
Salinity (‰)	10	15	20	25	30
Salinity variation (‰)	9.9–10.1	14.8–15.0	19.7–21.1	24.3–25.5	27.7–30.6

[a]Reached 13.3 C but was corrected within 4 hr.
[b]Fell to 8.9 C but was corrected within 4 hr.

resulted in abrupt temperature changes: the 10.0 C water-bath temperature reached 13.3 C and the 13.9 C water-bath temperature dropped to 8.9 C However, both problems were corrected within 4 hr. With the exception of the temperature drop, there was no overlap of temperature or salinity levels. A total of 625 larvae were tested during the study, 25 at each combination of the above factors. The assignment order of larvae to flasks and flask position in the baths was not randomized because the circular bath shape and periodic rotation of flask position was considered adequate to prevent survival and growth variations. Five zoeae were placed in each flask and were not acclimatized to test temperatures or salinities. Salt water in the flasks was changed three times a week for the first 30 days and at least twice a week thereafter. *Balanus glandula* nauplii were used for food. Zoeae were exposed to fluorescent lights for 9 hr each day. Survival and growth of zoeae were studied at test temperatures and salinities to the appearance of the megalops instar but were not studied further because of cannibalism.

RESULTS

FOOD STUDY

The survival of two groups of zoeae fed different diets was compared on the 26th day of identical treatment. A diet of *B. glandula* nauplii provided good survival (50%) whereas the larvae of *M. edulis* resulted in poor survival (10%) (Fig. 2).

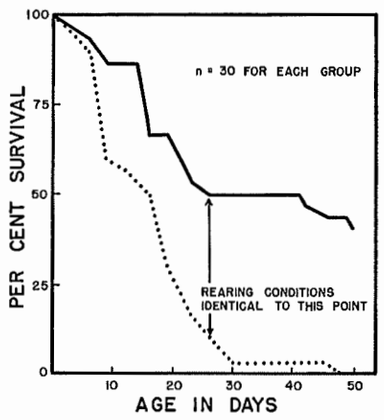

FIG. 2. Survival of *Cancer magister* larvae fed *Balanus glandula* nauplii (*solid line*) and *Mytilus edulis* larvae (*dotted line*).

Survival was analyzed on day 26 because differences in culture methods between diet groups were introduced thereafter. The differences were a maximum temperature variation of 1 C, a maximum salinity variation of 2‰, and a longer interval between saltwater changes for the *M. edulis* diet group. Culture of the two groups with their respective diets was continued beyond the 26th day for the duration of larval development. The *B. glandula* diet group began metamorphosis to the megalops instar on the 48th day with 43% survival and the first postlarval instar appeared on the 76th day with 13% survival. The difference in survival between these stages was caused almost entirely by cannibalism of newly metamorphosed megalopae on the remaining zoeae in each flask. No attempt was made to prevent cannibalism. Zoeae fed the *M. edulis* diet never attained the megalops instar and all died by the 48th day.

Temperature–Salinity Study

Salient features of the study were apparent from a graphical summary of zoeal survival at the 25 temperature–salinity combinations (Fig. 3).

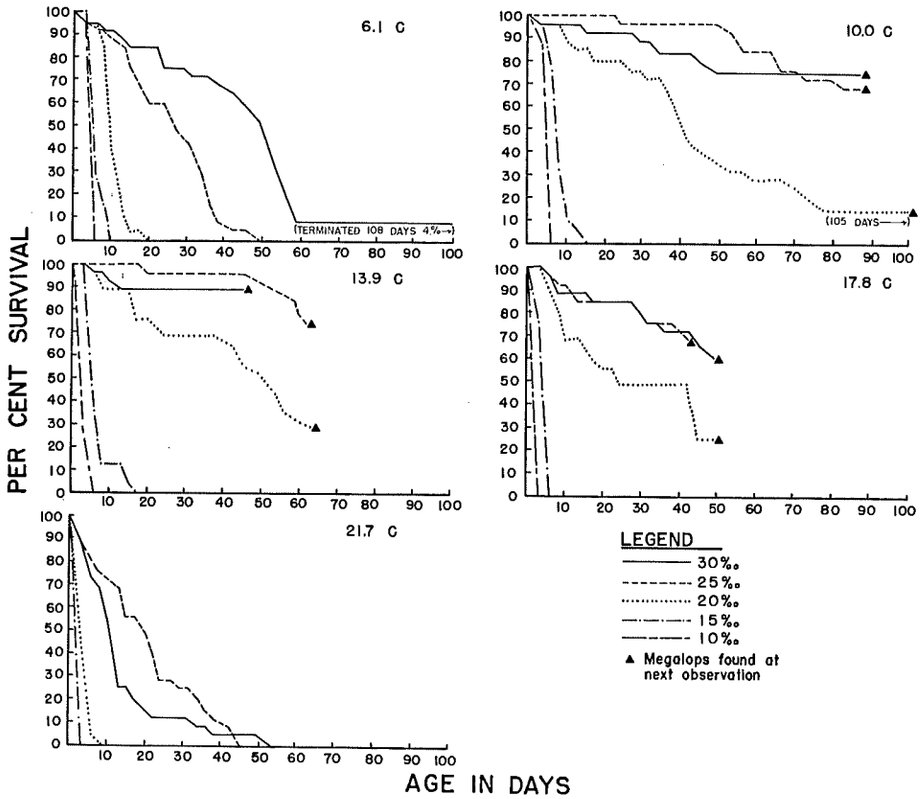

Fig. 3. Survival of *Cancer magister* zoeae cultured at different temperatures and salinities.

Survival at 6.1 C was poor and zoeae were noticeably less active at this temperature. By 48 days zoeae in all salinities except 30‰ had died. After 60 days survival at 30‰ salinity was only 8%. None of the zoeae completed their development at 6.1 C after 108 days of rearing.

Survival at 10.0 and 13.9 C was good at salinities of 25 and 30‰. Zoeae under these conditions reached the megalops instar with 68–88% survival. Zoeae reared in 20‰ reached the megalops instar, but survival was below 30%. Salinities below 20‰ killed zoeae rapidly. Development time to the megalops instar was nearly 90 days at 10.0 C but was reduced to less than 65 days at 13.9 C.

Zoeae cultured at 17.8 C in salinities of 25 and 30‰ survived well. Survival at 20‰ was below 25%. Zoeae cultured below 20‰ died within 6 days. Development time to the megalops instar was reduced to less than 50 days in contrast to cooler temperatures. At 21.7 C all zoeae died within 53 days and none reached the megalops instar.

To appraise the significance of differences in zoeal survival and to test for interaction between temperature and salinity, an analysis of variance was performed on the data at 30 days of culture. The 30-day survival point was selected because survival trends were well established and no megalopae had appeared. The effects of temperature, salinity, and temperature–salinity interaction on survival were all significant (Table 2).

TABLE 2. Analysis of variance of *Cancer magister* larval survival at different temperature–salinity combinations after 30 days of culture.

Variation due to:	Sum of squares	df	Mean square	F[a]
Temp	24,282.0	4	6,070.5	38.4
Salinity	82,378.6	4	20,594.6	130.2
Temp–salinity interaction	20,110.5	16	1,256.9	7.9
Error	15,815.5	100	158.2	
Total	142,586.5	124		

[a] $F_{.05} = 2.5$ with 4 and 100 df; 1.8 with 16 and 100 df.

Survival was ranked by a multiple range test to evaluate the significance of specific differences and define the optimum combination of temperature and salinity. In the following discussion, when two temperatures or two salinities are combined, there was no significant difference in zoeal survival; any stated difference of zoeal survival between temperatures or between salinities was significant. Survival at 10.0 and 13.9 C was better than at any other temperature. Survival at 17.8 C was better than survival at 6.1 or 21.7 C. Survival was poorest at 21.7 C. Salinities of 25 and 30‰ resulted in the best zoeal survival. Survival at 20‰ was poorer than at higher salinities and the poorest survivals occurred at 15 and 10‰. There was a significant in-

teraction between the two factors with salinity buffering temperature. At favorable temperatures, unfavorable salinities resulted in complete mortality whereas favorable salinities at unfavorable temperatures allowed some survival.

The growth rate of zoeae was directly related to temperature within the temperature range 6.1–13.9 C (Fig. 4). As temperature decreased the growth

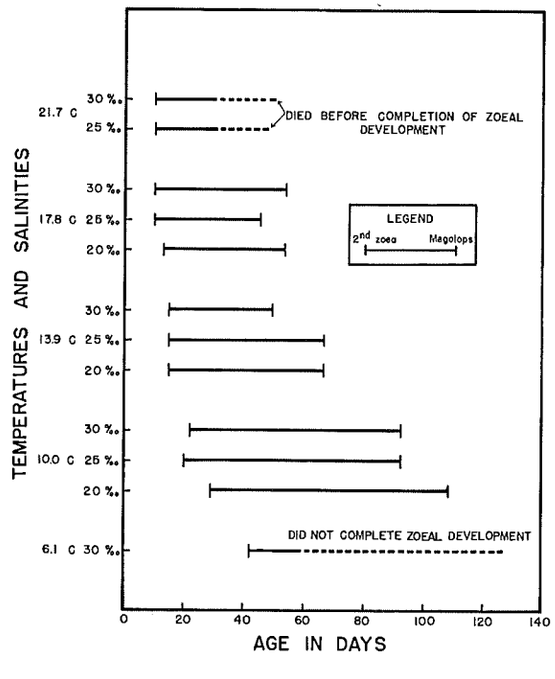

FIG. 4. First appearance of second zoea and duration of zoeal development to appearance of the megalops instar at different temperatures and salinities.

rate decreased; more time was required for both the first appearance of the second zoea and the duration of zoeal development to the first appearance of the megalops instar. Larvae cultured at 21.7 C died before reaching the megalops instar. Some larvae cultured at 6.1 C and 30‰ salinity survived for 125 days but did not complete zoeal development. Zoeae cultured at some temperature–salinity levels (21.7 C at 20, 15, and 10‰; 17.8 C at 15 and 10‰; 13.9 C at 15 and 10‰; 10.0 C at 15 and 10‰; and 6.1 C at 25, 20, 15, and 10‰) did not become second stage zoea. The time required for completed zoeal development ranged from 45 days at 17.8 C and 25‰ to 108 days at 10.0 C and 20‰ salinity. Salinities between 20 and 30‰ appeared to have little effect on the growth rate of zoeae.

It is interesting that the most obvious effect on the growth rate occurred at temperatures that resulted in the best survival (10.0 and 13.9 C). A 3.9 C temperature decrease resulted in zoeal development ranging from 26 to 43 days longer at corresponding salinities. These temperatures are within observed ocean ranges within 40 miles of the Oregon coast during the larval period (9–15 C).

DISCUSSION

Food Study

The results of the food study indicated *B. glandula* nauplii were a suitable food organism for *C. magister* zoeae. Results of the temperature–salinity study supported this evidence with survival of over 80% at optimum conditions through zoeal development. *Mytilus edulis* larvae per se were not suitable food as zoeae died within 48 days and did not complete zoeal development. However, recent work suggested unfed zoeae will not survive longer than 14 days, therefore zoeae must have received some nutritional benefit from *M. edulis* larvae.

Although a diet of *A. salina* nauplii was suspected to be a factor in initial culture failure, further study discounted this idea. *Artemia salina* nauplii were initially cultured for 1 day after hatching began. At the time of feeding, nauplii averaged 752 μ in maximum length compared with 2.1 mm (tip of dorsal to tip of rostral spine) for newly hatched zoeae. By controlling culture temperature at 10 C immediately after hatching *A. salina*, the duration of the first nauplius (475 μ in total length) can be prolonged for 24 hr. If size is a criterion for food preference of zoeae, *A. salina* nauplii (475 μ) compare favorably with *B. glandula* nauplii (ranging from 370 to 420 μ in maximum length) and may be a suitable food organism.

Temperature–Salinity Study

This study shows the optimum ranges of temperature and salinity for laboratory-cultured *C. magister* zoeae are 10.0–13.9 C and 25–30‰, respectively. The significant interaction between temperature and salinity dictates caution when making statements about either variable independent of the other. Zoeal growth was directly related to ocean temperatures observed within 40 miles of the Oregon coast during the larval period. Salinities that favored survival generally had little effect on zoeal growth (exceptions were 25‰ at 17.8 C, 30‰ at 13.9 C, and 20‰ at 10.0 C).

Survival was not significantly affected by temperatures and salinities approximating ocean ranges of these variables found within 40 miles of the Oregon coast during the zoeal period. Thus, the effects of temperature and salinity alone on *C. magister* zoeae appear unable to cause large fluctuations in zoeal survival. The effect of reduced temperature and resulting prolonged zoeal development combined with current transport may be a determining factor in survival of postlarval *C. magister*.

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