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A Response-Surface Approach to the Combined Effects of Temperature and Salinity on the Larval Development of *Adula californiensis* (Pelecypoda: Mytilidae). I. Survival and Growth of Three and Fifteen-Day Old Larvae

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Abstract

Laboratory experiments of a factorial design were used to examine the combined effects of temperature and salinity on the survival and growth of early and late-stage larvae of *Adula californiensis* (Phillippi, 1847). Response-surface curves were generated to predict optimal conditions for survival and growth in order to better understand the successful recruitment of this species within the Yaquina Bay estuary (Oregon, USA). Three-day old cultured larvae were more sensitive to reduced salinity than were 15-day old larvae. However, the 15-day old larvae showed a narrower temperature tolerance than the 3-day old larvae. *A. californiensis* larvae survived over a wider range of temperatures near optimum salinities than at salinities near their lower tolerance limit, and conversely. Temperature and salinity ranges for maximum survival (10 ° to 15 °C, 31 to 33 ‰) were narrower than the ranges which occur within the estuary where the adult populations exist. Larval size did not increase markedly during the 15-day rearing period, and was not greatly affected by temperature or salinity. No statistically significant temperature-salinity interaction was found for either survival or growth.

Introduction

The importance of the combined effects of temperature and salinity on an organism, and the need to consider these factors jointly, has been emphasized by Kinne (1963, 1964, 1970). Salinity may modify the effects of temperature and change the temperature range of many biological processes; likewise, temperature can modify the effects of salinity. The combined effects of these factors on bivalve larval development was first observed by Amemiya (1928), who found that growing *Crassostrea gigas* larvae at the lower end of their temperature range narrowed their range of salinity tolerance. More recently, Bayne (1965) found that the optimum salinity range varied with temperature for *Mytilus edulis* larvae. Davis and Calabrese (1964) were the first investigators to look at the combined effects of temperature and salinity on bivalve larval development using factorially designed experiments. They found that the larvae of *Mercenaria mercenaria* and *Crassostrea virginica* grew over a wider range of temperatures near optimum salinities than near the lower limit of their salinity tolerance. Similar studies of the combined effects of temperature and salinity on the early development of bivalve larvae have been performed by Brenko and Calabrese (1969) for *Mytilus edulis*, Calabrese (1969) for *Mulinia lateralis*, and Lough and Gonor (1971) for *Adula californiensis*. However, these studies only examined the effects of temperature and salinity by inspection of the data, and not by a critical statistical analysis.

Statistical methods developed by Box and Youle (1955) have made it possible to estimate the response of larvae under a greater variety of environmental conditions than is possible through experiments in the laboratory, and to determine which factors are significantly contributing to the response variation. This response-surface technique has opened a new approach to the study of larval ecology (see Costlow and Bookhout, 1964), and has been applied to brachyuran larvae by Costlow *et al.* (1960, 1962, 1966) and Costlow (1967); and to English sole, petrale sole, and Pacific cod larvae by Forrester and Alderdice (1966) and Alderdice and Forrester (1968, 1971a, b). The use and evaluation of response-surface techniques in marine ecology has been reviewed in detail by Alderdice (1972). These techniques have not been previously used on the larvae of any other marine invertebrates except Brachyura.

Our study examined the combined effects of temperature and salinity on an early and a late stage of development of the bivalve mollusc *Adula californiensis* in laboratory culture, by using response-surface techniques to predict survival and growth in nature. Part II of this study will compare early larval respiration with long-term larval survival and growth, and will attempt to relate these findings to recruitment to the adult population under natural conditions in its Yaquina Bay, Oregon, habitat.

Materials and Methods

The methods used to obtain adult *Adula californiensis*, secure gametes from them, and handle embryos, glassware and seawater, are as described previously (Lough and Gonor, 1971).

Preliminary Larval-Density Experiment

A suitable density of larvae for the culture of *Adula californiensis* was determined by growing larvae in densities of approximately 25 to 400/ml culture water. To estimate the density of the original egg suspension and the amount of error introduced by the pipetting method, 0.25 ml egg samples were pipetted into 40 vials of formalin with a 1 ml 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 ml-capacity Mini-pet syringe into duplicate cultures to produce the desired density. One culture of low larval density (50 larvae/ml) contained 50,000 units/l Penicillin G and 100 mg/l Streptomycin. The antibiotic mixture was made with 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* larvae cultures without adverse effects on development.

All densities of larvae were reared in 100 ml of 33.1‰ S seawater at 15° ± 0.01 °C. After 72 h development at 15 °C, the larvae had developed shells and could be handled without damage. The larvae were sieved with a 54 µm mesh nylon screen, washed into a graduated cylinder, diluted to 100 ml, agitated with a plunger, and a 5 ml aliquot taken from each culture with a 1 ml Mini-pet syringe. The 5 ml 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. The remaining larvae in the graduated cylinder were washed back into the culture flask with fresh seawater of the same salinity. A sample was taken for counting, and the water changed every 24 h until the larvae had developed for 120 h.

Immediately after changing the water, all cultures were fed *Isochrysis galbana* every other day, starting from 72 h of development. Algal cultures were grown at room temperature (ca. 20 °C) in Matthiessen 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 densities were determined by counting in a haemocytometer. Every 24 h, the pH value was determined for all cultures. The pH values remained in the range of 7.70 to 7.91 during the experiments.

The results and analysis of the larval density experiment and the effect of the antibiotic treatment are given in Tables 1 and 2, respectively, in which the number of larvae per milliliter are converted to percent survival. The greatest number of larvae surviving in any culture at a given density over the 5 days of rearing was selected as 100% survival. An analysis of variance indicated a significant difference in survival between the density levels (Table 2). The least-significant difference (LSD) test among treatment means indicated that cultures of the lowest density had the poorest survival. There was no significant difference among the other density levels. Since survival at all higher densities was the same, the apparent poor survival in the lowest density cultures probably resulted from sampling error associated with the small numbers of larvae in these cultures. The effect of the antibiotic mixture on larval survival was determined to be not significant using raw percent survival or transformed percent (arcsin transformation) data in one-way analyses of variance.

Table 1. *Adula californiensis. Effect of varying densities of larvae on survival in replicate cultures reared in 100 ml sea water of 33.1‰ S at 15° ± 0.01 °C*

Number at fertilization (eggs/ml)	% Surviving 72 h	96 h	120 h	Mean
25	60.8	59.4	48.3	74.6
	100	78.7	74.8	
50	100	84.1	94.7	90.6
	83.7	84.1	83.7	
50[a]	92.5	87.5	88.3	90.8
	83.3	81.9	100	
99	100	90.7	94.1	95.8
	97.1	94.9	91.9	
199	88.3	90.2	89.8	93.1
	100	90.9	91.2	
398	100	100	97.1	93.4
	77.7	71.7	89.5	

[a] Cultured with antibiotics: concentration of 50,000 units/l Penicillin G plus 100 mg/l Streptomycin.

Table 2. *Adula californiensis. Analysis of variance of the effect of different densities of larvae on survival over 5 days of rearing, using arcsin transformed percentage survival data. DF: Degrees of freedom; SS: sum of squares; MS: mean square*

Source	DF	SS	MS	F	Significance level
Density	4	1172.41	293.10	5.12	5%
Time	2	469.55	234.78	4.10	Not significant
Density × time	8	457.98	57.25	0.48	Not significant
Duplicate cultures (experimental error)	15	1792.74	119.52		
Total	29	3892.74			

Least significant difference (5% level) = ± 9.4%.
Least significant difference (1% level) = ± 16.8%.

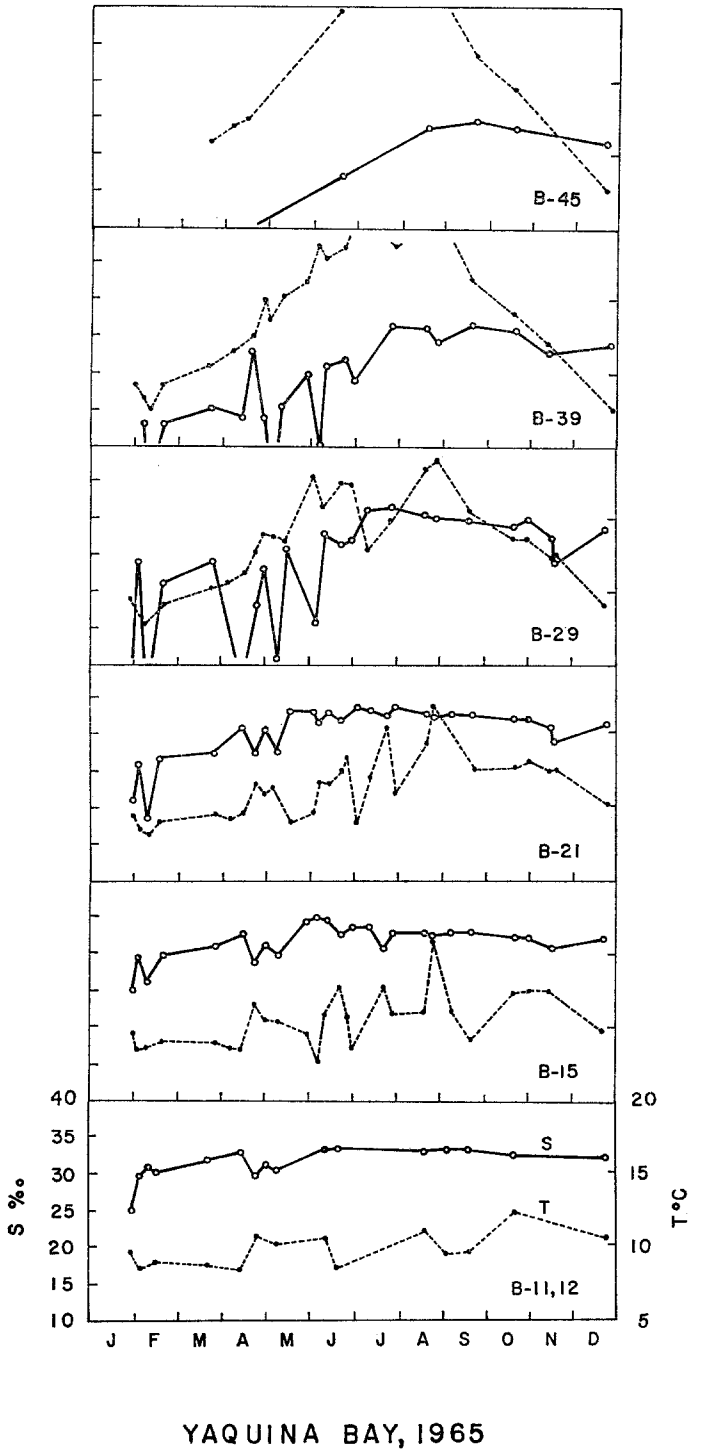

Fig. 1. Bottom salinity (solid line) and temperature (dotted line, T) of Yaquina Bay, Oregon, for year 1965, at selected navigational buoy (B) stations, where the larvae of *Adula californiensis* might be expected to be found in the plankton. Adult populations occur at Stations B-11 and B-15. Distances in nautical miles from bay mouth to stations are: B-11,12: 1.4; B-15: 2.8; B-21: 4.3; B-29: 6.6; B-39: 8.0; B-45: 9.2

Factorial Experiments on Survival and Growth

Using conditions known to occur within the extremes found in Yaquina Bay (Fig. 1), a 4×4 factorial experiment was conducted 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‰. The experiment was carried out twice.

In the first set of duplicate cultures, unequal volumes of eggs were pooled from more than 5 females to insure a response representative of the population. After the pooled egg-volume was estimated and adjusted, the eggs were fertilized with pooled sperm. One milliliter of the fertilized egg suspension was pipetted to each of the experimental salinity cultures to produce a density of approximately 100 larvae/ml, and transferred to the proper temperature. In this and the subsequent experiment, larvae were cultured in 200 ml of water at densities of about 100 larvae/ml. From previous work, it did not appear to make any difference whether eggs were transferred into low salinities directly or by slow dilution. The antibiotic mixture described above was initially added to each culture.

After 72 h development, the water was changed in each culture and the larvae were fed equal concentrations of *Isochrysis galbana* every other day. The algal concentrations used at each feeding varied from 1,220 to 1,430 cells/ml seawater in the culture flask. A 5 ml sample was taken from each culture at the end of the 3rd and 15th day, preserved, and the total number of larvae in the sample counted. The 3 and 15-day percent survival was calculated by regarding the highest survival among all of the temperature-salinity combinations of the combined duplicate cultures as 100% survival. The mean length of larvae after 3 and 15 days under the experimental conditions was determined by measuring the long axis of 15 larvae from each culture with an ocular micrometer to the nearest 1.5 μm.

The second set of duplicate cultures differed from the first in two ways: (1) equal volumes of eggs from 4 females were pooled; (2) all the larvae were fed starting 24 h after fertilization instead of after 72 h. The first modification was made to control any variability between egg batches from different females. 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 h. The algal concentrations at each feeding varied from 1,060 to 1,220 cells/ml seawater in the culture flask. During both sets of experiments, variations in salinity between water changes were ±0.5‰. The percent survival and the mean body length at the end of the 3rd and 15th day were averaged for both sets of experiments.

Estimation of Experimental Error

Larval survival within a particular temperature-salinity cell of these factorial experiments has three components of error: sampling, counting, and response error due to inherent biological variability. In order to provide a basis for determining if the response in one cell was significantly different from that in another cell, the 95% confidence intervals for a single observation have been estimated by the 4 following methods.

A three-factor (temperature, salinity, duplicate cultures) analysis of variance was computed for larvae surviving to 15 days (Table 3). The 95% confidence intervals for a single observation were estimated as ±14.5% by taking the square root of the mean square error (MSE = 54.4225) and multiplying by 1.96.

The second method used 3-day old survivors of 32 identical cultures reared under the optimum conditions of 15 °C, 33.2‰ S, with a larval density estimated at 100/ml. Computation of the 95% confidence intervals for a single observation based on the mean were estimated at ±17.4%.

The third and fourth methods were based on the larval density experiment. From Table 2, the 95% confidence intervals for a single observation were estimated to be ±13.4% ($1/\sqrt{119.516}\ 1.96$). Using arcsin transformed percent-survival data from Table 1, the 95% confidence intervals of a single observation were estimated as ±17.3% of the mean.

Therefore, the 95% confidence intervals of a single observation in our experiments lie between approximately ±13 to 18%.

Statistical Treatment of Experimental Data

Our experiments were of a factorial design, in which the treatments consisted of all combinations of different levels of a set of factors. Such experiments provide a greater precision for estimating overall factor effects, permit the interactions between different factors to be explained, and allow the range of validity of the conclusions to be extended by the insertion of additional factors (Cox, 1958). The fitting of a response surface to a factorial experiment may greatly add to our understanding, by suggesting the form of the underlying factor combinations that are of most significance for the system. A response surface is produced by plotting the treatment effect against variables (temperature and salinity in this case) in a three-dimensional diagram. The statistical calculations involved are a special example of the method of multiple linear regression.

The computer program *STEP, a stepwise multiple linear regression-analysis program discussed by M. A. Efroymson (Ralston and Wilf, 1960) was used for the analysis. It was assumed that the nature of the response surface could be described by a regression equation of the form:

$$Y = b_0 + b_1 x_1 + b_2 x_2 + b_{11} x^2_1 + b_{22} x^2_2 + b_{12} x_1 x_2 ,$$

where Y = percent survival or mean length, x_1 = temperature (°C), x_2 = salinity (‰), and b_0 = 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 effects between temperature and salinity (Box and Youle, 1955).

Regression coefficients (b's) were calculated by the computer from the experimental points by the method of least squares. A t test was made, indicating the equality of the individual regression coefficients to zero and the level of significance. Those factors below the 10% significance level were not considered important.

F-levels were set equal to zero to enter and remove variables. This allowed all variables to come into the equation by a forward selection procedure, their order of insertion being determined by using the partial correlation coefficient as a measure of their importance. The contribution a variable makes in reducing the variance of the equation can also be considered by looking at various values given as the program proceeds. One of the more useful is the square of the multiple correlation coefficient, R^2, defined as the sum of squares due to regression, b_0/total sum of squares, corrected for the mean. It is often stated as a percentage, 100 R^2. The larger it is, the better the fitted equation explains the variation in the data. Values of R^2 can be compared at each stage of the regression program.

The calculated regression coefficients from a particular equation were fitted by computer to a full quadratic equation in temperature and salinity, in order to print a contour diagram of the response surface. The computer program was designed to print 20% survival contour intervals, wide enough to approximate the 95% confidence interval calculated for single observations.

Results

Survival after 3 and 15-Day Rearing Periods at Different Temperatures and Salinities

Since a two-tailed t test indicated that there was no significant difference at the 5% level in mean percent survival at 3 or at 15 days between the two sets of duplicate experiments, the results were averaged for each time period. The averaged survival data for the *Adula californiensis* larvae after 3 and 15 days in the experimental temperature and salinity combinations are presented in Table 3. Pooling equal volumes of eggs from 4 adults in contrast to pooling unequal volumes of eggs from many adults produced comparable results. Larvae that were initially fed 24 h after fertilization showed no significant difference in survival at any temperature from those fed initially at 72 h.

Maximal survival for the 3-day larvae occurred in 32.9‰ S and at temperatures of 11° and 15 °C (Table 3). There was approximately a 10 to 20% 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‰ S and 7 °C, 26.2‰ S 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‰ S (Table 3). The optimum temperature for survival in all salinities appeared to be 11 °C. Below 26.2‰ S, there was a sharp decrease in survival to virtually no survivors at 20.3‰ S at any temperature. Temperatures of 7° and 20 °C narrowed the range of salinities for larval survival; the higher temperature producing a greater mortality.

Table 3. *Adula californiensis. Percentage of larvae surviving in 16 temperature-salinity[a] combinations*

Salinity (‰)	Temperature 7 °C	11 °C	15 °C	20 °C
		After 3 days		
32.9	29.2[b]	100.0	98.3	78.5
26.2	51.3[b]	88.4	82.5	85.7
23.2	61.7	62.1	71.8	61.8
20.3	0	41.4	52.8	34.1
		After 15 days		
32.9	79.1	99.2	98.5	44.4
26.2	21.8	93.8	82.6	13.3
23.2	0	66.8	48.3	0.3
20.3	0	0.5	0.4	0

[a] Averaged salinity values ±0.5‰.
[b] Because of difficulties in counting, these values were not included in the statistical analysis.

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 4) 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% of the variation:

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

The 15-day statistical analysis (Table 4) 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

Table 4. *Adula californiensis. Statistical analysis of larval survival in 16 temperature-salinity combinations. S, S^2 and T, T^2: Linear and quadratic effects of salinity and temperature, respectively; DF: degrees of freedom*

Regression step no.	Variable	R-square	*t* value (8 DF)	Significance level
		After 3 days		
1	S	0.632	4.96	1%
2	S^2	0.803	4.04	1%
3	T	0.810	3.86	1%
4	T^2	0.908	2.46	5%
5	$T \times S$	0.932	1.68	Not significant
		After 15 days		
1	S	0.543	2.07	10%
2	T^2	0.588	4.59	1%
3	T	0.831	4.08	1%
4	S^2	0.859	1.47	Not significant
5	$T \times S$	0.873	1.05	Not significant

important at the 10% significance level based on *t* test, but was ranked first by partial correlation coefficients. The resulting equation incorporating all variables explained 87.3% of the variation:

$$Y_{\%\,\text{survival}} = -608.43 + 29.25\,(S) + 33.72\,(T) - 0.21\,(T \times S) - 0.38\,(S^2) - 1.06\,(T^2)\,.$$

The significance of the differences between the fitted equations of the effect of temperature and salinity on survival at Days 3 and 15 was tested using the method described by Ostle (1963, p. 205) to test the significance of the difference between two polynomials. The results of this analysis of variance, summarized in Table 5, indicate a significant difference between the equations at the 1% level.

Table 5. *Adula californiensis. Comparison, by analysis of variance, of the polynomial equations for 3 and 15 days survival. Hypothesis: no significant difference between 3 and 15-day survival polynomials. DF: Degrees of freedom; SSE: sum of squares error; MSE: mean square error*

Source	DF	SSE	MSE	F
Polynomial 1: 3-day survival	8	666.91		
Polynomial 2: 15-day survival	10	3119.85		
Total: Polynomial 1 & 2	18	3786.76	210.38	
Polynomial 3: Combined 3 & 15-day survival	24	11816.15		
Difference: between Polynomial 3 and Total	6	8029.39	1338.23	6.36[a]

[a] Significant at 1% level. $F_{(6,18,.99)} = 4.01$.

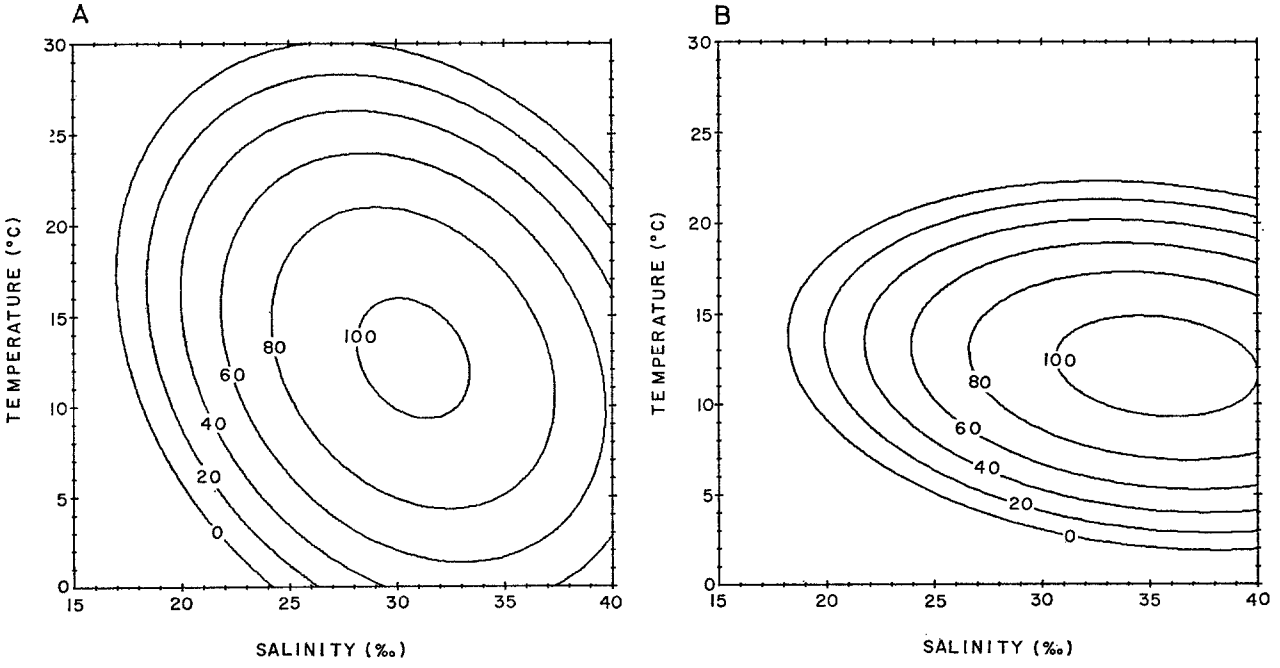

Fig. 2. *Adula californiensis.* Response-surface estimation of percent survival of larvae after (A) 3 days, (B) 15 days development at 16 different temperature and salinity combinations

Graphical estimations of percent survival for the 3 and 15-day larvae based on the fitted response-surface to survival under 16 different combinations of temperature and salinity are presented in Fig. 2. Assuming the survival estimations apply to the temperature-salinity conditions in nature, and assuming optimal nutrition, the following predictions can be made.

One hundred percent survival for the 3-day larval stage (Fig. 2A) can be expected between salinities of 28 and 33‰ 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. The nearly circular shape of the fitted contours indicates the absence of any significant interaction effect.

Maximal survival for the 15-day larvae (Fig. 2B) can be expected between salinities and temperatures of 31 and 40‰ and 9.5° and 15 °C. No survival is predicted in salinities below 18‰ and at temperatures outside of the range 2° to 22 °C.

Growth of Larvae after 3 and 15 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 Table 6. Both the greatest 3-day and greatest 15-day mean length occurred at 20 °C and 32.9‰ S. Mean length decreased with decreasing salinity, while the standard deviations show that the size range of the individuals increased.

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 (Table 7). The 3-day analysis showed the linear and quadratic effects of salinity to be significant only at the 10% level. The resulting equation explained a significant 93.9% of the variation for the 3-day survival data:

$$Y_{\text{mean length}} = -241.75 + 23.63\,(S) - 3.05\,(T) + 0.11\,(T \times S) - 0.40\,(S^2) + 0.004\,(T^2)\,.$$

None of the variables were significant for the 15-day mean-length analysis (Table 7). Only 37.4% of the variation in the data was explained by the equation incorporating all the variables.

Table 7. *Adula californiensis. Statistical analysis of mean length for larvae surviving in 16 temperature-salinity combinations. Abbreviations as in Table 4*

Regression step no.	Variable	R-square	*t* value (3 DF)	Significance level
		After 3 days		
1	S	0.802	2.41	10%
2	S^2	0.915	2.37	10%
3	$T \times S$	0.919	1.06	Not significant
4	T	0.939	0.51	Not significant
5	T^2	0.940	0.02	Not significant
		After 15 days		
1	T	0.041	0.94	Not significant
2	T^2	0.108	0.98	Not significant
3	$T \times S$	0.112	0.33	Not significant
4	S	0.130	1.28	Not significant
5	S^2	0.374	1.25	Not significant

Table 6. *Adula californiensis. Mean length (μm) and standard deviation of larvae surviving after development in 16 temperature-salinity combinations*

Salinity (‰)	Temperature 7 °C[a]	11 °C	15 °C	20 °C
		After 3 days		
32.9	—	106.4 ± 3.1	107.8 ± 3.1	111.2 ± 3.0
26.2	—	98.0 ± 6.1	95.1 ± 6.3	103.3 ± 6.9
23.2	—	84.0 ± 8.1	86.8 ± 7.4	77.3 ± 13.1
20.3	—	—	—	—
		After 15 days		
32.9	117.4 ± 4.5	116.9 ± 4.1	117.1 ± 2.9	120.1 ± 7.1
26.2	110.2 ± 9.0	113.9 ± 4.7	107.9 ± 6.8	120.2 ± 10.0
23.2	—	112.1 ± 8.4	109.1 ± 11.7	—
20.3	—	—	—	—

[a] No shell development at this temperature by 3 days.

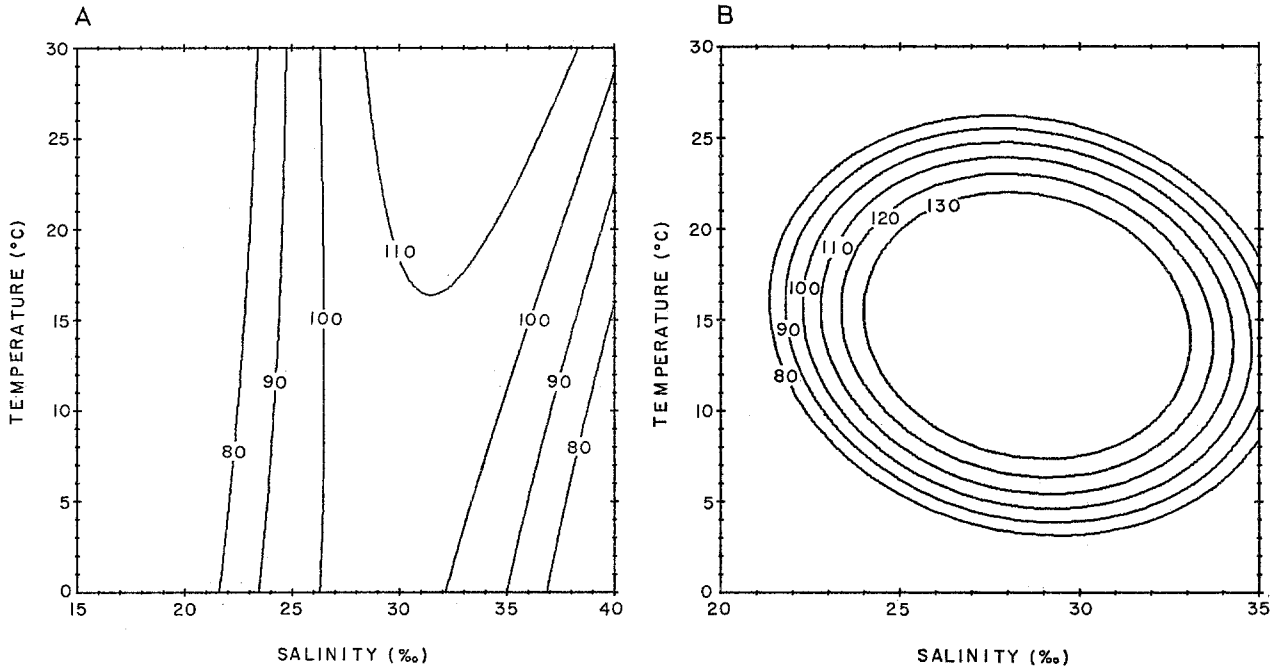

Fig. 3. *Adula californiensis*. Response-surface estimation of mean length (μm) of larvae after (A) 3 days, (B) 15 days development at 16 different temperature and salinity combinations

The computer output of the variables, based on partial 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. The linear and quadratic effects of salinity made the greatest contribution to R-square.

The 3-day mean-length response surface (Fig. 3A) estimates the experimental points well, despite the fact that none of the variables in the fitted polynomial were highly significant. The greatest mean length is estimated to occur at temperatures above 15 °C and at salinities between 28 and 38‰. The ridge of maximum mean length is slightly skewed towards the higher salinities, indicating a slight interaction effect, i.e., greater mean length at higher temperatures is coupled with higher salinities.

The 15-day mean-length response surface (Fig. 3B) contours are nearly circular, indicating the absence of a significant interaction effect between temperature and salinity. Maximum mean length is estimated to occur between temperatures and salinities of 8° and 22 °C and 23 and 33‰, respectively.

Discussion

In *Adula californiensis* larvae, maximum survival after 3 days of development occurred in a much narrower range of conditions than did fertilization (Lough and Gonor, 1971). Salinities between 28 and 33‰ and temperatures from 9° to 16 °C are required for maximum estimated survival (Fig. 2A). This indicates that success of early development in *Adula californiensis*, from fertilization to veliger stage, is limited to the normal range of inshore oceanic conditions rather than the broader range of conditions possible in the estuary. Young (1941) showed that salinities less than 29.6‰ also decreased larval survival of *Mytilus californianus*, another common mytilid on the Pacific coast.

Conditions for maximum survival of 15-day old veliger larvae (9.5° to 15 °C, 31 to 40‰ S, Fig. 2B) were estimated to be approximately the same as for the 3-day old larvae. However, the temperature range for survival to 15 days (2° to 22 °C) is considerably narrower than for survival to 3 days (0° to 30 °C). The response surfaces for the two sampling periods were shown to be significantly different by analysis of covariance. This suggests that the 3 and 15-day old larvae may have different survival tolerances, despite the fact that the two sampling periods were within the same experiment and not independent. Larvae reared to 3 days under the experimental conditions appear to be more sensitive to small salinity changes than the older larvae, while these same larvae reared to 15 days appear to have become more sensitive to extremes of temperature. It could also be argued that the major effect of temperature and salinity on development occurred within the initial 3 days, but that the mortality response was not manifest until Day 15.

From our experience with laboratory rearing of *Adula californiensis*, it is doubtful that larvae reared from fertilization would survive to settling stage outside the 80% survival contour range of 7° to 12 °C and 26 to 40‰ S (Fig. 2B). Survival decreases rapidly in temperature and salinity conditions more extreme than the 80% survival contour. The response-surface technique used here to investigate temperature-salinity relationships should be considered to provide only estimations, and not precise limits.

Adula 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. 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 *A. californiensis* larvae for the 3 and 15-day survival data.

Since larval size remained about the same in all temperature and salinity combinations where there was survival, it appears to be relatively independent of temperature and salinity. The normal range of oceanic conditions appears to have only a slight effect on the size of the veliger larvae.

Successful recruitment of young to the adult populations within the estuary is apparently dependent upon the relatively limited period when the essentially oceanic conditions required for successful larval development occur within the lower reaches of Yaquina Bay. The ability of larvae initially reared at optimal conditions to withstand subsequent salinity and temperature changes was also examined in other experiments. The results of these experiments and the effect of these conditions on larval respiration, as well as the implications of these results for larval survival within the estuary, will be given in a subsequent paper.

Summary

1. Larvae of *Adula californiensis* (Phillippi, 1847) were cultured in the laboratory from fertilization to 15 days of development, in factorial experiments designed to investigate the effects of temperature and salinity on survival and growth.

2. A 4 × 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‰. Maximal survival after 3 and 15 days of rearing under experimental conditions occurred at 11° and 15 °C, 32.9‰ S. Below 26.2‰ S, survival decreased sharply to virtually no survivors at 20.3‰ S at any temperature.

3. Percent survival to 3 and 15 days were analyzed by multiple-regression methods. The linear and quadratic effects of salinity were indicated by the statistical analysis to be the more important factors affecting larvae reared from fertilization to 3 days of development. Survival of the same larvae to 15 days was most affected by the linear and quadratic effects of temperature.

4. Survival response surfaces were estimated for larvae reared to 3 and 15 days. Maximum survival was estimated to be approximately the same for both the 3 and 15-day old larvae (10° to 15 °C, 31 to 33‰ S), but the temperature range for any survival to 15 days (2° to 22 °C) was considerably narrower than for survival to 3 days (0° to 30 °C).

5. Our experiments indicated that the 3 and 15-day old larvae have different tolerances to temperature and salinity; a reflection of their different stages of development. Larvae reared to 3 days, from fertilization to veliger stage, appeared to be more sensitive to salinity changes, while the same veliger larvae reared to 15 days appeared to be more sensitive to temperature extremes.

6. Larval growth was not significant over 3 and 15 days of rearing, and only small differences in mean lengths were measured for larvae in any temperature-salinity combination where there was survival. A multiple-regression analysis of the 3 and 15-day mean lengths showed no single variable to be highly significant.

7. *A. californiensis* larvae survived and grew larger over a wider range of temperatures near optimum salinities than at salinities near their lower tolerance limit. However, a statistically significant temperature-salinity interaction was not found for either survival or size.

8. The early life stages of *A. californiensis* through 15 days of development require essentially oceanic conditions.

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Literature Cited

Alderdice, D. F.: Factor combinations — responses of marine poikilotherms to environmental factors acting in concert. *In:* Marine ecology, Vol. 1. pp 1659—1722. Part 3. Ed. by O. Kinne. London: Wiley-Interscience 1972.

— and C. R. Forrester: Some effects of salinity and temperature on the early development and survival of the English sole (*Parophrys vetulus*). J. Fish. Res. Bd Can. 25, 495—521 (1968).

— — Effects of salinity and temperature on embryonic development of the petrale sole (*Eopsetta jordani*). J. Fish. Res. Bd Can. 28, 727—744 (1971a).

— — Effects of salinity, temperature, and dissolved oxygen on early development of the Pacific cod (*Gadus macrocephalus*). J. Fish. Res. Bd Can. 28, 883—902 (1971b).

Amemiya, I.: Ecological studies of Japanese oysters with special reference to the salinity of their habitats. J. imp. Coll. agric. Univ. Tokyo **9**, 334—382 (1928). [Reviewed by H. P. Sherwood. J. Cons. perm. int. Explor. Mer **3**, 407—409 (1928)].

Bayne, B. L.: Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.). Ophelia **2**, 1—47 (1965).

Box, G. E. P. and P. V. Youle: The exploration and exploitation of response surfaces; an example of the link between the fitted surface and the basic mechanism of the system. Biometrics **11**, 297—323 (1955).

Brenko, M. Hrs. and A. Calabrese: The combined effects of salinity and temperature on larvae of the mussel *Mytilus edulis*. Mar. Biol. **4**, 224—226 (1969).

Calabrese, A.: Individual and combined effects of salinity and temperature on embryos and larvae of the coot clam *Mulinia lateralis* (Say). Biol. Bull. mar. biol. Lab., Woods Hole **137**, 417—428 (1969).

Costlow, J. D., Jr.: The effect of salinity and temperature on survival and metamorphosis of megalops of the blue crab *Callinectes sapidus*. Helgoländer wiss. Meeresunters. **14**, 84—97 (1967).

— and C. G. Bookhout: An approach to the ecology of marine invertebrate larvae. *In:* Symposium on experimental marine ecology, Occ. Publs Narragansett mar. Lab., Univ. Rhode Isl. **2**, 69—75 (1964).

— — and R. Monroe: The effect of salinity and temperature on larval development of *Sesarma cinereum* (Bosc) reared in the laboratory. Biol. Bull. mar. biol. Lab., Woods Hole **118**, 183—202 (1960).

— — — Salinity-temperature effects on the larval development of the crab *Panopeus herbstii* Milne-Edwards, reared in the laboratory. Physiol. Zoöl. **35**, 79—93 (1962).

— — — Studies on the larval development of the crab, *Rhithropanopeus harrisii* (Gould). I. The effects of salinity and temperature on larval development. Physiol. Zoöl. **39**, 91—100 (1966).

Cox, D. R.: Planning of experiments, 308 pp. New York: John Wiley & Sons 1958.

Davis, H. C. and A. Calabrese: Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. Fishery Bull. Fish Wildl. Serv. U.S. **58**, 643—655 (1964).

Forrester, C. R. and D. F. Alderdice: Effects of salinity and temperature on embryonic development of the Pacific cod (*Gadus macrocephalus*). J. Fish. Res. Bd Can. **23**, 319—340 (1966).

Kinne, O.: The effects of temperature and salinity on marine and brackish water animals. 1. Temperature. Oceanogr. mar. Biol. A. Rev. **1**, 301—340 (1963).

— The effects of temperature and salinity on marine and brackish water animals. II. Salinity and temperature-salinity combinations. Oceanogr. mar. Biol. A. Rev. **2**, 281—339 (1964).

— (Ed.) Marine ecology. Vol. 1. Environmental factors, Part 1. 681 pp. New York: Wiley-Interscience 1970.

Lough, R. G. and J. J. Gonor: Early embryonic stages of *Adula californiensis* (Pelecypoda:Mytilidae) and the effect of temperature and salinity on developmental rate. Mar. Biol. **8**, 118—125 (1971).

Matthiessen, G. C. and R. C. Toner: Possible methods of improving the shellfish industry of Martha's Vineyard, Duke's County, Massachusetts, 138 pp. Edgartown, Mass.: Marine Research Fdn Inc. 1966.

Ostle, B.: Statistics in research; basic concepts and techniques for research workers, 2nd ed. 585 pp. Ames: Iowa State University Press 1963.

Ralston, A. and H. S. Wilf (Eds.): Mathematical methods for digital computers, 293 pp. New York: John Wiley & Sons 1960.

Stickney, A. P.: Salinity, temperature and food requirements of soft-shell clam larvae in laboratory culture. Ecology **45**, 283—291 (1964).

Young, R. T.: The distribution of the mussel (*Mytilus californianus*) in relation to the salinity of its environment. Ecology **22**, 379—386 (1941).

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