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Title: THE EFFECTS OF TEMPERATURE AND SALINITY ON THE EARLY DEVELOPMENT OF ADULA CALIFORNIENSIS (PELECYPODA-MYTILIDAE)

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Jefferson J. Gonor

Adula californiensis, which inhabits mudstone along the open coast and in Yaquina Bay, Oregon, is subjected to extremely varying conditions. Laboratory experiments of a factorial design were used to investigate the effects of temperature and salinity on survival, growth and respiration during early development.

The rate of development to the veliger stage increased from 7° to 20°C. A retardation of developmental rate and increase in abnormalities and mortality occurred with decreasing salinity from 33.2 to 20.4 ppt.

Optimum conditions for survival were estimated by response surface techniques to occur between temperatures and salinities of 9° - 16°C and 29-32 ppt for 3 day larvae, and 9° - 15°C and 31-36 ppt for 15 day larvae. Statistical analysis indicated that the linear and

quadratic effects of salinity were the more important factors affecting larval survival to 3 days of development, while survival of larvae to 15 days of development was most affected by the linear and quadratic effects of temperature.

Preliminary survival data combined with the above 3 and 15 day data produced a better fitted response surface. Optimum conditions for survival were estimated to occur between 12°-16.5°C and 28-32 ppt for 3 day larvae; and between 10°-15.5°C and 29-34 ppt for 13, 15 day larvae. Both the linear and quadratic effects of temperature and salinity were nearly equal in affecting survival to 3 and 13, 15 days of development.

Larvae were reared to 3 days of development at optimum conditions (15°C, 32.2 ppt) and then transferred to experimental temperature-salinity combinations to determine the effect of these factors on later larval development. After 22 days of rearing, survival generally decreased with decreasing salinity but at a greater rate at the higher temperatures. There were no survivors at 20°C at any salinity and at 13.3 ppt at any temperature.

Larval growth was not significant over 22 days of rearing and only small differences in mean lengths were measured for larvae reared in any temperature-salinity combination where there was survival.

Oxygen consumption determined for 72 hour veliger larvae at

various temperatures and salinities generally increased from 7° to 18°C and then sharply decreased from 18° to 21°C. The linear and quadratic effects of temperature were indicated by statistical analysis to be the more important factors affecting oxygen consumption.

Since the early development of A. californiensis requires temperature and salinity conditions near oceanic for survival, successful recruitment of this species in Yaquina Bay depends upon the release of a large number of eggs at a critical period of optimum conditions.

THE EFFECTS OF TEMPERATURE AND SALINITY ON THE EARLY DEVELOPMENT OF ADULA CALIFORNIENSIS (PELECYPODA-MYTILIDAE)

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THE EFFECTS OF TEMPERATURE AND SALINITY ON THE EARLY DEVELOPMENT OF ADULA CALIFORNIENSIS (PELECYPODA-MYTILIDAE)

INTRODUCTION

Adula californiensis (Phillippi) 1847, is a common boring bivalve found both intertidally and subtidally along the west coast of North America from the San Juan Islands, Washington to San Diego, California (Soot-Ryen, 1956). Two populations of this species are present in the lower reaches of Yaquina Bay, Oregon. One is found in an intertidal bench of soft mudstone approximately 3 to 4 miles up Yaquina Bay at Coquille Point (Figure 1). The second population is on the north side of the bay entrance, where at low tide an extensive mudstone area is uncovered. A. californiensis also is found in intertidal mudstone at Boiler Bay, a shallow embayment open to the sea; and small individuals have been found in pieces of mudstone dredged from Yaquina Reef, just offshore from Newport. Since the adults occur in widely varying habitats, they must be capable of adapting to varying conditions.

Recruitment of young to adult bivalve populations is contingent upon the survival and successful settling of the planktonic larval stage. It has been shown by Davis (1958), Loosanoff et al. (1951), and others that the early embryonic stages of molluscs are more

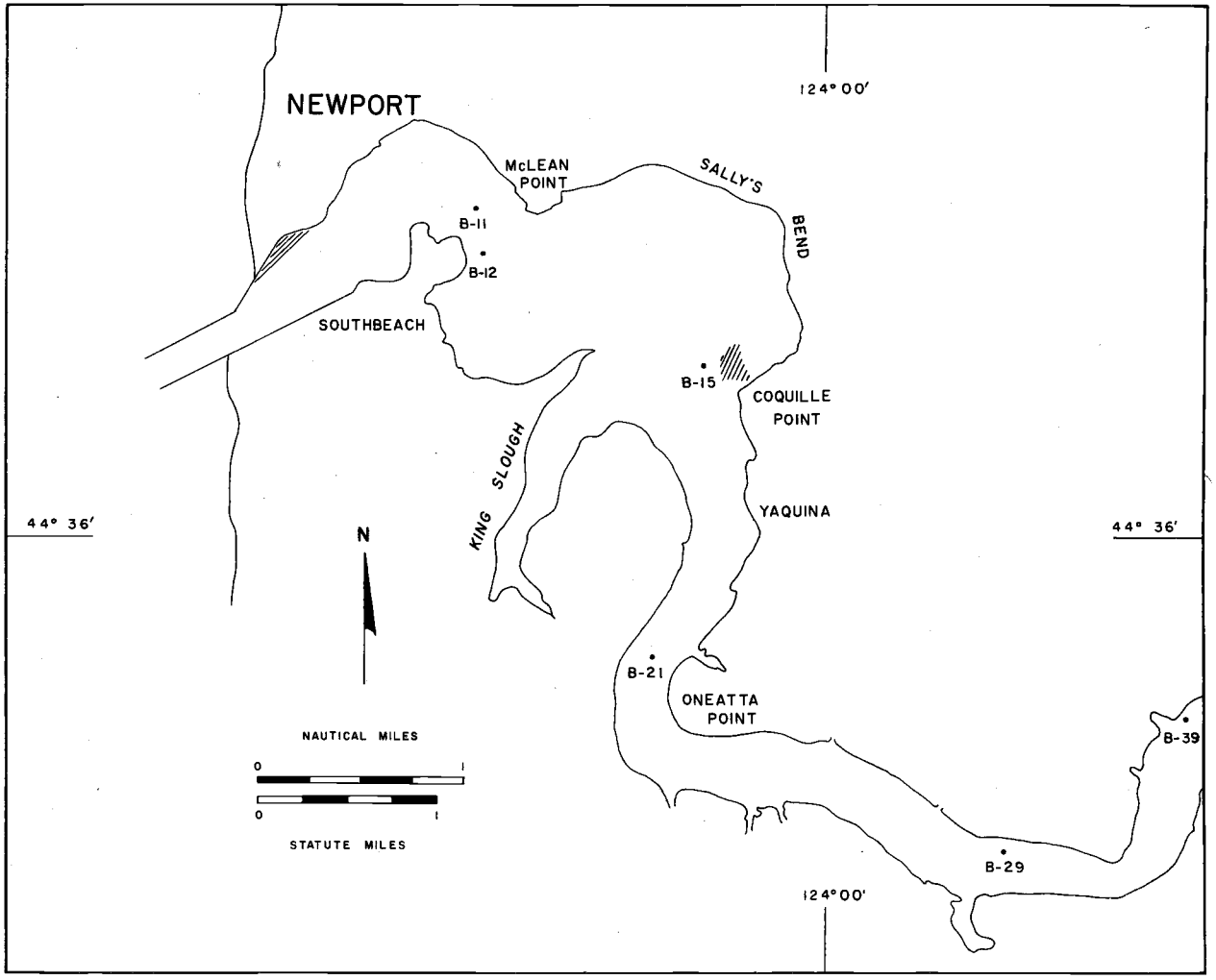

Figure 1. Yaquina Bay, Oregon, showing adult Adula californiensis populations (hatched lines) and buoy stations (B).

sensitive to environmental conditions than the adults making the larval stage the critical link in the life-cycle.

Planktonic larvae originating within Yaquina Bay are probably scattered widely throughout the estuary and many may be flushed out to sea before settlement and metamorphosis can occur. Those larvae retained within the estuary are subjected to extremely varying conditions of temperature and salinity.

This study is an investigation of how temperature and salinity affect the survival, growth and respiration of A. californiensis during its larval development in laboratory culture. Also, an attempt was made to correlate survival and growth with respiration under the same temperature-salinity conditions used for culturing.

Temperature is a major ecological factor regulating the developmental rate, length of pelagic life, and mortality of marine invertebrate larvae. Attempts have been made to relate seasonal sea temperatures to growth and development of molluscan larvae occurring in the plankton (Medcof, 1939; Needler, 1940; Korringa, 1941; Thorson, 1946, 1950; and Carriker, 1961, pp. 212-213).

Because field analysis is not a very precise method, investigators have tried to determine the effects of temperature under controlled laboratory conditions. Seno, Hori and Kusakabe (1926) observed the effect of temperature on the development of Crassostrea gigas from the time of fertilization to the early shelled larvae.

Clark (1935) subjected oyster eggs, sperm and larvae to a temperature range wider than normally found in the environment. With the development of adequate techniques for growing mass larval cultures from fertilization to settlement (Allen and Nelson, 1911; Bruce et al., 1940), it was possible to determine experimentally the effects of environmental conditions. Davis (1949) reared the larvae of Ostrea lurida under controlled temperatures until setting. Loosanoff et al. (1940) and Loosanoff (1951) using Mercenaria mercenaria (Venus mercenaria) were the first to demonstrate the effect of temperature on development throughout the pelagic life of a bivalve. Since then, laboratory studies demonstrating the effect of temperature on development have been made by Dehnel (1955) on prosobranch gastropods, Stickney (1964) on Mya arenaria, Walne (1965) on Ostrea edulis, Bayne (1965) on Mytilus edulis, Sheltema (1962, 1967) on the prosobranch gastropod Nassarius obsoletus, and Davis and Calabrese (1969) on Ostrea edulis.

The relationship of salinity to survival and development of commercially important bivalves has been investigated by field studies (Ryder, 1885; Nelson, 1921, 1931; Nelson and Perkins, 1930a, b; Hopkins, 1931; Rao, 1951; Carriker, 1951, 1961), and more recently by detailed laboratory observations. Bivalves display extremely wide differences in their salinity requirements and in their ability to withstand changes in salinity (Loosanoff and Davis, 1963). Seno,

Hori and Kusakabe (1926) found lowered salinities to have a retarding effect on the developmental processes of Crassostrea gigas. The retarding effect of reduced salinity has been further brought out in studies by Amemiya (1922, 1926, 1928) on the commoner species of Japanese oysters, Clark (1935) on Crassostrea virginica, Rao (1951) on Ostrea madrasensis, and by Nikitin and Turpaeva (1957) on six species of molluscs from the Black Sea. Davis (1958) made the first observations over the entire pelagic larval period on the effects of a wide range of salinity using Mercenaria mercenaria and Crassostrea virginica. Loosanoff et al. (1951) have shown that the eggs and larvae of Mercenaria mercenaria are not able to tolerate as wide a range of salinity as the later stage, a conclusion also reached by Davis (1958) on the same species. Other studies on the tolerance of molluscan larvae to salinity have been made by Young (1941) on Mytilus californianus, Nagabushanam (1961) on Martesia striata, Wells (1961) on various oyster bed fauna, Davis and Ansell (1962) on Ostrea edulis, Stickney (1964) on Mya arenaria, Sheltema (1962; 1965) on Nassarius obsoletus, Bayne (1965) on Mytilus edulis and Wilson (1968) on Xenostrobus securis.

Little experimental work has been done concerning the effects of salinity upon larval behavior. A few investigators have shown that salinity variations may play an important role in vertical movements of the larvae within an estuary (Nelson and Perkins, 1930a, 1931; Prytherch, 1934; Turner and George, 1955; Haskins, 1964;

Harder, 1968).

Recently the importance of the combined effects of these two environmental factors has been emphasized. Kinne (1963, 1964) emphasized the ecological and physiological importance of temperature and salinity, and thus the need to consider these factors jointly. Salinity may modify the effects of temperature and change the temperature range of many biological processes of an organism and, likewise, temperature can modify the effects of salinity. Amemiya (1928) found that growing Crassostrea gigas larvae at the lower end of their temperature range narrowed their range of salinity tolerance. Davis and Calabrese (1964) found that at near optimum salinities the larvae of Mercenaria mercenaria and Crassostrea virginica survived and grew over a wider range of temperatures than near the lower limit of their salinity tolerance. Bayne (1965) found the optimum salinity range varied with temperature for Mytilus edulis larvae.

Early respiratory studies of molluscan larvae by Spark (1929, 1936) and Thorson (1936) were concerned with the role of temperature in metabolic adaptation. The first comprehensive study of respiration in pelagic molluscan larvae was made by Zeuthen (1947). Using Cartesian diver techniques he quantitatively assessed the respiratory rates of several classes of molluscs at different activity levels. Recent investigators have attempted to relate larval metabolism to the ecology of the animal. The rate of oxygen consumption

was used as an index of physiological condition for Teredo bartschii (Clapp) larvae by Lane, Tierney and Hennacy (1954). Jørgensen (1952) calculated high growth efficiencies based on oxygen uptake and growth rates for two different stages of Mytilus edulis and for veligers of two marine gastropods. Walne (1965) estimated the percent of food used in growth and respiration by Ostrea edulis larvae; and found that respiration studies only indicate about half or less of the total food requirement. Millar and Scott (1967) correlated oxygen consumption with the decrease in protein, carbohydrate and lipid content of starved Ostrea edulis larvae.

Both resistance adaptation and capacity adaptation of tropical and temperate zone fiddler crab (Uca) larvae have recently been studied by Vernberg and Vernberg (1964) and Vernberg and Costlow (1966). However, they were only concerned with the influence of one environmental factor, i.e., temperature, upon larval metabolism. Within an estuarine system the interaction of several factors would seem to be important in modifying larval metabolism and thereby be important in determining the distribution of the species. The only study available concerning the interaction of several factors upon larval metabolism appears to be that of Engel and Angelovic (1968) on the influence of temperature and salinity upon the respiration of brine shrimp nauplii.

METHODS

General methods for the culture of marine bivalve larvae are discussed by Loosanoff and Davis (1963), Walne (1964) and Costello (1957). Rearing of bivalve larvae requires sea water that is of proper salinity and free of substances that may interfere with normal development. Because of the sensitivity of larvae to traces of dissolved substances, culturing containers and sea water must be handled with care.

Clean, high-salinity sea water was collected at high tide from Yaquina Bay and filtered through a 40-60 micron fritted glass funnel to remove coarse suspended material. The sea water was then filtered again through Millipore membrane filter (1.2 micron pore diameter) or a 2 micron Whatman Filter Tube to remove clay particles and to reduce the bacterial population. Sea water of reduced salinity was made by diluting the filtered water with distilled water. The final salinity after dilution was determined by the method of Schales and Schales (1941). The filtered sea water was stored in autoclaved 5-gallon glass jugs at 10°C. Pyrex glass flasks of 500 milliliter capacity were used for culturing the larvae. These were soaked in sea water, rinsed in hot tap water and distilled water, capped with disposable beakers, autoclaved and finally rinsed with the experimental sea water just before use.

Four constant temperature water baths were used for holding flasks with larvae. Three of the water baths held the water temperature to ±0.01°C and the fourth water bath used for the lowest temperature held the temperature to ±0.5°C. Temperatures within the culture flasks corresponded to the water bath temperatures. All water baths were located so that only diffused sunlight reached the cultures. Experiments by Walne (1966) have shown light to have little effect on growth of Ostrea edulis larvae, but to have a stimulating effect on spatfall. Except for normal laboratory lighting at night, cultures were exposed to the natural period of day light during the months of June through October, 1968, when the experiments were being performed at the Oregon State University Marine Science Center, Newport, Oregon.

Adult A. californiensis to be used for spawning were collected from the bay mouth population. Shells of the adults were scrubbed to reduce external contamination and placed in individual dishes of filtered full-strength sea water. A 100 watt bulb was used to gradually warm the animals to induce spawning. A majority of the animals spawned when the water temperature reached 17° to 18°C. This temperature corresponded to burrow temperatures taken in the field on a sunny day. A significantly greater number of adults, both male and female, spawned quicker under the 100 watt bulb than those adults allowed to slowly warm to laboratory air temperature. Since the

temperature-salinity tolerances of larvae may be influenced by the conditions at which the adults are living (Davis, 1958; Stickney, 1964), the adults were spawned within 24 hours after being brought into the laboratory. As soon as spawning started the eggs were pipetted from the spawning dishes into beakers of filtered full-strength sea water near 33 parts per thousand (ppt) at 15°C. Preliminary work showed that salinities around 33 ppt and temperatures close to 15°C produced optimum development. The eggs in suspension were then poured through a 120 micron mesh nylon screen to remove detritus and fecal matter. Then the eggs were washed by successive decanting and refilling with filtered sea water of the same salinity. Fertilization was accomplished by adding a few drops of fresh sperm suspension to the eggs, stirring and allowing the gametes to remain undisturbed for 20 minutes at 15°C before being transferred to the experimental conditions.

Preliminary Larval Concentration Experiment

A thick layer of eggs on the bottom of a rearing vessel may result in a sufficient depletion of oxygen and accumulation of catabolic products to retard further development. Over-crowded larvae are also more susceptible to disease. Loosanoff and Davis (1963) found that development of overcrowded bivalve embryos stops at the shell gland stage. The rate of growth in over-crowded cultures is

slower and the time of metamorphosis is correspondingly delayed.

A suitable concentration of larvae for the culture of A. californiensis was determined by growing larvae in concentrations of approximately 25 to 400 per milliliter of culture water. Eggs from a single adult were collected and prepared for fertilization in the manner described above. To estimate the concentration of the original egg suspension, one fourth milliliter egg samples were pipetted into forty vials of formalin with a 1 milliliter tuberculin syringe. The total number of eggs in each of the samples was counted in a Sedgewick-Rafter cell under a 40 power microscope objective. The appropriate quantity of egg suspension was then pipetted with a 1 milliliter capacity Mini-pet into duplicate cultures to produce the desired concentration. One culture of low larval concentration (50 larvae per milliliter) contained 50,000 units per liter of Penicillin G and 100 mg per liter of Streptomycin. The antibiotic mixture was made in water of the same salinity as that used in the experimental cultures. This mixture and concentration of antibiotics was found by Stickney (1964) to be effective in preventing bacterial contamination of Mya arenaria cultures without adverse affects on development.

All concentrations of larvae were reared in 100 milliliters of 33.1 ppt salinity sea water at $15^{\circ} \pm 0.01^{\circ}C$. After 72 hours development at $15^{\circ}C$ the larvae had developed shells and could be handled

without damage. The larvae were sieved with a 54 micron mesh nylon screen, washed into a graduated cylinder, diluted to 100 milliliters, agitated with a plunger, and a 5 milliliter aliquot was taken from each culture with a 1 milliliter Mini-pet syringe. The 5 milliliter sample was preserved with formalin and a total count of normal larvae was later made in a Sedgewick-Rafter cell under a 40 power microscope objective. Larvae in the graduated cylinder were washed back into the culture flask with fresh sea water of the same salinity. A sample was taken for counting and the water changed every 24 hours until the larvae had developed for 120 hours. Immediately after changing the water all cultures were fed Isochrysis galbana every other day starting from 72 hours of development. Algal cultures were grown at room temperature in Malthiessen and Toner (1966) medium. The different larval concentrations received varying concentrations of I. galbana to make the final ratio of algal cells to larvae about 23 to 1. Algal concentrations were determined by counting in a haemocytometer. Every 24 hours the pH value was determined for all cultures. The pH values remained in the range of 7.70 to 7.91.

Over the 5 day rearing period survival was virtually the same for concentrations of larvae ranging from 25 to 400 larvae per milliliter (Table 1). The concentrations of antibiotics used did not appear to have any effect on survival. Therefore, in all further experiments

Table 1. Effect of varying concentrations of larvae on survival in culture.*

Fertilization eggs/ml	72 Hours larvae/ml	96 Hours larvae/ml	120 Hours larvae/ml
24.8	17.4	17.0	13.8
24.8	28.6	22.5	21.4
49.7	52.8	44.4	50.0
49.7	44.2	44.4	44.2
99.4	91.8	86.8	87.6
99.4	82.6	81.2	99.2
198.8	195.8	177.6	184.2
198.8	190.2	185.8	180.0
397.6	385.2	393.4	391.8
397.6	436.25	396.4	397.8
49.7**	54.6	54.6	53.0
49.7**	42.4	38.8	48.6

*All cultures were reared in 100 mls sea water of 33.1 ppt salinity at 15° ± 0.01°C.

**Cultured with antibiotics: concentration of 50,000 units per liter Penicillin G plus 100 mg per liter Streptomycin.

an initial dose of these antibiotics was used in the cultures, and concentrations of 100 larvae per milliliter was considered an adequate number of larvae for survivor counts in the experimental conditions.

Preliminary Survival Experiment

Temperatures of 9°, 12°, 15° and 18°C and salinities of 19.4, 25.8 and 33.1 ppt, producing a total of 12 different environmental combinations, were used to investigate survival of A. californiensis larvae. These conditions occur within the extremes found in Yaquina Bay (Figure 11). Eggs were pooled from 5 adults to insure a response representative of the population. After the pooled egg volume was estimated and adjusted, the eggs were fertilized. One milliliter of the fertilized egg suspension was pipetted to each of the experimental salinity cultures to produce a concentration of approximately 100 larvae per milliliter and transferred to the proper temperature. In this experiment and all future experiments larvae were cultured in 200 milliliters of water at concentrations of about 100 larvae per milliliter. From previous work it did not appear to make any difference whether eggs were transferred into low salinities or by partial dilution. The antibiotic mixture described above was initially added to each culture. All cultures were grown in duplicate.

After 72 hours development the water was changed in each

culture and the larvae were fed equal concentrations of I. galbana every other day. The algal concentrations used at each feeding varied from 2, 680 to 3, 080 cells per milliliter of sea water in the culture flask. A 5 milliliter sample was taken from each culture at the end of the 3rd and 13th day, preserved, and the total number of larvae counted. The 3 and 13 day percent survival was calculated by letting the greatest number of survivors in any of the temperature-salinity combinations of the combined duplicate cultures be 100 percent survival.

The temperature and salinity ranges were not wide enough to produce the graded survival response necessary for the desired statistical analysis (Tables 2 and 3).

Survival and Growth Methods

A 4 x 4 factorial approach was used to produce a graded survival response to temperatures of 7°, 11°, 15° and 20°C and salinities of 20.3, 23.2, 26.2 and 32.9 ppt.

In the first set of duplicate cultures, unequal volumes of eggs were pooled from more than 5 adults. A 5 milliliter sample was taken from each culture at the 3rd and 15th day of development and used to determine survival and growth by the methods used in previous experiments. The mean length after 3 and 15 days in the experimental conditions was determined by measuring the long axis of 15

Table 2. Percentage of larvae surviving after 3 days of development in 12 temperature-salinity combinations.

	9°C	12°C	15°C	18°C
33.1 ppt	84.7*	100.0	93.8	91.3
25.8 ppt	75.6*	95.0	94.6	88.6
19.4 ppt	58.9	35.6*	65.2*	41.4*

Table 3. Percentage of larvae surviving after 13 days of development in 12 temperature-salinity combinations.

	9°C	12°C	15°C	18°C
33.1 ppt	95.0	96.1	96.1	92.9
25.8 ppt	89.4	100.0*	85.3	84.1
19.4 ppt	2.0	3.1	2.5	0

*Only one value.

larvae from each culture under 400X magnification with an ocular micrometer to the nearest 1.53 microns. The algal concentrations used at each feeding varied from 1,220 to 1,430 cells per milliliter of sea water in the culture flasks.

The second set of duplicate cultures differed from the first run in two ways: (1) Equal volumes of eggs from 4 adults were pooled, and (2) all the larvae were fed starting 24 hours from fertilization instead of after 72 hours. The first modification was made to control the variability between egg batches. The second change was made to determine whether the larvae were starving, since larvae at the higher temperatures were reaching the feeding stage before 72 hours. The algal concentrations at each feeding varied from 1,060 to 1,220 cells per milliliter of sea water in the culture flask. During both sets of experiments, variations in salinity between water changes were only ±0.5 ppt. The percent survival and the mean length at the end of the 3rd and 15th day were averaged for both sets of experiments.

An experiment was designed to determine if the later larval stages of A. californiensis would be able to better tolerate a range of conditions once they had passed through early development under optimal conditions. Larvae were cultured from fertilization in 200 milliliters of 32.2 ppt salinity sea water at 15°C, at a concentration of 100 larvae per milliliter. Equal volumes of eggs from 5 adults

were pooled for the experiment. After 72 hours of development, the larvae were transferred to one of four experimental salinities and distributed among four different temperatures. Salinities of 13.3, 19.9, 25.9 and 32.7 ppt and temperatures of 7°, 11°, 15° and 20°C were used to produce a total of sixteen different environmental combinations. All cultures were grown in duplicate. After the 3rd day of development, the cultures were fed algal concentrations varying between 980 to 1,620 cells per milliliter of sea water in the culture flasks. Before each culture was transferred to the experimental conditions and after 22 days in the experimental temperature-salinity conditions, a 5 milliliter sample was removed and preserved for counting. The percentage of larval survival at 22 days was calculated for each temperature-salinity combination using the corresponding 3 day count as 100 percent survival. The percent survival for the duplicate cultures was averaged. The growth increment was calculated by subtracting the mean length of 45 3-day larvae from the mean length of 15 larvae from each experimental condition.

An experimental error of ±8.9 percent survival, including sampling, counting and biological variation, was calculated by taking one standard deviation of the mean survivor count of 32 identical 3 day cultures.

Fertilization and Early Development Experiments

An experiment was done to determine if fertilization would occur under the experimental conditions. Eggs from several adults were pipetted into small dishes of sea water at the same temperature and salinity conditions used for the survival and growth experiments. The eggs were allowed to remain for 15 minutes before one drop of concentrated sperm suspension was added and the culture stirred. Eggs from each culture were checked for cleavage stages 5 and 24 hours after fertilization.

The rate and condition of development to the veliger stage was determined. Eggs from several adults were fertilized at standard conditions (33.2 ppt, 15°C for 20 minutes) and then transferred to the same temperatures and salinities used above. The stage of development and the condition of each culture was periodically checked.

Larval Respiration

The effect of temperature and salinity on survival and growth also may be reflected in respiration of the early veliger larvae. A respiratory experiment was designed to match the 22 day survival-growth experiment. The experiment was a 4 x 6 factorial design employing four salinity levels of 20 percent decreasing dilution and six temperature levels from 7° to 20°C.

Larvae to be used for respiratory studies were cultured to the veliger stage in concentrations suitable for normal development, using 33.0 ± 1.0 ppt filtered sea water at a temperature of 15°C. Eggs were pooled from at least five individuals. After 72 hours of development the veligers were concentrated with a screen and transferred to one of the four experimental salinities of 13.0, 19.8, 26.2 or 32.9 ppt. The maximum range for each salinity was ±0.5 ppt.

A Gilson differential respirometer with a shaking rate of 80 cycles per minute was used for respiration determinations. Three or four milliliters of each larval suspension were then transferred to a respirometry reaction flask containing 0.5 milliliters of 10 percent KOH in a side arm. After a 15 minute equilibration period respiration values were taken every hour for 5 hours at one of the experimental temperatures of 7°, 9°, 12°, 15°, 18° or 21°C. Each run consisted of three flasks of larval suspension of each experimental salinity at one of the experimental temperatures. Only one run was done at each temperature. All larvae were checked for viability before and after each run.

At the end of each respiration run the larvae were preserved with formalin and later washed with glass distilled water to remove sea water. They were then dried at 68°C to constant weight and weighed on a semimicro analytical balance to the nearest 0.01 mg. An experimental weight error of ±22 percent, probably due to sea

water salt residue, resulted from weighing three samples of a known number of larvae. The results are expressed as microliters of oxygen consumed per hour per 10,000 veligers or 1.23 mg dry weight. All oxygen consumption values were corrected to standard temperature and pressure.

Statistical Treatment of Experimental Data

As the experiments were of a factorial nature; a response surface of survival, mean length and respiration could be made to determine which variables were contributing significantly to the variation of the system. It was presumed that the nature of the response surface could be described by a regression equation of the form:

$$Y = b_o + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2$$

where Y = percent survival, mean length or oxygen consumption; x_1 = temperature in ^oC, x_2 = salinity in ppt, and b_o = constant, b_1 = linear effects of temperature, b_2 = linear effects of salinity, b_{11} = quadratic effects of temperature, b_{22} = quadratic effects of salinity, b_{12} = interaction effect between temperature and salinity (Box and Youle, 1955). An analysis was also made for the linear and quadratic effects of oxygen by time, the effect of temperature by time, and the effect of salinity by time, for the 5 hour oxygen consumption experiments. Regression coefficients (b's) were calculated by computer

from the experimental points by the method of least squares. A computer program was designed to print 20 percent contour intervals based on the least squares surface. A 20 percent contour interval was chosen to exclude the ±8.9 percent experimental error calculated for survival.

The polynomial expressions resulting from the analysis of survival, mean length, and oxygen consumption were used to explain which factors may be contributing significantly to variation in the data. Individual regression coefficients were tested using a t-test and the level of significance noted. Those factors below the 10 percent significance level were not considered important.

By using these statistical methods it is possible to estimate the response of the larvae under a greater variety of environmental conditions than is possible through experiments in the laboratory. This response surface technique was developed by Box and Youle (1955) and applied to brachyuran larvae by Costlow, Bookhout and Monroe (1960, 1962, 1966) and Costlow (1967); and to English Sole larvae by Alderice and Forrester (1968).

A survival response surface of this type was estimated for A. californiensis larvae of an early and a late stage of development. The calculated regression coefficients were fitted to a full quadratic equation in temperature and salinity to predict survival over the temperature-salinity range for Yaquina Bay.

RESULTS

Normal Development and Behavior of Larvae

In order to follow the rate and condition of development in the experimental cultures, easily recognizable stages of development at 15°C and 33.2 ppt salinity were selected as guidelines for normal development.

Early development of A. californiensis is basically the same as that of most bivalves with yolk-rich eggs. The eggs are 70 to 80 microns in diameter, pink to orange in color, and very yolky. The spindle fibers can only rarely be seen during cleavage due to the heavy yolk. A fertilization membrane is not apparent.

A few minutes after fertilization a small polar body forms at the animal pole. Cleavages are holoblastic, unequal and spiral. The first polar lobe forms within 1 hour at the vegetal pole, and at the end of 1 1/2 hours has been reabsorbed leaving the CD blastomere larger than the AB. The second polar lobe is formed and reabsorbed by the end of 2 1/2 hours at the completion of second cleavage. Second cleavage is characterized by a large D blastomere and three smaller A, B and C blastomeres of equal size.

Third cleavage begins the characteristic spiral formation of micromeres. Within the next few hours successive cleavages

produce a more or less solid, stereoblastula. Cilia appear in the late blastula stage evenly distributed over the surface. Swimming begins as a slow clockwise roll which gradually becomes stronger and more directional as development proceeds.

Gastrulation probably begins with an epibolic extension of the micromeres, after which an invagination takes place. The stomodael invagination becomes the site of the larval mouth and future adult mouth. The embryo at 15 hours from fertilization has become an early trochophore larvae. The cilia have become regionalized into an anterior trochal ring and a single apical flagellar tuft. A small posterior protuberance with stereocilia has developed at this stage and can be followed through the late veliger stage. No information in the literature could be found concerning this structure so that its nature and function is not known. The trochophore is able to move at different speeds in a straight line, rotating clockwise on its axis. In simple light and dark experiments, no phototaxis was shown by the trochophore or any other larval stage. It was also observed that upon contact with each other or other objects the trochophores increased swimming speed, thereby avoiding the obstacle by rapidly moving away from it.

During the late trochophore stage (31 hours) a rudimentary shell gland has formed opposite the stomodael invagination. The shell gland side of the larva has become flattened and the cilia have

moved anterior to begin functioning as a velum. The small posterior bump and stereocilia appear to have increased slightly in size.

An experiment was conducted to determine if the trochophore larvae prefer one salinity over another, or if they would congregate at an interface as reported for some molluscan larvae by Harder (1968). Water of various salinities (33.2, 29.8, 25.4, 23.2, 19.5 ppt) was pipetted into long narrow vials and water of 33.2 ppt containing 46 hour trochophores was carefully pipetted into the bottom of the vial, displacing the lighter sea water. The entire experiment was conducted at 10°C and in the dark. At the end of 12 hours, the closer the salinity matched the salinity at which the trochophores were grown, the more even was the distribution of larvae in the upper and lower layers. There were no larvae in the 19.5 ppt sea water and there was an even distribution of larvae throughout the column of 33.2 ppt matching sea water.

When the shell begins to enclose the soft parts, the larva has reached the veliger or straight-hinge stage. The trochal ring has developed into a powerful swimming organ, the velum. A small strip of shorter cilia has developed below the long velar cilia and this region is believed to metamorphose into the adult foot. By this time the posterior bump is reduced and not seen in most specimens. It is at this stage that the larvae appear to have two main orientation movements in culture vessels. They normally are observed to

rapidly move directly upward and congregate at the surface film. This behavior may allow them to take advantage of small microorganisms that also congregate in surface films, since feeding of the larvae was also first observed at this stage. The larvae are able to swim directly downward at a rapid rate but show little lateral movement.

By the end of 72 hours, the shell of most of the veliger larvae has completely enclosed the soft body parts. Most of the larvae would eventually rest on the bottom of the culture flask, but would swim when agitated. Environmental conditions determined the time at which this behavior began. Metamorphosis was not observed to occur during 25 days of rearing. Substrate experiments with the veliger larvae were unsuccessful. Substrate selections included algae, mudstone, sandstone and pieces of adult shell. Only a few larvae attached themselves to both sides of Ulva sp., while the majority settled randomly on the bottom of the glass dish. There appeared to be some gregarious behavior by those larvae settling on the bottom of the glass dish.

Effects of Temperature and Salinity on Early Development

Fertilization was found to occur in all temperature-salinity combinations used except for the 7°C and 20.4 ppt combination, where about 60 percent of the eggs did not appear to be fertilized

at the end of 5 and 24 hours. In all other temperature-salinity combinations 100 percent fertilization occurred.

The rates of development for larvae grown in the experimental temperatures and salinities are graphically represented in Figures 2 through 5. Four easily recognizable stages were selected to serve as guidelines of development. A qualitative judgment was made on the predominant larval stage and degree of developmental normality. If more than 50 percent of the sample was at a particular stage, the sample was counted to be at that stage. The ordinate scale was determined by the length of time required to reach each stage of development at 15°C, expressed as a percentage of the total time.

An analysis of these results shows that the rate of development of A. californiensis increased with increased temperature at all salinities. As the temperature increased from 7° to 20°C, the time of development to the veliger stage decreased from 88 to 25 hours in sea water of 33.2 ppt. At 20.4 ppt salinity, the time of development to the veliger stage decreased from 90 hours at 11°C to 40 hours at 20°C. The difference in rate of development was greater between 7°C and 11°C than between 15° and 20°C. With decreasing salinity there was a retardation in developmental rate and an increase in abnormalities and mortality. The retardation of the developmental rate in the lower salinities was greater at the higher temperatures. In salinities below 26.3 ppt a marked increase in abnormalities

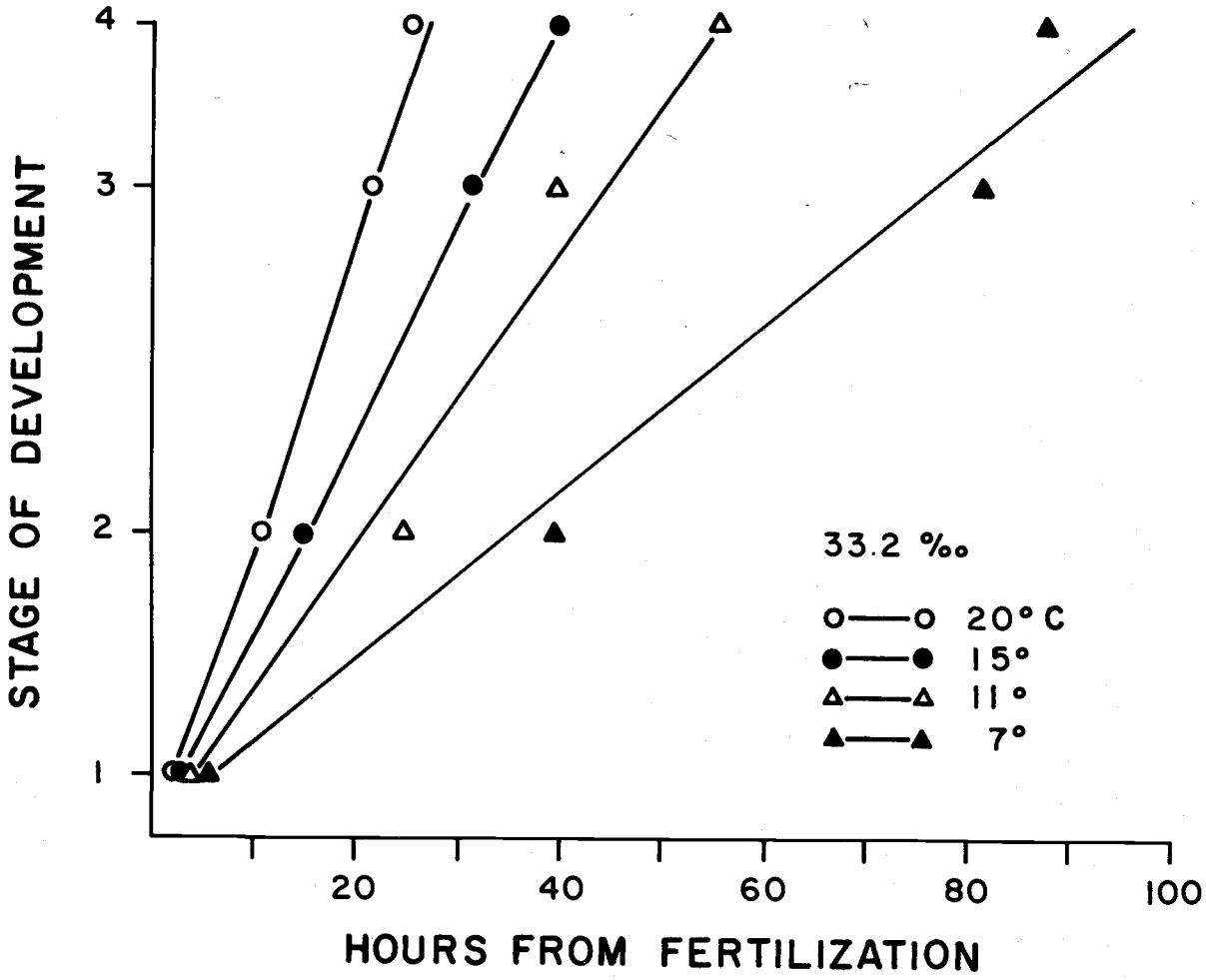

Figure 2. Effect of temperature on the rate of early development of Adula californiensis in 33.2 ppt salinity. Stage 1, second cleavage; stage 2, early trochophore; stage 3, late trochophore; stage 4, veliger larvae.

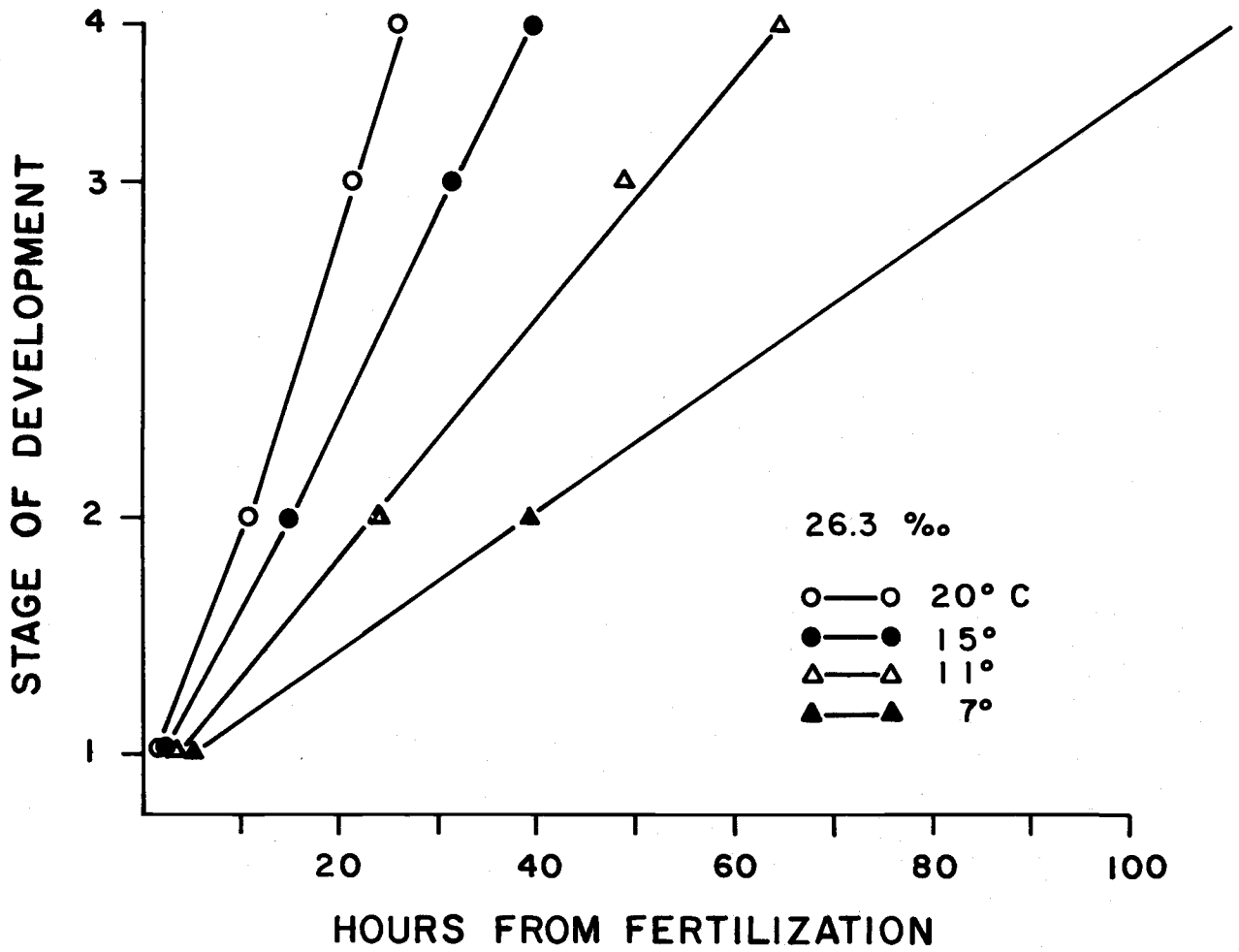

Figure 3. Effect of temperature on the rate of early development of Adula californiensis in 26.3 ppt salinity. Stage 1, second cleavage; stage 2, early trochophore; stage 3, late trochophore; stage 4, veliger larvae.

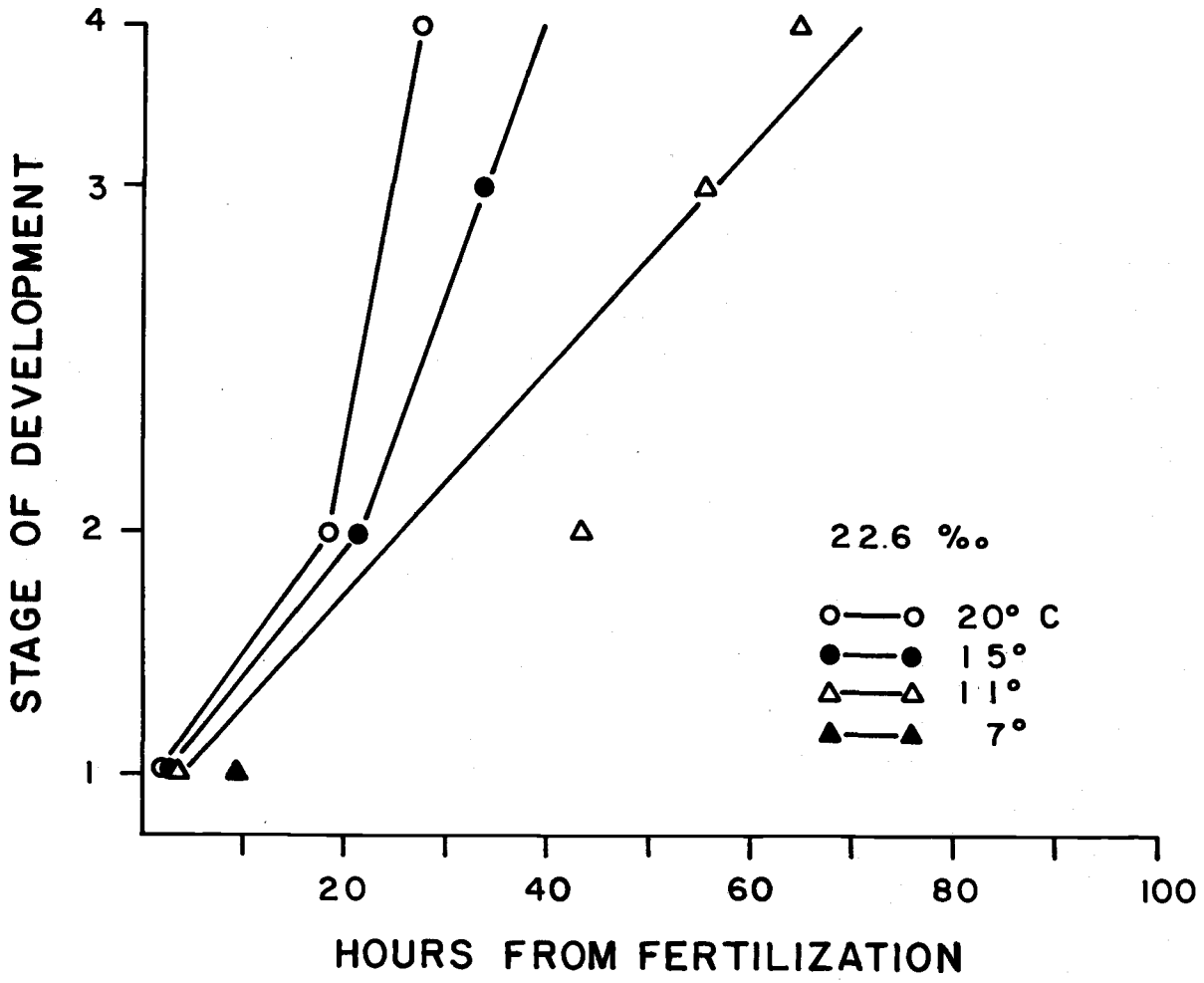

Figure 4. Effect of temperature on the rate of early development of Adula californiensis in 22.6 ppt salinity. Stage 1, second cleavage; stage 2, early trochophore; stage 3, late trochopore; stage 4, veliger larvae.

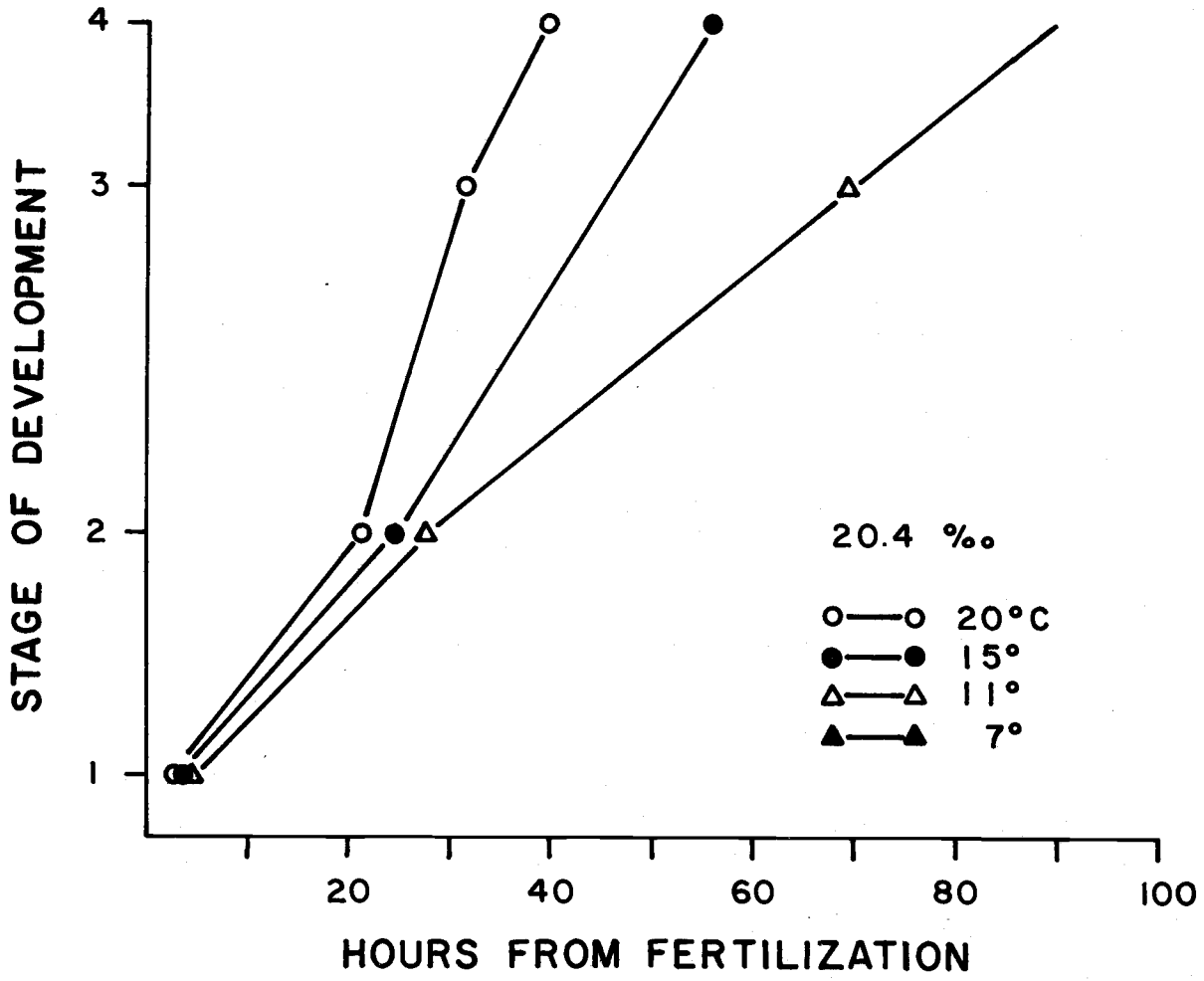

Figure 5. Effect of temperature on the rate of early development of Adula californiensis in 20.4 ppt salinity. Stage 1, second cleavage; stage 2, early trochophore; stage 3, late trochopore; stage 4, veliger larvae.

occurred such as failure to cleave, asynchronous cleavage, irregular elongate polar lobe formation, irregular cell formation in general, and abnormally formed larvae. The stage of development in 22.6 and 20.4 ppt salinity was very difficult to discern after the initial cleavages. Development at 22.6 ppt and 7°C could only be followed to the second cleavage stage. All swimming larvae appeared to be more active at 26.3 ppt salinity than in the other salinities at all temperatures.

Survival After 3 and 15 Day Rearing Periods at Different Temperatures and Salinities

Survival data for the larvae after 3 and 15 days in the experimental temperature and salinity combinations are presented in Tables 4 through 7 and Figure 6. Since there was no significant difference in survival between the two sets of duplicate experiments the percent survival was averaged. Pooling equal volumes of eggs from four adults in contrast to pooling unequal volumes of eggs from many adults produced comparable results, indicating that there were no differences between egg batches. Larvae that were fed 24 hours after fertilization showed no significant difference in survival at any temperature from those fed at 72 hours.

Maximal survival for the 3 day larvae occurred in 32.9 ppt salinity and at temperatures of 11° and 15°C (Table 4). There was

Table 4. Percentage of larvae surviving after 3 days of development in 16 temperature-salinity* combinations.

	7°C	11°C	15°C	20°C
32.9 ppt	29.2**	100.0	98.3	78.5
26.2 ppt	51.3**	88.4	82.5	85.7
23.2 ppt	61.7	62.1	71.8	61.8
20.3 ppt	0	41.4	52.8	34.1

**Because of difficulties in counting, these values were not included in the statistical analysis.

Table 5. Percentage of larvae surviving after 15 days of development in 16 temperature-salinity combinations.

	7°C	11°C	15°C	20°C
32.9 ppt	79.1	99.2	98.5	44.4
26.2 ppt	21.8	93.8	82.6	13.3
23.2 ppt	0	66.8	48.3	0.3
20.3 ppt	0	0.5	0.4	0

*Averaged salinity values ±0.5 ppt.

Table 6. Statistical analysis of larval survival after 3 days of development in 16 temperature-salinity combinations.

Variable	t-Value (8 D. F.)	Significance level
T	3.86	1%
T^2	2.46	5%
S	4.96	1%
S^2	4.04	1%
T x S	1.68	not significant

Table 7. Statistical analysis of larval survival after 15 days of development in 16 temperature-salinity combinations.

Variable	t-Value (10 D. F.)	Significance level
T	4.08	1%
T^2	4.59	1%
S	2.07	10%
S^2	1.47	not significant
T x S	1.05	not significant

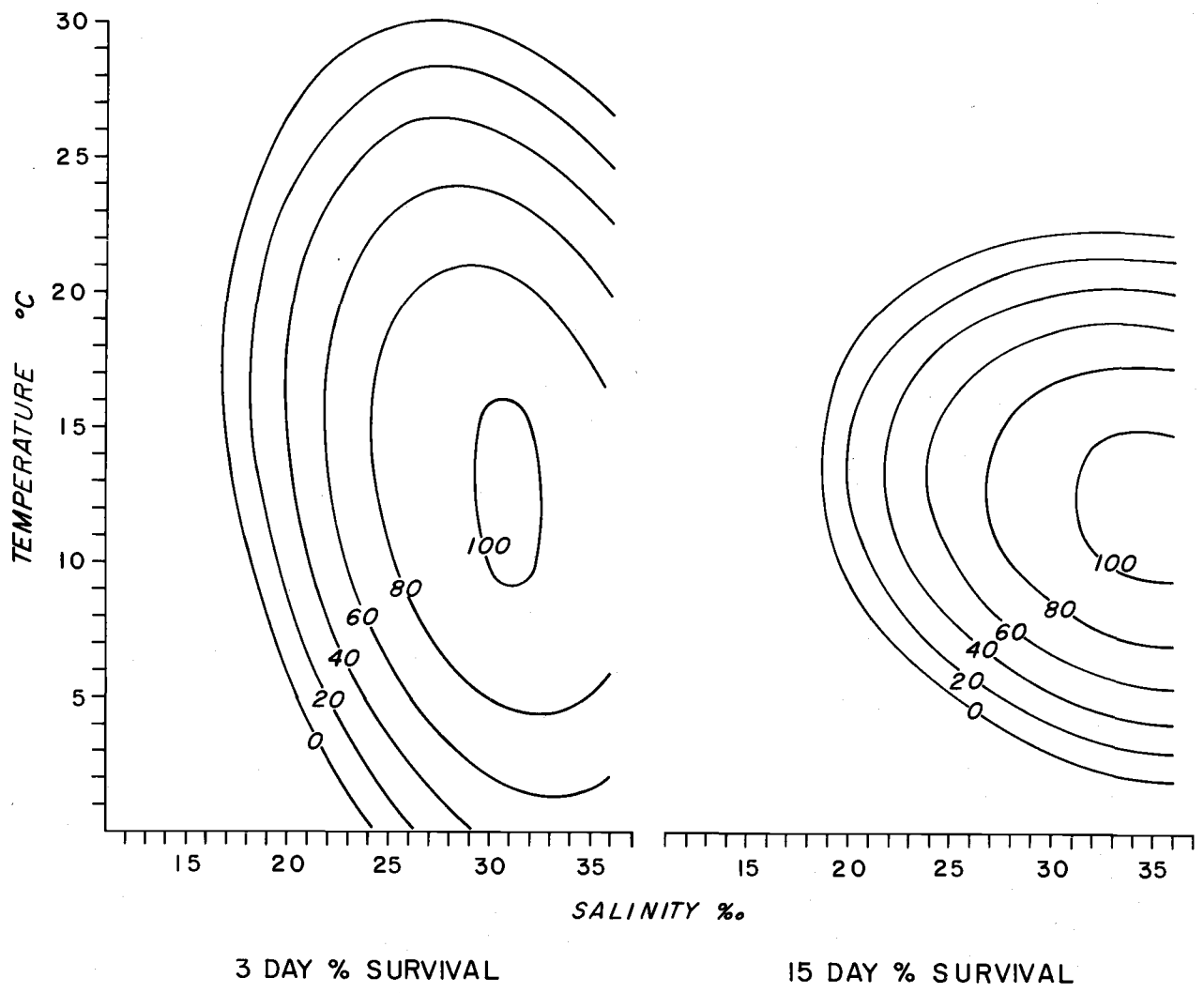

Figure 6. Response surface estimation of percent survival of Adula californiensis after 3 and 15 days of development in 16 different temperature-salinity combinations.

approximately a 10 to 20 percent decrease in survival for each level of decreasing salinity. Because of difficulties encountered in counting larvae without shells, survival values for the temperature-salinity combinations of 7°C, 32.9 ppt and 7°C, 26.2 ppt are considered to be inaccurate and were not used in the statistical analysis.

After 15 days under the experimental conditions maximal survival still occurred at 11° and 15°C and 32.9 ppt salinity (Table 5). The optimum temperature for survival in all salinities appeared to be 11°C. Below 26.2 ppt salinity there was a sharp decrease in survival to virtually no survivors at 20.3 ppt at any temperature. Temperatures of 7° and 20°C narrowed the range of salinities for larval survival; the higher temperature producing a greater mortality.

A multiple regression analysis was applied to the 3 and 15 day survival data to determine the linear and quadratic effects of temperature (T, T^2), the linear and quadratic effects of salinity (S, S^2), and the interacting effects of temperature and salinity ($T \times S$).

The 3 day statistical analysis (Table 6) shows that all variables except the interacting effects of temperature and salinity significantly contributed to variation in the survival data. The linear and quadratic effects of salinity were the two most significant factors. A polynomial expression incorporating all the variables explained 93.2 percent of the variation:

$$Y_{\%\ Survival} = -603.95 + 39.16(S) + 16.72(T) - 0.24\ (T \times S) - 0.59(S^2) - 0.37(T^2)$$

The 15 day statistical analysis (Table 7) indicated that only the linear and quadratic effects of temperature were contributing significantly to variance in the survival data. The linear effect of salinity was only important at the 10 percent significance level. The resulting equation incorporating all variables explained 87.3 percent of the variation:

$$Y_{\%\ Survival} = -608.43 + 29.25(S) + 330.72(T) - 0.21(T \times S) - 0.38(S^2) - 1.06(T^2)$$

Graphical estimations of percent survival for the 3 and 15 day larvae based on the fitted response surface to survival under sixteen different combinations of temperature and salinity are presented in Figure 6. Assuming the survival estimations apply to the temperature-salinity conditions in nature, the following predictions can be made.

One hundred percent survival for the 3 day larval stage can be expected between salinities of 29 and 32 ppt and between temperatures of 9° and 16°C. Survival decreases with lower or higher temperatures and salinities. Some survival is predicted at temperatures as high as 30°C and as low as 0°C; however, from experience with

laboratory rearings it is doubtful that larvae could survive through early cleavages below 5°C.

Maximal survival for the 15 day larvae can be expected between salinities and temperatures of 31 and 36 ppt and 9° and 15°C. No survival is predicted in salinities below 18 ppt and at temperatures wider than 3° to 23°C.

Analysis of Combined Survival Data for 3 and 13, 15 Day Rearing Periods

The preliminary survival experiment of 3 and 13 days and the 3 and 15 day survival data were combined (Tables 8 through 11) to produce a better fitted response surface (Figure 7). The survival data for 15°C in both experiments were combined and averaged. It was assumed there was little or no difference in survival between the 13 day survivor sample of the preliminary experiment, and the 15 day survivor sample.

All variables, except for the interacting effects of temperature and salinity, were significant at the 1 percent level for both the 3 day survival and the 13, 15 day survival (Tables 10 and 11). The linear and quadratic effects of salinity were still ranked first and second respectively by the correlation coefficients as being more important in explaining 3 day survival. The resulting equation explained 88.5 percent of the variation for the 3 day survival data:

Table 8. Percentage of larvae surviving after 3 days of development in 28 temperature-salinity* combinations.

	7°C	9°C	11°C	12°C	15°C	18°C	20°C
32.9 ppt	--	84.7**	100.0	100.0	96.1	91.3	78.5
26.1 ppt	--	75.6**	88.4	95.0	88.6	88.6	85.7
23.2 ppt	61.7	--	62.1	--	71.8	--	61.8
20.1 ppt	0	58.9	41.1	35.6**	59.0	41.4**	34.1

Table 9. Percentage of larvae surviving after 13, 15 days of development in 28 temperature-salinity combinations.

	7°C	9°C	11°C	12°C	15°C	18°C	20°C
32.9 ppt	79.1	95.0	99.2	96.1	97.2	92.9	44.4
26.1 ppt	21.8	89.4	93.8	100.0**	83.5	84.1	13.3
23.2 ppt	0	--	66.8	--	48.3	--	0.3
20.1 ppt	0	2.0	0.5	3.1	1.5	0	0

*Averaged salinity values ±0.7 ppt.

**Only one value.

Table 10. Statistical analysis of larval survival after 3 days of development in 28 temperature-salinity combinations.

Variable	t-Value (17 D. F.)	Significance level
T	3.63	1%
T^2	3.32	1%
S	5.37	1%
S^2	4.66	1%
T x S	0.75	not significant

Table 11. Statistical analysis of larval survival after 13, 15 days of development in 28 temperature-salinity combinations.

Variable	t-Value (19 D. F.)	Significance level
T	4.55	1%
T^2	5.17	1%
S	4.23	1%
S^2	3.47	1%
T x S	0.91	not significant

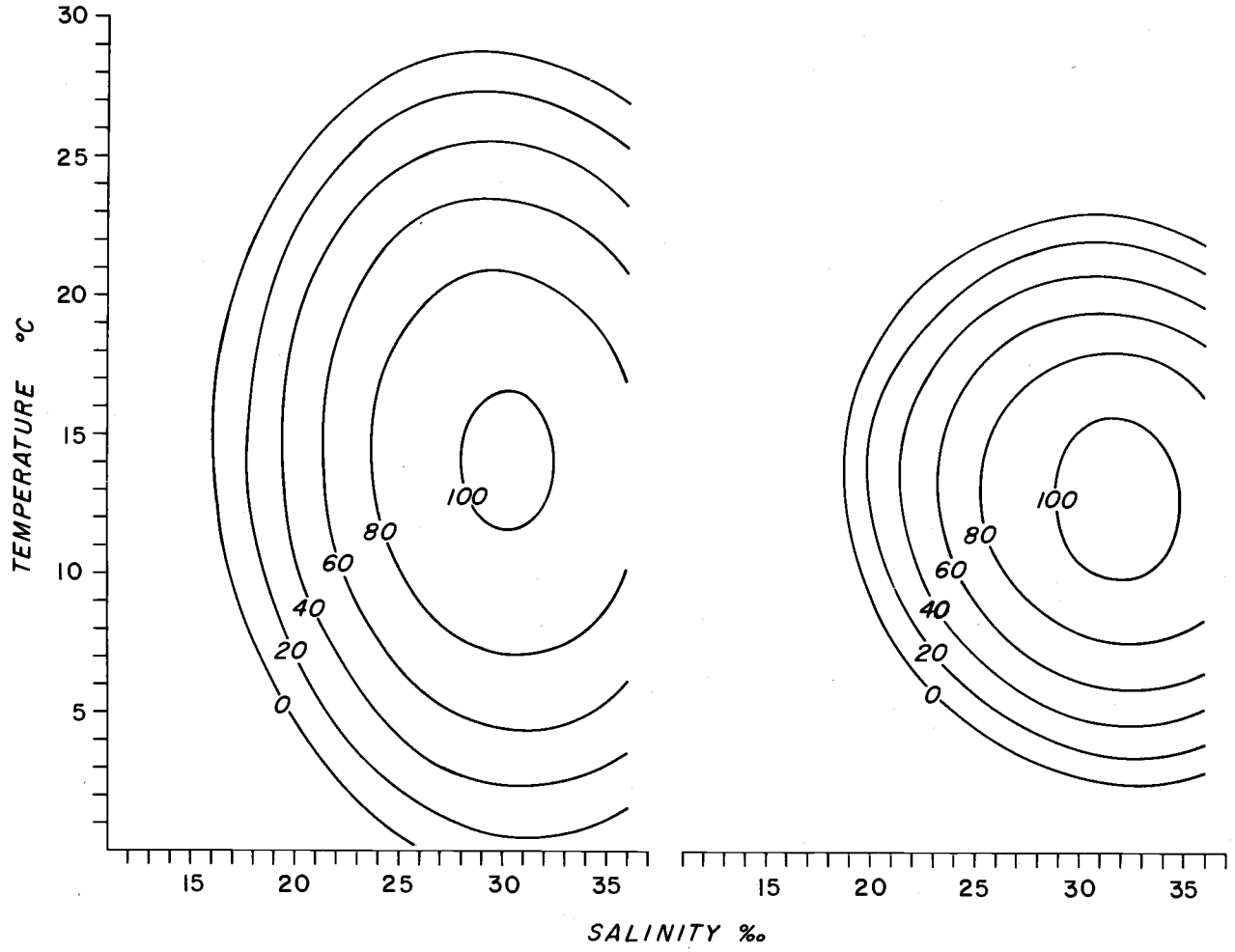

Figure 7. Response surface estimation of percent survival of Adula californiensis after 3 and 13, 15 days of development in 28 different temperature-salinity combinations.

$$Y_{\%\ Survival} = -504.63 + 33.06(S) + 15.40(T) - 0.08(T \times S) - 0.53(S^2) - 0.47(T^2)$$

For the 13, 15 day survival, the quadratic effect of temperature followed by the linear effect of temperature were ranked first and second in importance by simple correlation coefficients. The resulting equation explained 87.4 percent of the variation:

$$Y_{\%\ Survival} = -771.38 + 42.59(S) + 31.59(T) - 0.11(T \times S) - 0.64(S^2) - 1.06(T^2)$$

Survival response surfaces based on 28 different temperature and salinity combinations were estimated for larvae reared to 3 and 13, 15 days (Figure 7). The survival contours are nearly circular for both rearing periods verifying the statistical analysis that all variables except for the interacting effects of temperature and salinity were significant. Maximal survival of larvae reared to 3 days is predicted between salinities of 28 and 32 ppt and temperatures between 12° and 16.5°C. No survival is predicted below 17 ppt salinity and at temperatures above 29°C. Optimum conditions for larvae reared to 13, 15 days is estimated to occur in salinities and temperatures of 29 to 34 ppt and 10° to 15.5°C. No survival is predicted beyond the temperature range of 3° to 23°C and below salinities of 19.0 ppt.

Survival of the Veliger Larvae After 22 Day Rearing Period at Different Temperatures and Salinities

Survival data for veliger larvae after 22 days at experimental temperatures and salinities are presented in Tables 12 and 13. Between the 12th and 14th day of the experiment all the larvae died in 13.3 ppt salinity at all temperatures and at 20°C in all salinities. At 7°, 11° and 15°C, survival decreased with decreasing salinities but at a greater rate at the higher temperatures. There was little difference in percent survival except for larvae in salinities of 25.9 and 19.9 ppt at 15°C. At 25.9 ppt and 15°C, a 5 percent survival occurred; however, a 24.8 percent survival occurred at the lower salinity of 19.9 ppt at 15°C. There was approximately an 8 percent mortality (i.e., 92% survival) at optimum conditions (32.7 ppt, 11° and 15°C) over the 22 day period.

Statistical analysis of the survival data produced a polynomial equation explaining 78.9 percent of the variation:

$$Y_{\%\ \text{Survival}} = -173.89 + 14.61(S) + 9.03(T) - 0.33(T \times S) - 0.15(S^2) - 0.26(T^2)$$

However, when computed together none of the variables were shown to be highly significant (Table 13). The linear effect of salinity and the interaction effects of temperature and salinity were both only significant at the 10 percent level. The greater portion of the

Table 12. Percentages of larvae surviving after 22 days in 16 temperature-salinity combinations.

	7°C	11°C	15°C	20°C
32.7 ppt	92.7	93.6	92.3	0
25.9 ppt	96.7	86.8	5.0	0
19.9 ppt	86.4	79.7	24.8	0
13.3 ppt	0	0	0	0

*Percentages are based on the combined percentage of each duplicate culture. The duplicate percentage was calculated from the 3-day count of each culture.

Table 13. Statistical analysis of larval survival after 22 days in 16 temperature-salinity combinations.

Variable	t-Value (10 D. F.)	Significance level
T	0.90	not significant
T^2	0.77	not significant
S	2.00	10%
S^2	1.01	not significant
$T \times S$	1.83	10%

survival data (69.6%) could best be explained by the quadratic effects of temperature and the linear effects of salinity; both at the 1 percent level:

$$Y_{\%\ \text{Survival}} = 7.87 + 3.26(S) - 0.21(T^2)$$

Growth of Larvae After 3, 15 and 22 Days of Rearing at Experimental Temperatures and Salinities

Mean length data of the larvae after 3 and 15 days rearing at the experimental temperatures and salinities are given in Tables 14 through 17. The greatest 3 day mean length occurred at 20°C and 32.9 ppt. Mean length decreased with decreasing salinity while the standard deviations show that the size range of the individuals increased. The same comments can be applied to the 15 day mean length.

Due to the small difference in size between the larvae in any temperature-salinity combination, the statistical analysis did not show any single variable to be highly significant. The 3 day analysis showed the linear and quadratic effects of salinity to be significant only at the 10 percent level (Table 16). The resulting equation explained a significant 94 percent of the variation for the 3 day survival data:

$$Y_{\text{Mean Length}} = -241.75 + 23.63(S) - 3.05(T) + 0.12(T \times S) - 0.41(S^2) + 0.01(T^2)$$

Table 14. Mean length in microns and standard deviation from larvae surviving after 3 days of development in 16 temperature-salinity combinations.

	7°C*	11°C	15°C	20°C
32.9 ppt	-	106.4 ± 3.1	107.8 ± 3.1	111.2 ± 3.0
26.2 ppt	-	98.0 ± 6.1	95.1 ± 6.3	103.3 ± 6.9
23.2 ppt	-	84.0 ± 8.1	86.8 ± 7.4	77.3 ± 13.1
20.3 ppt	-	-	-	-

*No shell development at this temperature by 3 days.

Table 15. Mean length in microns and standard deviation for larvae surviving after 15 days of development in 16 temperature-salinity combinations.

	7°C	11°C	15°C	20°C
32.9 ppt	117.4 ± 4.5	116.9 ± 4.1	117.1 ± 2.9	120.1 ± 7.1
26.2 ppt	110.2 ± 9.0	113.9 ± 4.7	107.9 ± 6.8	120.2 ± 10.0
23.2 ppt	-	112.1 ± 8.4	109.1 ± 11.7	-
20.3 ppt	-	-	-	-

Table 16. Statistical analysis of mean length for larvae surviving after 3 days of development in 16 temperature-salinity combinations.

Variable	t-Value (3 D. F.)	Significance level
T	0.51	not significant
T^2	0.21	not significant
S	2.41	10%
S^2	2.37	10%
T x S	1.06	not significant

Table 17. Statistical analysis of mean length for larvae surviving after 15 days of development in 16 temperature-salinity combinations.

Variable	t-Value (4 D. F.)	Significance level
T	0.94	not significant
T^2	0.98	not significant
S	1.28	not significant
S^2	1.25	not significant
T x S	0.33	not significant

None of the variables were significant for the 15 day mean length analysis (Table 17). Only 37.4 percent of the variation in the data was explained by the equation incorporating all the variables. The computer output of the variables, based on simple correlation coefficients, ranked the linear effect of temperature followed by the quadratic effect of temperature as first and second respectively in importance; although they were not significantly important.

Mean length and growth increments of the veliger larvae after 22 days of rearing at experimental temperatures and salinities are given in Tables 18, 19 and 20. In general, there were only small differences in mean length and growth increments in all the temperature-salinity combinations where there was survival. At 15°C and in salinities of 25.9 and 19.9 there was a greater mean length and growth increment than in the other experimental combinations. However, there was a greater range in size of individuals at the reduced salinities.

Statistical analysis of the 22 day mean length data produced an equation explaining 76.4 percent of the variation:

$$Y_{\text{Mean Length}} = 35.82 + 7.43(S) - 2.21(T) - 0.12(T \times S) - 0.12(S^2) + 0.29(T^2)$$

No single variable was found to be highly significant (Table 20). An equation for mean length could best be described by the quadratic

Table 18. Mean length in microns and standard deviation for larvae surviving after 22 days in 16 temperature-salinity combinations.

	7°C	11°C	15°C	20°C
32.7 ppt	121.1 ± 3.4	120.5 ± 3.0	118.9 ± 3.5	-
25.9 ppt	121.7 ± 3.6	121.1 ± 3.4	137.2 ± 11.0	-
19.9 ppt	120.3 ± 4.7	119.2 ± 4.4	129.9 ± 13.3	-
13.3 ppt	-	-	-	-

Table 19. Growth increment in microns for larvae surviving after 22 days in 16 temperature-salinity combinations.*

	7°C	11°C	15°C	20°C
32.7 ppt	10.9	10.3	8.7	-
25.9 ppt	11.5	10.9	27.0	-
19.9 ppt	10.1	9.0	19.7	-
13.3 ppt	-	-	-	-

*Grown increment based on 3-day mean length of 110.2 ± 2.9 microns determined from 45 larvae grown in 33.2 ppt salinity, 15°C.

Table 20. Statistical analysis of mean length for larvae surving after 22 days in 16 temperature-salinity combinations.

Variable	t-Value (3 D. F.)	Significance level
T	0.41	not significant
T^2	1.33	not significant
S	1.62	not significant
S^2	1.43	not significant
T x S	1.26	not significant

Table 21. Respiration of 72 hour larvae under 24 temperature-salinity combinations. *

	13.0 ppt	19.8 ppt	26.2 ppt	32.9 ppt
7°C	1.01 ± 0.09(15)	1.10 ± 0.10(15)	1.15 ± 0.10(15)	1.08 ± 0.11(15)
9°C	1.30 ± 0.05(10)	1.43 ± 0.10(15)	1.24 ± 0.06(15)	1.55 ± 0.06(15)
12°C**	1.38 ± 0.20(3)	2.02 ± 0.67(3)	1.27 ± 0.02(3)	1.58 ± 0.18(3)
15°C	1.44 ± 0.15(15)	1.65 ± 0.16(15)	1.92 ± 0.15(15)	1.54 ± 0.13(15)
18°C	2.63 ± 0.33(15)	2.09 ± 0.26(10)	2.17 ± 0.24(10)	2.01 ± 0.19(10)
21°C	1.50 ± 0.18(15)	1.26 ± 0.16(15)	1.51 ± 0.17(10)	1.58 ± 0.14(15)

*Mean values are expressed as microliters O_2 per hour per 10,000 larvae (1.23 mg). Each mean is followed by the standard error and the number of determinations in parenthesis.

**Only first hour values. All other values are from five hour readings.

effect of temperature and the interacting effects of temperature and salinity:

$$Y_{\text{Mean Length}} = 121.81 - 0.03(T \times S) + 0.86\ (T)^2$$

The quadratic effect of temperature was significant at the 10 percent level and the interacting effects of temperature and salinity only at the 50 percent level. The computer output of the variables, based on simple correlation coefficients, also showed the quadratic effect of temperature to be the most important factor contributing to variation in the data.

Effects of Temperature and Salinity on Oxygen Consumption of 72 Hour Veliger Larvae

Data and graphs for oxygen consumption of the 72 hour veliger larvae are presented in Tables 21 through 23 and Figures 8 and 9. The 12°C oxygen consumption values were not represented in the graphs and statistical analysis because only one reading was taken. In general, oxygen consumption increased with increasing temperatures from 7° to 18°C and decreased from 18° to 21°C at all salinities (Figure 8). At 32.9 and 19.8 ppt salinity the greatest rate of oxygen consumption occurred from 7° to 9°C. The Q_{10} values for these data are given in Table 22. At 26.2 ppt the greatest rate of oxygen consumption occurred from 9° to 15°C. In the lowest

Table 22. Q_{10} values of respiration for 72 hour larvae.

	13.0 ppt	19.8 ppt	26.2 ppt	32.9 ppt
7°-9°C	3.15	3.71	1.46	6.10
9°-15°C	1.38	1.27	2.08	*
15°-18°C	7.98	2.20	1.51	2.43
18°-21°C	*	*	*	*

*Decrease in oxygen consumption

Table 23. Statistical analysis of respiration for 72 hour larvae under 20 temperature-salinity combinations.

Variable	t-Value (9 D.F.)	Significance level
T	4.86	1%
T^2	5.70	1%
S	0.08	not significant
S^2	0.47	not significant
T x S	0.62	not significant
t	1.52	not significant
t^2	0.43	not significant
T x t	3.07	2%
S x t	0.22	not significant

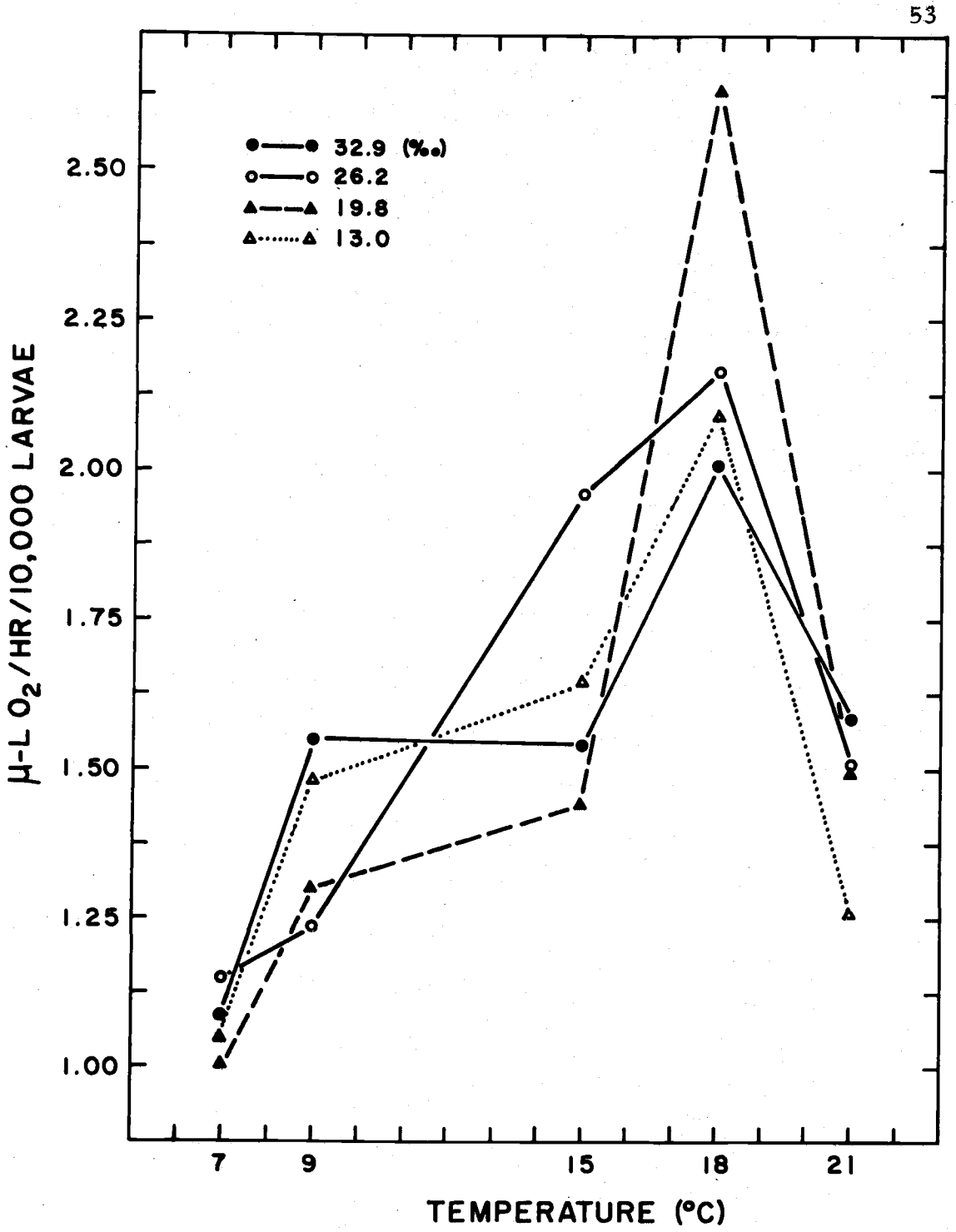

Figure 8. Respiration of 72 hour Adula californiensis larvae under 20 different combinations of temperature and salinity represented as a function of temperature. Respiration values are expressed as microliters oxygen consumed per 10,000 veliger larvae.

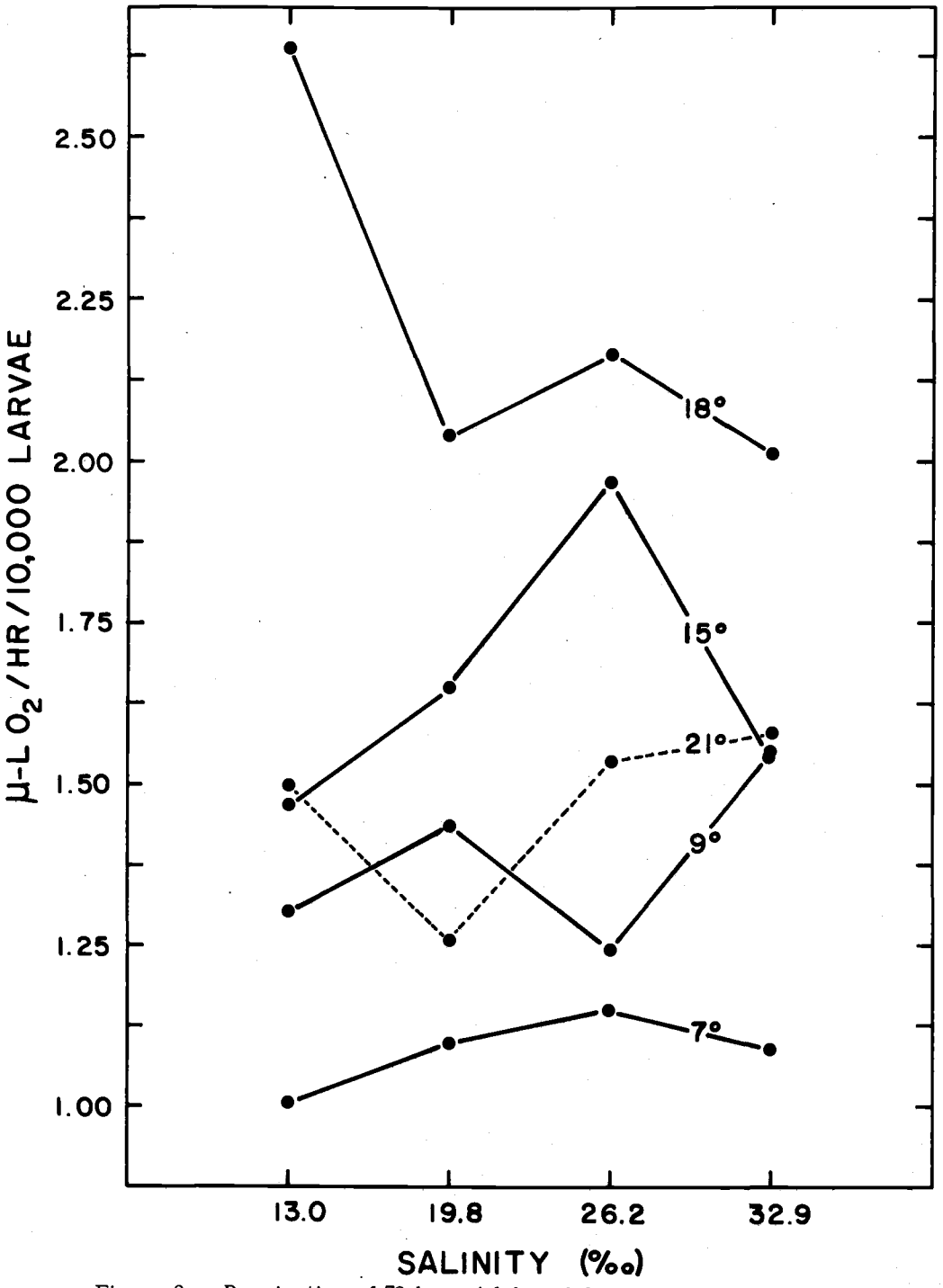

Figure 9. Respiration of 72 hour Adula californiensis larvae under 20 different combinations of temperature and salinity represented as a function of salinity. Respiration values are expressed as microliters oxygen consumed per hour per 10,000 veliger larvae.

dilution of 13.0 ppt the greatest rate of oxygen consumption was from 15° to 18°C.

The relationship of larval respiration to salinity is not clear. In 26.2 and 19.8 ppt salinity at temperatures of 7°, 15° and 18°C there was an increase in oxygen consumption above that at 32.9 ppt. The greatest increase occurred at 15°C (24.7%) and the least increase at 7°C (6.5%) in 26.2 ppt salinity. In the lowest salinity of 13.0 ppt the oxygen consumption was below that of 32.9 ppt at 7° and 15°C, however, a 31.7 percent increase occurred at 18°C. At 9° and 21°C there was a general decrease in oxygen consumption with decreasing salinities.

Regression coefficients were determined for the linear and quadratic effects of temperature (T, T^2), the linear and quadratic effects of salinity (S, S^2), the interacting effects of temperature and salinity ($T \times S$), the linear and quadratic effects of oxygen consumption by time (t, t^2), the effects of temperature by time ($T \times t$), and the effects of salinity by time ($S \times t$).

The polynomial expression produced for larval oxygen consumption explained only 35.6 percent of the data variation:

$$Y_{\text{oxygen consumption}} = -7.21 - 5.36(S) + 4.92(T) - 4.34(t) - 9.77(T \times S) - 1.30(S \times t) - 2.51(T \times t) + 6.51(S^2) - 1.87(T^2) + 1.58(t^2)$$

The linear and quadratic effects of temperature at the 1 percent level were the most significant factors affecting oxygen consumption of the larvae (Table 23). Simple correlation coefficients used by the computer to order the variables in importance for additive regression verified the importance of these factors. Temperature by time (T x t) was significant at the 2 percent level. All other variables contributed little to explaining variation in the data.

DISCUSSION

Effects of Temperature and Salinity on Fertilization

Fertilization of A. californiensis eggs occurred at temperatures from 7° to 20°C and salinities from 20.4 to 33.2 ppt except for the 7°C, 20.4 ppt combination. Forty percent of the eggs inseminated under the latter conditions were found to undergo irregular cleavages. The remaining 60 percent either were not fertilized or were unable to cleave.

Young (1941), working with the mussel, Mytilus californianus, clearly showed the deleterious effect on fertilization of dilutions greater than 21.5 ppt. Gametes of M. californianus held at various salinities for periods of up to 3.6 hours produced the same results as those fertilized immediately. Fertilization for M. edulis occurred at all temperatures from 5° to 22°C and in all salinities from 15 to 40 ppt (Bayne, 1965).

Effects of Temperature and Salinity on Early Development

Bayne (1965) found that normal cleavage for M. edulis occurred only in the 8° to 18°C temperature range. Cleavage did not occur at 5°C and was abnormal at 20° to 22°C. Salinities in the 30 to 40 ppt range were necessary for successful development to the trochophore

stage. This reduction of tolerance after fertilization has also been shown by Young (1941), Schecter (1956) and in this study for A. californiensis. The polar body stage of Spisula solidissima was shown by Schecter (1965) to be highly sensitive to reduced salinities.

A comparable increase in the rate of early development with increased temperature was also shown by Bayne (1965) for M. edulis. The linear relationship for each temperature indicates an equal portion of the time was spent at each stage (also see Walne, 1965). Bayne also found that the effect of temperature on developmental rate was greatest at the lower temperatures for M. edulis larvae, as it is for A. californiensis larvae.

The retardation of developmental rate with reduced salinities has previously been observed for various bivalves by many investigators (Seno, Hori and Kusakabe, 1926; Amemiya, 1922, 1926, 1928; Clark, 1935; Rao, 1951; Nikitin and Turpaeva, 1957). The fact that a greater retardation of developmental rate of A. californiensis occurred at the higher temperatures seems reasonable since any retarding effect would most likely be emphasized by increasing temperature.

Effects of Temperature and Salinity on Survival

Maximal survival at the end of 3 days of development occurred in a much narrower range of conditions than did fertilization. The

requirement of salinities between 28 and 32 ppt and temperatures from 12° to 16.5°C for maximum estimated survival (Figure 7) indicate that the early development of A. californiensis is limited to the normal range of inshore oceanic conditions. Young (1941) showed that salinities less than 29.6 ppt decreased survival of M. californianus larvae. Although both temperatures and salinities within the range of Yaquina Bay estuarine conditions affect A. californiensis survival, the effects of reduced salinity were more significant than the extremes of temperature in producing mortality for development to 3 days or to the veliger stage.

Once development has proceeded to the veliger stage, the effects of extreme temperatures delimit survival as shown by the 15 day statistical analysis. Thus, extreme temperatures produce a greater rate of mortality in those larvae harmed by reduced salinity during the first 3 days of development. It is doubtful larvae reared from fertilization would survive to maturity outside the 80 percent survival contour range of 7° to 17.5°C and 28 to 36 ppt (Figure 7). Survival rapidly decreases in temperature and salinity conditions more extreme than the 80 percent survival contour. The surface response technique used to investigate temperature-salinity relationships should only be considered as an estimation and not exact limits.

Survival data from the 22 day rearing experiment demonstrates

that the later stage veliger larvae are able to tolerate dilution as low as 19.9 ppt at lower temperatures of 7° and 11°C. Although the statistical analysis indicated a possible interacting effect of temperature and salinity on survival, the major effects were brought about by the quadratic effect of temperature and the linear effect of salinity. The detrimental effect of high temperature is shown at 20°C, where no larvae survived to 14 days at any salinity. The number of larvae surviving at 15°C at lower salinities was markedly reduced. It is doubtful that larvae of any advanced stage would be able to tolerate salinities below 19.9 ppt indefinitely, as shown by these experiments.

A. californiensis larvae are able to survive over a wider range of temperatures at near optimum salinities than at salinities near the lower limit of their tolerance. This also was found by Davis and Calabrese (1964) in their study of the combined effects of temperature and salinity on Mercenaria mercenaria and Crassostrea virginica. A. californiensis larvae also are able to survive over a wider range of salinities near the optimum temperatures. At optimum temperatures between 9° and 15°C, a few larvae were able to survive in all salinities as low as 20.1 ppt (Table 9). Conversely, at optimum salinities (32.9 ppt) larvae were able to survive temperature extremes of 7° and 20°C for 15 days. A true temperature-salinity interaction, where the direction and magnitude of change in one level would be required to maintain a maximum response at a particular

level of another factor, was not found for the 3 and 15 day survival data. A temperature-salinity interaction was found to be significant at only the 10 percent level for the veliger larvae after 22 days of rearing. Survival in the lower salinities decreased with increased temperature. Maximal survival with decreasing salinity is coupled with decreasing temperature. The beneficial effect of such a combination of factors on survival is reported by Kinne (1964) and Alderice and Forrester (1968).

Effects of Temperature and Salinity on Growth

The size of those larvae measured at the end of 3 days of development was reduced at lower salinities (Tables 14 and 16). After 15 days of development under experimental conditions the mean lengths were relatively unaffected by either temperature or salinity over the range at which survival occurred (Tables 15 and 17). The 15 day results indicate that larvae whose size was reduced by diluted salinities at the end of 3 days, either regained their loss or died, leaving only the larger individuals. Since larval size remains about the same in all temperature and salinity combinations where there was survival, it appears to be genetically determined and relatively independent of temperature and salinity. The range of oceanic conditions would have only a slight effect on the size of the veliger larvae.

Only small differences in mean lengths were observed for

larvae reared for 22 days in various temperature and salinity combinations (Tables 18 and 19). Those few larvae surviving at 15°C and 25.9 and 19.9 ppt reached a slightly greater size than larvae reared in the other temperature-salinity combinations. No larvae were found in any other temperature-salinity combinations as large as those found in 15°C and 25.9 and 19.9 ppt. This particular temperature and salinity apparently enabled a few individuals to reach a very large size compared to the normal population. Increasing temperatures and decreasing salinities on the larval population increase the size range of the individuals, as indicated by the standard deviations.

Statistical analysis of the 22 day mean length indicates a possible temperature-salinity interaction effect (Table 20). The size of the veliger larvae after 22 days of rearing is more likely to be relatively unaffected by temperature and salinity as noted for the 15 day mean length. It is unusual that A. californiensis larvae did not grow significantly during the course of the experiment. Most bivalve larvae have been reported at least to double in size during the pelagic phase before metamorphosis and to be affected by temperature, salinity and availability of food (see introduction). Sheltema (1965) concluded that salinity affects larval growth less than any other factor in the environment, while the species and concentration of food usually affect growth more than any other factor.

Isochrysis galbana has been shown to be a suitable food species without harmful side effects (Davis, 1953; Walne, 1956, 1963, 1965, 1966; Davis and Guillard, 1958; Loosanoff and Davis, 1963; Bayne, 1965). A. californiensis larvae were observed to have ingested I. galbana at all temperature and salinity combinations where the larvae developed normally past the veliger stage. There were no harmful effects noted with any of the concentrations of algae used in the experiments. The concentrations of algae were great enough that food was left over before the next feeding. Bayne (1965) found the growth of Mytilus edulis larvae reared on I. galbana generally increased with increased concentration of cells up to a point. In experiments with A. californiensis, reducing the concentration of algae (from 2,680-3,080 cells per milliliter in the preliminary experiment to 980-1,620 cells per milliliter in the later experiments) did not affect survival. Therefore, concentrations of I. galbana used for A. californiensis were considered more than adequate. A. californiensis larvae are able to survive for a considerable period without feeding; more than 10 days at optimum conditions. Bayne (1965) reported Mytilus edulis larvae are able to survive without food for more than 26 days. The species of algae used may not have had the right food value nor trace elements necessary for growth. Various investigators have found that a mixture of flagellates will support a faster growth than a single species (Bayne, 1965; Davis and Guillard,

1958; Davis and Calabrese, 1964).

To what extent A. californiensis larvae are able to utilize I. galbana is not known. Walne (1965) estimated the percent of total food assimilated that is used in growth and respiration by Ostrea edulis larvae and found it to be only 13 to 50 percent of the food caught. The work of Davis and Calabrese (1964) implied that certain digestive enzymes may not be functional at low temperatures. A. californiensis survived at 7°C and grew to lengths equal to those larvae at higher temperatures; this tends to rule out their theory in this case. Larvae at high temperatures (20°C) may have been metabolizing at a greater rate than they were physically capable of ingesting and assimilating algae at any concentration and therefore starving to death.

Minimal growth of the A. californiensis veliger larvae and failure of metamorphosis over 25 days of culture suggests two possibilities: (1) The culture conditions were inadequate, that is, proper food was not provided for normal growth; or (2) culture conditions were adequate, suggesting the larvae do not normally remain in the pelagic phase for a long time, but begin settling as soon as the veliger stage is reached.

Perhaps the larvae delay any measurable growth until settlement and metamorphosis. The structural reorganization upon metamorphosis may allow them to filter and assimilate a greater quantity

of food. The change in habitat from the planktonic to benthic would permit a change in types of digestive enzymes and their activity to accommodate a greater variety of foods.

Effects of Temperature and Salinity on Larval Respiration

Oxygen consumption of 72 hour veliger larvae was determined under various temperature and salinity combinations. The measurement and evaluation of oxygen consumption by molluscan veliger larvae has been discussed by Zeuthen (1947), who has shown that below a certain oxygen tension there is a marked reduction in the activity of larvae resulting in a decreased oxygen consumption.

Shaking rate and oxygen tension are known to effect respiration under experimental conditions. Oxygen tension may have been reduced by using a continuously closed system over a 5 hour interval, which could have caused a decrease in oxygen consumption of the larvae. Oxygen consumption values for A. californiensis larvae, however, were not observed to decrease over the 5 hour period; instead, the statistical analysis shows a trend of increasing oxygen consumption (see Table 23).

Oxygen consumption values were based on a standard weight of 1.23 mg equal to 10,00 larvae as all larvae in the experiments were cultured under identical conditions. The experimental weight error of ±22 percent was not considered important because the

differences in oxygen consumption between the different temperature-salinity combinations was of a greater magnitude.

The concentration of larvae and their level of activity are two important biological factors that affect oxygen consumption. At high concentrations of larvae, oxygen consumption is depressed (Walne, 1966; Millar and Scott, 1967). The latter found that for Ostrea edulis, as the concentration of larvae increased from about 25 to 250 larvae per milliliter, the oxygen consumption decreased from 5.4 to 0.2 micro-L O_2/hr/1000 larvae respectively. The 0.2 micro-L O_2/hr/1000 larvae (=2.0 micro-L O_2/hr/10,000 larvae) at 250 larvae per milliliter is comparable to oxygen consumption values obtained for A. californiensis larvae at 8 to 16 times this concentration of larvae. It was necessary to use such high concentrations of A. californiensis larvae for the oxygen consumption values to register on the respirometer. The oxygen consumption rate did not appear to be greatly altered when the concentration of larvae was doubled from 16,000 to 30,000 larvae per milliliter in the reaction flasks.

From Table 21, the rate of oxygen consumption for 72 hour veliger larvae at 15°C and 32.9 ppt is 1.54 micro-L O_2/hr/10,000 larvae or 0.00074 ml O_2/48 hr/100 larvae. Full salinity sea water at 15°C and normal pressure has an oxygen content of 5.79 ml/L or 0.0579 ml/ml (Harvey, 1966). Assuming that there was no gaseous exchange with the atmosphere, the consumption data suggests that

larvae reared under culture conditions of full salinity sea water, 15°C, and at concentrations of approximately 100 larvae per milliliter would only use about 14 percent of the available oxygen every 48 hours, the time interval between water changes. The oxygen content of all the other experimental temperature and salinity combinations would also be above 5 ml/O_2/L. The oxygen content of the cultures can therefore be said to not be a limiting factor. Similarly, since the calculated oxygen content was not found to be limiting, values obtained in the respiration experiments would appear to be reasonable.

Activity levels of molluscan veliger larvae are important because, unlike larvae of other phyla, the veliger can close its shell and live anaerobically for short periods of time when conditions are unfavorable. However, Zeuthen (1947) concludes that the work of floating and swimming requires less than 50 percent, and in some cases only a small fraction, of the total metabolic energy; so that the swimming and resting states of the larvae have comparable respiratory rates. The averaged oxygen consumption values for A. californiensis larvae therefore should be indicative of their "normal activity."

A. californiensis larvae can survive for a long period of time before feeding because they have yolky eggs. However, Millar and Scott (1967) found that as the nutritional reserves of Ostrea edulis

larvae were depleted the rate of respiration declines. A. californiensis larvae used in the experiments were starved approximately 32 hours from the time they were observed to ingest I. galbana. Although starved larvae may experience a greater stress at suboptimum conditions than well-fed larvae, the 32 hour starvation interval does not appear to affect their vitality.

Oxygen consumption in poikilotherms generally increases with increasing temperature up to some critical level and then decreases sharply (Kinne, 1964; Nicol, 1967). Oxygen consumption for A. californiensis larvae increases from 7° to 18°C and declines sharply between 18° to 21°C. Nicol (1968) reports Q_{10} values for poikilotherms of 10 to 11 between 0° and 5°C. A high Q_{10} of 6.1 occurred between 7° and 9°C for A. californiensis larvae in 32.9 ppt salinity (Table 22). There was little change in oxygen consumption between 9° and 15°C at oceanic salinity (32.9 ppt). A Q_{10} of 2.43 occurred between 15° and 18°C at 32.9 ppt. From this information it seems that the larvae have the enzymatic system(s) or adequate concentration of enzymes to enable them to compensate their metabolic rate between 9° and 15°C. At the extremes of their temperature range there might be a shift in the enzymatic system(s) or sufficient activation energy to allow an increase in metabolic rate. In other words, the larvae are biochemically well adapted to oceanic rather than estuarine temperatures and salinities.

In the lower salinities the interpretation of the Q_{10} values becomes difficult because the rates are modified by such factors as salinity and dissolved oxygen. Kinne (1964, p. 286) states that changes in salinity may bring about effects through changes in total osmo-concentration, relative proportion of solutes, coefficients of absorption and saturation of dissolved gases, and through changes in density and viscosity. Salinities beyond the tolerated range always result ultimately in reduced rates of metabolism. Stenohaline species which suffer from osmotic damage whenever the salinity deviates significantly from the normal usually have a lower metabolism in both sub- and supra-normal salinities. Kinne warns against over-emphasizing Q_{10} values as a physiological tool in studying complex phenomena, such as metabolism. In general, at lower salinities the larvae do not appear to be compensating their metabolism to any consistent degree at any temperature.

The interaction of temperature and salinity in modifying the oxygen consumption of A. californiensis larvae is difficult to ascertain. Salinity may alter the effects of temperature and temperature may alter the effects of salinity. The statistical analysis of the oxygen consumption data only accounts for 35.6 percent of the variance. However, an analysis of this type tends to mask some of the important variation.

At temperatures of 7°, 15° and 18°C oxygen consumption tends

to increase in salinities of 26.2 and 19.9 ppt compared to that of 32.9 ppt (Figure 9). The greatest increase in oxygen consumption (24.7%) occurred at 15°C and 26.2 ppt. A temperature of 15°C would exert the least amount of temperature stress since the larvae were cultured and acclimated at 15°C. Therefore, any marked effect of salinity on oxygen consumption would probably appear maximal at the acclimating temperature. Temperatures beyond the range of those in which the larvae normally develop would tend to mask the effects of salinity, at least down to a dilution of 19.9 ppt. This generalization may be going beyond the reliability of the data, but in three of the five temperature levels there is an increased respiration at 19.9 and 26.2 ppt salinity. The fifth temperature, 21°C, can be ignored because it is lethal. In the lowest salinity of 13.0 ppt, temperatures of 15°C and below tend to lower the respiratory rate; while temperatures slightly above 15°C tend to drastically raise the respiratory rate.

The rise in respiratory rate at 19.9 and 26.2 ppt salinity may possibly be attributed to the increased metabolic demands caused by increased activity of the larval protonephridial organs. The larvae may be able to regulate in an environmental dilution of 20 to 40 percent but be incapable of meeting the demands of a greater osmotic stress. The regulation of internal concentration may only occur for a short period acting as a brake to sudden dilutions as speculated by

Wilson (1968) for the bivalve Xenostrobus securis. This implies that the larval form has osmoregulating abilities but loses this ability upon metamorphosis thereby becoming an osmoconformer as are most bivalves (Prosser et al., 1961). This possibility exists as the larvae are quite differently adapted animals than the adults and well developed protonephridial structures are present in bivalve veligers (Raven, 1966). Krishnamoorthi (1951) concluded from his work on a polychaete larva that the protonephridial organs were responsible for its osmoregulating ability in reduced salinity.

There is the possibility that all of the increased oxygen demand may be used for the general increased activity observed for A. californiensis larvae in 26.2 ppt salinity at all temperatures.

Relationship of Respiration to Survival and Growth

Since temperature and salinity both affect metabolism it is expected they would also affect survival and growth. A comparison of respiration, mean length and growth; at 7° and 15°C in four experimental salinities is made in Figure 10. It is believed that after 22 days of culturing under experimental conditions, the major effects of temperature and salinity can be shown and a definite response trend can be established for both survival and growth.

The greater mean length and growth increments at 19.9 and 26.1 ppt salinity (compared to that of 32.8 ppt) correspond to the

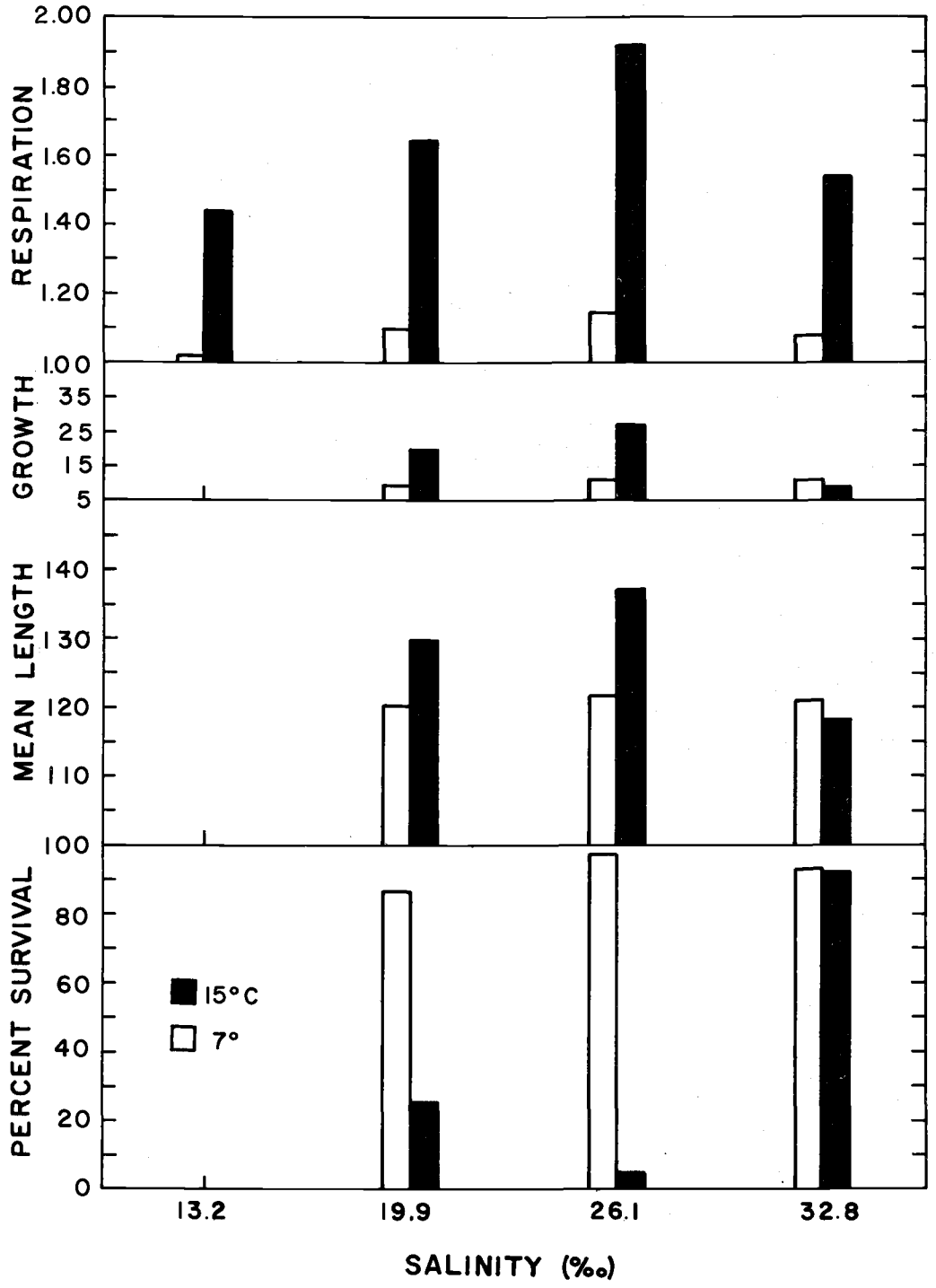

Figure 10. A comparison of percent survival, mean length, growth increment of Adula californiensis larvae after 22 days and respiration of 72 hour larvae in salinities of 13.2, 19.9, 26.1, 32.8 ppt and temperatures of 7° and 15°C. Mean length and growth units are given in microns and respiration values are expressed as microliters oxygen consumed per hour per 10,000 veligers.

increased respiration rates at the same salinities. Small differences in growth, mean length and survival at 7°C, 19.9 and 26.1 ppt salinity are reflected in small differences of respiration values at the same conditions. At 15°C the relationship of survival to respiration is exactly the opposite. The unexpected decrease in survival at 15°C and 26.1 ppt salinity below that of 19.9 and 32.8 ppt is significantly greater than the ±8.9 percent error calculated for the survival counting methods. Both duplicate cultures also showed this trend. The slight increase in survival at 7°C, 26.1 ppt salinity over 32.8 ppt cannot be considered highly significant. All responses were greater at 15°C than at 7°C. Where the respiration rate dropped below that at 32.8 ppt there was no survival to the end of 22 days.

It appears that the increased respiration in the 19.9 to 26.1 ppt salinity range may produce an accelerated growth but result in an increased mortality at the higher temperature (15°C). This increased respiration may possibly be attributed to the added metabolic work required for osmoregulation and/or general increased activity.

Ecological Implications of Results

The results of this study allow inferences to be drawn concerning the survival, growth and metabolic response of A. californiensis larvae to temperature and salinity conditions found in Yaquina Bay. During the summer months along the Oregon coast upwelled cold, high

salinity water is carried several miles into estuary entrances with the tidal influx. Yaquina Bay has a moderately large salinity difference between bottom and surface water during late winter to spring (type B estuary), but has small differences between bottom and surface during summer and fall (type D) (Burt and McAlister, 1959). Under Pritchard's (1955) system for Oregon estuaries, type B is a partly mixed estuary and type D is a vertically homogenous or well mixed estuary. Bottom temperature and salinity curves for locations within the estuary (Figure 11) show minimal variation around the entrance and maximal variation upstream (Manske, 1968).

Spawning of A. californiensis in Yaquina Bay has been observed from June through October. Mass spawning occurred on the incoming tide after the adults had been exposed at low tide to heat and light from the sun.

Once spawning takes place and the eggs are fertilized, the developing larvae are either swept out to sea or upstream. Studies by Burt and Marriage (1957) indicate that it is possible for materials introduced into Yaquina Bay at any point to eventually be distributed throughout the length of the estuary. Maximal survival for A. californiensis during the first three days of development has been estimated by the surface response method to be limited to temperatures ranging from 12° to 16.5°C and salinities from 28 to 32 ppt (Figure 7). Those larvae swept out of the bay would be favored by the oceanic

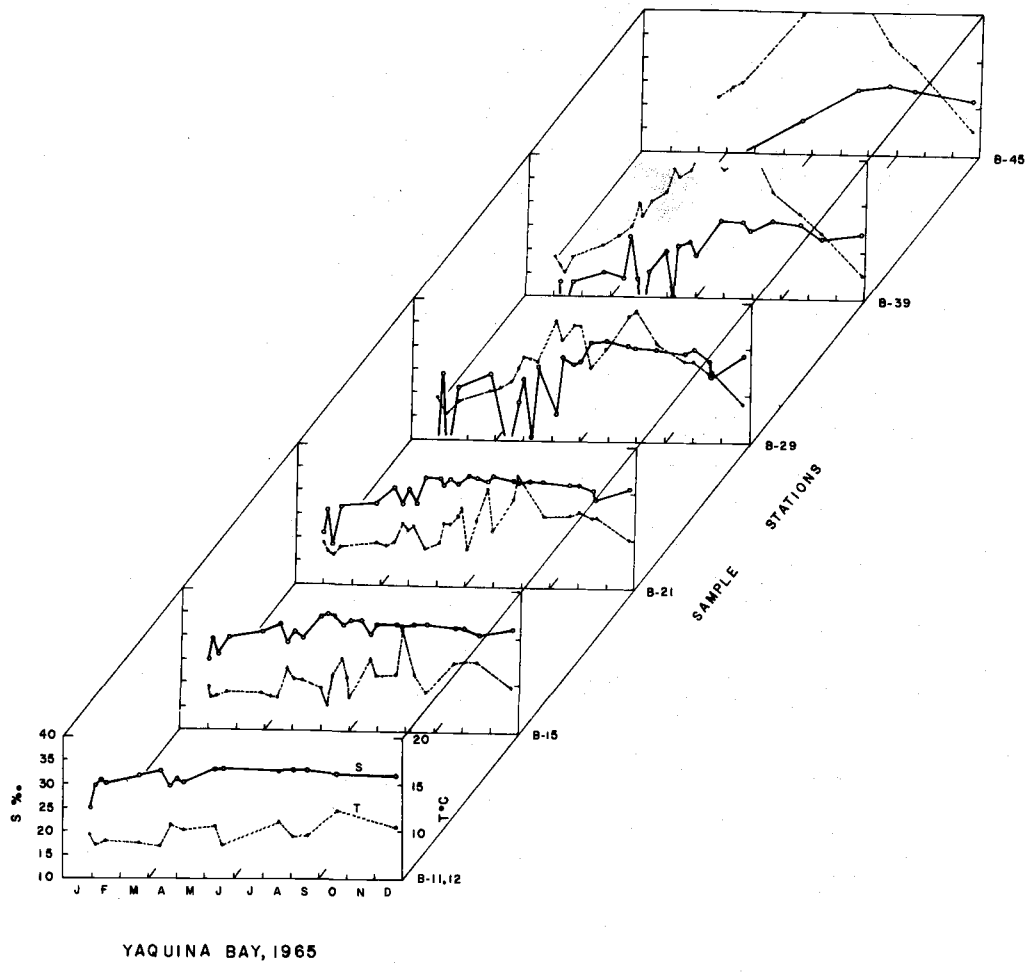

Figure 11. Bottom salinity (solid line) and temperature (dotted line) of Yaquina Bay for the year 1965 at stations B-11, B-15, B-29, B-39, B-45. Salinity and temperature values reflect tidal as well as seasonal variations at each station (adapted from Manske, 1968).

conditions prevailing outside. Within two to four tidal cycles the larvae would have developed to the veliger stage (depending on water temperature) and be capable of feeding. Under constant conditions of approximately 19 ppt salinity, no larvae would survive to 15 days. Experiments indicate that the majority of those larvae successfully reaching the veliger stage could tolerate constant salinity conditions as low as 19.9 ppt up to 22 days in water of 7° to 11°C. The effect of higher temperatures found upstream (15°C and above), when combined with reduced salinity, would drastically increase mortality.

Larvae transported upstream during the months from June through October would probably be able to tolerate conditions found as far as 8 miles up the bay where temperatures range from 12° to 18°C and salinities from 25 to 32 ppt (Figure 11) at that time. The oceanic zooplankton population found in Yaquina Bay has been reported by Frolander (1964) to be limited in distribution to salinity conditions greater than 19.6 ppt. Salinities of 19.6 ppt and greater correspond to the "life zone" area estimated for A. californiensis larvae.

The 3 day and older veliger larvae may have enhanced survival and growth at a combination of low temperatures and a salinity of about 26 ppt as indicated by the respiration results and correlated survival experiment. Perhaps by this time in development the veligers have acquired the capacity to tolerate some dilution and thereby be somewhat adapted to estuarine conditions. Davis (1958)

and Stickney (1964) have noted that the salinity tolerance of larvae of various bivalve species is determined in part by the salinity at which the parents were kept prior to spawning. A. californiensis larvae settling near the bay entrance where temperatures are low and salinity somewhat reduced have the most favorable environment for survival and growth. This does not take into consideration other factors such as suitable substrate, food and predators.

Since there was little growth during 25 days of culture, and metamorphosis was not observed, perhaps the larvae normally do not have a long planktonic life but quickly settle out of the water column upon reaching the shelled stage to assume a benthic-pelagic life until metamorphosis. The very yolky eggs of A. californiensis would place it in Thorson's classification as a species with a planktotrophic larvae and a short pelagic life. For this larval type, feeding on the plankton is of secondary importance while spreading of the larval stock is the main objective.

Many bivalve larvae are reported to be capable of searching for suitable substrate upon which to settle and metamorphose (Bayne, 1965; Cole and Knight-Jones, 1949; Walne, 1966; etc.). Experiments indicate A. californiensis larvae do not actively seek a suitable substrate prior to metamorphosis. The larvae may be able to crawl into small worm holes that permeate the surface of the mudstone since young adults on the order of 2 to 3 millimeters in length were

found burrowed into the surface to a depth of 3 or 4 centimeters. Only after metamorphosis would the larvae have some ability to select their location, as young adults of the small size mentioned above were observed to rapidly crawl with their foot when disturbed. Larger sized adults cannot crawl. This would enable the small clams to avoid coming in direct competition with the adults.

Larvae of oyster species limited to estuarine conditions have a behavioral mechanism which retains the larvae within the estuary (Carriker, 1951; etc.) by vertical migrations with tide changes. Since the larvae of A. californiensis require essentially oceanic temperatures and salinities for optimal survival, it is doubtful that they have such a mechanism. The behavior experiment with vials of layered water of different salinity indicates that A. californiensis trochophores normally distribute themselves throughout a water column as long as salinities remain around oceanic. In a water column of layered salinities the larvae may be able to selectively determine their position. However, during the summer upwelling this behavior would not have much adaptive value as the estuary is well-mixed. Preliminary studies of monthly gonad samples do not indicate a cessation in oogenesis at any time of the year, so it may be possible for spawning to occur during the winter months when the estuary is layered.

Most adult bivalves are able to withstand some salinity

fluctuations by (1) closing their valves, or (2) having tissues that are able to tolerate a high dilution. The larvae of A. californiensis are highly sensitive to dilute salinity conditions in early development but become progressively less sensitive later. If the larvae are able to spread and survive over an area as wide as the adults are found, then the larval form must either tolerate or avoid the extremes of salinity found at times in the adult habitat. The larval form may have the ability to regulate in a slight dilution of sea water, even if only for a short time. It seems more likely that the only mechanism for insuring the successful recruitment of A. californiensis is the critical period of spawning during the season of optimum conditions for larval survival and the production of a large number of eggs. Within the region of the bay with acceptable temperatures and salinities, the major limiting factor appears to be the availability of suitable substrate. Only two mudstone areas are known to exist in the estuary near the mouth and at Coquille Point; and both areas are heavily populated by the adults. Larvae settling out on the nearby tide flats without a suitable substrate would probably be preyed upon or buried under mud.

SUMMARY

1. Larvae of Adula californiensis were cultured in the laboratory from fertilization to 25 days of development. Experiments of a factorial design were used to investigate the effects of temperature and salinity on survival, growth and respiration.
2. Fertilization and the rate of early development were studied under 12 different environmental combinations of temperature (7°, 11°, 15°, 20°C) and salinity (20.4, 22.6, 26.3, 33.2 ppt). One hundred percent fertilization was found to occur in all temperature-salinity combinations except for the 7°C, 20.4 ppt combination where 60 percent of the eggs did not appear to be fertilized. The rate of development from fertilization to the veliger stage increased with increasing temperatures at all salinities. A retardation of developmental rate and an increase in abnormalities and mortality occurred with increasing salinity.
3. A 4 x 4 factorial approach was used to produce a graded survival response to temperatures of 7°, 11°, 15°, 20°C and salinities of 20.3, 23.2, 26.2, 32.9 ppt. Maximal survival after 3 and 15 days of rearing under experimental conditions occurred at 11° and 15°C, 32.9 ppt. Below 26.2 ppt survival decreased sharply to virtually no survivors at 20.3 ppt at any

temperature. The linear and quadratic effects of salinity were indicated by statistical analysis to be the more important factors affecting larvae to 3 days of rearing. Survival of the larvae to 15 days of rearing was most affected by the linear and quadratic effects of temperature. Survival response surfaces based on these 16 different combinations of temperature and salinity were estimated for larvae reared to 3 and 15 days. Maximal survival for larvae reared to 3 days was predicted to occur between salinities of 29 and 32 ppt and temperatures between 9° and 16°C. Maximal survival for 15 days of rearing was predicted to occur between 31 and 36 ppt and 9° and 15°C.

4. An analysis of preliminary survival data combined with the above 3 and 15 day data produced a better fitted response surface. Maximal survival of larvae reared to 3 days was estimated to occur between salinities of 28 and 32 ppt and temperatures between 12° and 16.5°C. Maximal survival for larvae reared to 13, 15 days was estimated to occur between 29 and 34 ppt and 10° and 15.5°C. Both the linear and quadratic effects of temperature and salinity were indicated by statistical analysis to be significantly affecting survival to 3 and 13, 15 days of rearing.

5. Larvae were reared for a 3 day period (to veliger stage) at 15°C, 32.2 ppt and then transferred to 16 combinations of

temperature (7°, 11°, 15°, 20°C) and salinity (13.3, 19.9, 25.9, 32.7 ppt) to determine the effects of these factors on later larval development. In general, survival decreased with decreasing salinity but at a greater rate at the higher temperatures. After 22 days of rearing no larvae survived at 20°C at any salinity and at 13.3 ppt at any temperature. Statistical analysis showed the quadratic effect of temperature and the linear effect of salinity to be the more important factors affecting survival.

6. Larval growth was not significant over 3, 15 and 22 days of rearing and only small differences in mean lengths were measured for larvae reared in any temperature-salinity combination where there was survival. Although the statistical analysis showed no single variable to be significant, the quadratic effect of temperature was generally the more important factor affecting growth. It is speculated that growth of the larvae during the planktonic stage is relatively temperature or salinity independent.

7. Oxygen consumption was measured for the 72 hour veliger larvae at salinity and temperature combinations of 13.0, 19.8, 26.2, 32.9 ppt and 7°, 9°, 12°, 15°, 18°, 21°C. Oxygen consumption generally increased from 7° to 18°C and then sharply decreased from 18° to 21°C. Statistical analysis showed that

the linear and quadratic effects of temperature were the more significant factors affecting oxygen consumption. The small change in oxygen consumption from 9° to 15°C at 32.9 ppt is believed to indicate that the larvae are well adapted to the normal range of oceanic conditions found off the Oregon coast.

8. A comparison of 72 hour larval oxygen consumption, 22 day mean length and growth at 7° and 15°C in all experimental salinities suggested a generally greater metabolism at 19.9 and 26.1 ppt than at 32.8 ppt.

9. Since the early development of A. californiensis requires temperature and salinity conditions near oceanic for survival, successful reproduction of this species depends upon the release of a large number of eggs at a critical period of optimum conditions. The planktotrophic larvae are believed to have a short pelagic life before settlement. Within the bay region where acceptable temperatures and salinities occur, the major limiting factor appear to be the availability of a suitable substrate.

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