A REEVALUATION OF THE COMBINED EFFECTS OF TEMPERATURE AND SALINITY ON SURVIVAL AND GROWTH OF BIVALVE LARVAE USING RESPONSE SURFACE TECHNIQUES

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ABSTRACT

The combined effects of temperature and salinity on larval survival and growth of *Crassostrea virginica*, *Mercenaria mercenaria*, and *Mulinia lateralis* as reported in the literature were critically examined using response surface techniques. The late veliger larvae generally have a greater tolerance to both temperature and salinity than the developing embryos. Each species shows its own characteristic change in temperature-salinity tolerance as it develops and approaches the range normally tolerated by the adults as it matures. Maximum growth of the veliger larvae required higher temperatures and somewhat higher salinities than maximum survival. Differences in temperature-salinity ranges estimated for maximum survival and growth were significantly different for all three species. In each case growth showed a significant temperature-salinity interaction. Response surface plots are given for early larval survival and late veliger survival and growth. Inferences of tolerance studies are made to the fields of pollution and aquaculture.

Recent studies of the combined effects of temperature and salinity on early development of bivalve larvae have been done by Davis and Calabrese (1964) for *Crassostrea virginica* and *Mercenaria mercenaria*, Brenko and Calabrese (1969) for *Mytilus edulis*, Calabrese (1969) for *Mulinia lateralis*, Lough and Gonor (1971, 1973a, b) for *Adula californiensis*, and Goodwin (1973) for *Panope generosa*. However, only Lough and Gonor (1973a, b) have critically examined the effects of temperature and salinity on bivalve larval life by multiple regression analyses and the fitting of response surfaces to survival, growth, and respiration of early and late stage larvae. The use and evaluation of this response surface technique in marine ecology has been reviewed in detail by Alderdice (1972). This technique not only facilitates the prediction of an organism's response to a wide range of untested conditions but also visually represents any change in its response at various stages of development. The experimental data from the above mentioned species have been critically analyzed by response surface techniques to reevaluate the combined effects of temperature and salinity on larval survival and growth. The results for *Crassostrea virginica, Mercenaria mercenaria*, and *Mulinia lateralis* are given in this paper.

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METHODS

The mathematical model used in the analyses was of the form:

$$Y = b_0 + b_1 (T) + b_2 (S) + b_3 (T^2) + b_4 (S^2) + b_5 (T \times S)$$

where Y = percentage survival or growth
b_0 = a constant
T = linear effect of temperature
S = linear effect of salinity
T^2 = quadratic effect of temperature
S^2 = quadratic effect of salinity
$T \times S$ = interaction effect between temperature and salinity

The coefficients in the model (b's) were estimated by a stepwise multiple regression computer program contained in the Oregon State University Statistical Program Library. 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 process, their order of insertion 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 the 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 divided by the total sum of squares corrected for the mean. It is often stated as a percentage, $100R^2$. The larger R^2 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. A *t*-test also is made indicating the equality of the individual regression coefficients to zero and their level of significance.

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 instructed to print 20% contour intervals, wide enough to exclude the approximate ±10% experimental error reported by the authors. Temperature and salinity scales on all plots were set to range from 0 to 40 in order to facilitate response comparison and to allow the overall form of the surface to be visualized. Contours extrapolated beyond the experimental data are given as dotted lines.

Analysis of covariance methods, as used in Lough and Gonor (1973a, b), were used to test the significance of the difference between the estimated polynomials for early and late larval survival and between late survival and growth.

RESULTS

Crassostrea virginica

Davis and Calabrese (1964) first reared the larvae for 2 days at six levels of temperature and nine levels of salinity to study the effect of these factors on early development, or the period from fertilization to approximately the veliger stage. To learn what effect these same combinations of temperature and salinity had on late larval development, larvae were initially reared from eggs for 2 days at normal seawater conditions (24.0°C, 27.5‰) and then transferred at the veliger stage to the experimental conditions.

Tables of the multiple regression analyses are given in the Appendix and will not be referred to in this section. Survival to 2 days of development was affected most by the linear and quadratic effects of salinity and the linear effect of temperature. Maximum survival of the 2-day-old larvae (80% survival contour) was estimated to occur at temperature and salinity conditions between 19° and 30.5°C and 19 and 30‰ (Figure 1), which is in good agreement with the experimental results.

The analysis of survival of 10-day-old larvae, after 8 days of rearing at experimental conditions, indicated that the linear and quadratic effects of temperature and the quadratic effect of salinity significantly affected survival. Maximum survival after 8 days (60% survival contour) was estimated to occur above 21°C and between 8 and 30.5‰ (Figure 2). The 10-day-old larvae showed a tolerance to much higher temperature and a wider salinity range than the 2-day-old larvae. Analysis of covariance showed a significant difference (1% level) between the 2- and 8-day survival polynomials further substantiating that the range of temperatures and salinities tolerated by the late veliger larvae were significantly different than that of the early embryos.

Growth of the larvae during 8 days was affected most by the interacting effect of temperature and salinity and the quadratic effect of salinity. Maximum growth (100% response contour) was estimated to occur at temperatures and salinities above 19°C and 33‰ (Figure 3).

There was a significant difference (1% level) between the polynomials estimated for 8-day

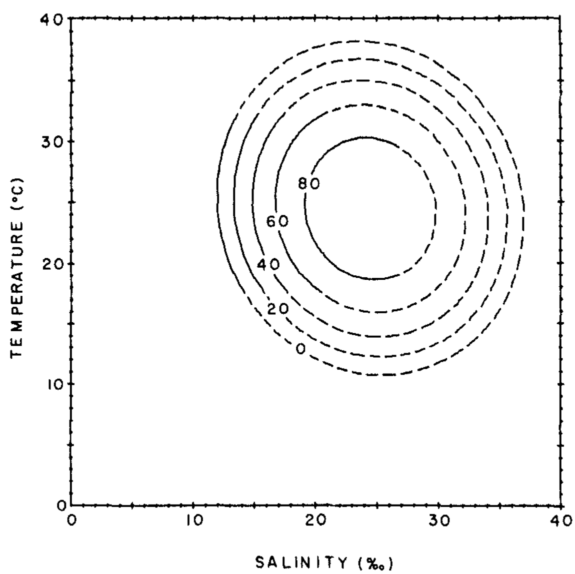

FIGURE 1.—Response surface estimation of percent survival of *Crassostrea virginica* larvae after 2 days of development at experimental temperature and salinity combinations given in Davis and Calabrese (1964).

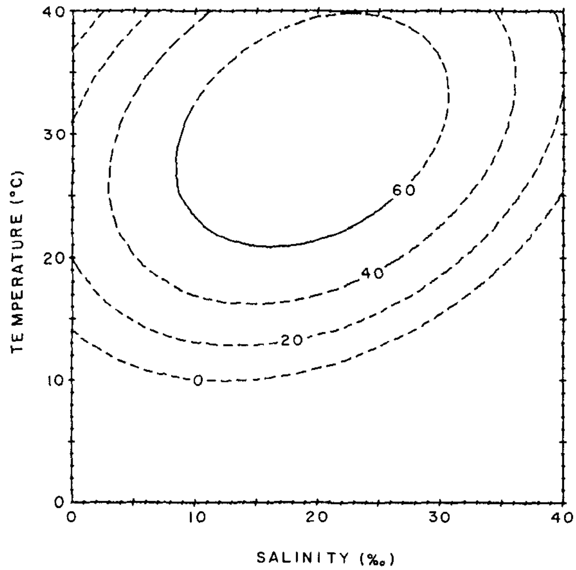

FIGURE 2.—Response surface estimation of percent survival of *Crassostrea virginica* veliger larvae after 8 days of development at experimental temperature and salinity combinations given in Davis and Calabrese (1964).

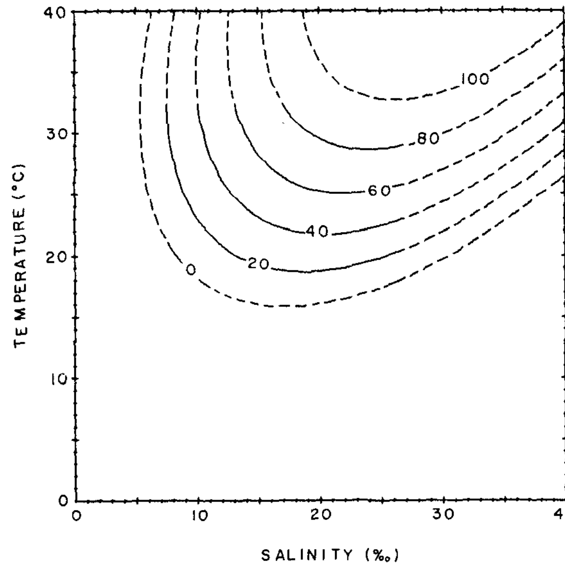

FIGURE 3.—Response surface estimation of percent growth of *Crassostrea virginica* veliger larvae after 8 days of development at experimental temperature and salinity combinations given in Davis and Calabrese (1964).

survival and growth indicating a significantly higher salinity range is required for optimum growth than is required for optimum survival.

An analysis of combined 8-day-survival and growth data indicated that the linear effect of temperature, the interacting effect of temperature and salinity, and the quadratic effect of salinity were the more important factors explaining the data. Optimum (80% contour) temperature and salinity conditions for maximizing both larval survival and growth was estimated at above 30°C and between 18 and 35‰.

Mercenaria mercenaria

The same experimental design with the exception of nine levels of temperature and six levels of salinity was used by Davis and Calabrese (1964) to study the larval tolerance of this species.

Survival to 2 days of development, or from fertilization to veliger stage larvae, was affected most by the quadratic effects of salinity and temperature, and the interacting effect of temperature and salinity. The response surface for 2-day-old larvae clearly shows the skewed contours resulting from the interaction effect (Figure 4). Maximum survival to 2 days of development (100% survival contour) was estimated to occur at temperatures and salinities above 7.2°C and 28‰. Their experimental data show maximum survival between 17.5° and 30°C at a salinity of 27‰.

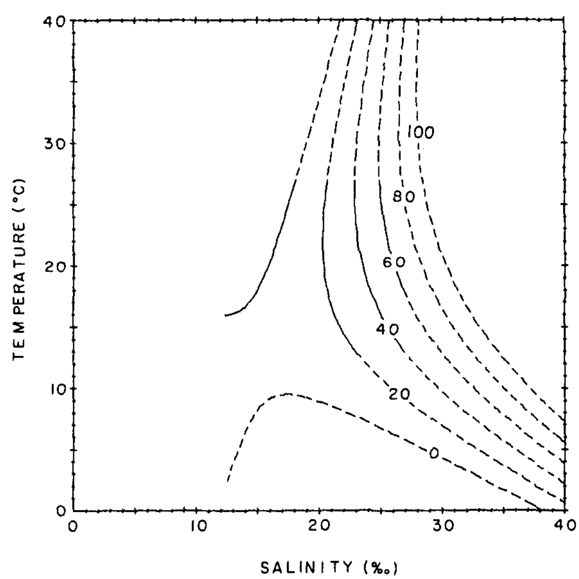

FIGURE 4.—Response surface estimation of percent survival of *Mercenaria mercenaria* larvae after 2 days of development at experimental temperature and salinity combinations given in Davis and Calabrese (1964).

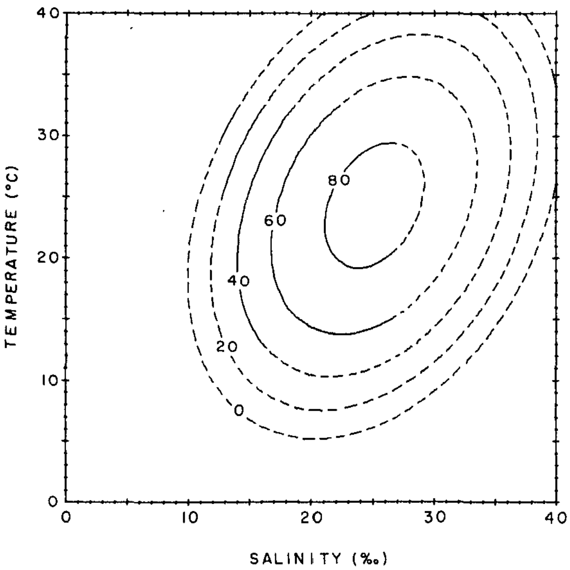

FIGURE 5.—Response surface estimation of percent survival of *Mercenaria mercenaria* veliger larvae after 10 days of development at experimental temperature and salinity combinations given in Davis and Calabrese (1964).

Late larval survival after 10 days of rearing at the experimental conditions indicated that the linear and quadratic effects of salinity and the interacting effect of temperature and salinity were the more important factors affecting survival. Maximum survival of these 12-day-old larvae (80% survival contour) was estimated to occur between temperatures and salinities of 19° and 29.5°C and 21 and 29‰ (Figure 5). Although the late larvae had a much narrower temperature tolerance than the developing embryos, the late larvae showed a significantly greater tolerance to low salinity. This difference in tolerance of these two life stages was further substantiated by the fact that there was a significant difference (1% level) between the 2- and 10-day survival polynomials.

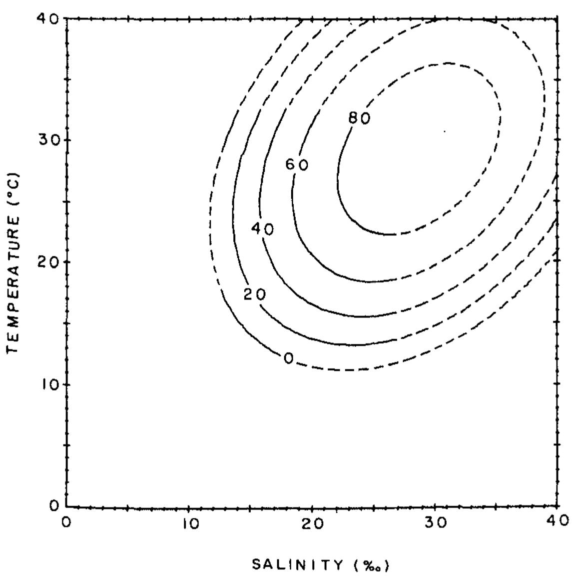

FIGURE 6.—Response surface estimation of percent growth of *Mercenaria mercenaria* veliger larvae after 10 days of development at experimental temperature and salinity combinations given in Davis and Calabrese (1964).

Growth of the larvae during the 10-day experimental period was most affected by the interacting effect of temperature and salinity and by the linear and quadratic effects of temperature, and the linear effect of salinity. Maximum growth (80% contour) was estimated to occur at temperatures and salinities between 22.5° and 36.5°C and 21.5 and 30‰ (Figure 6). There was a significant difference (1% level) between the polynomials estimated for 10-day survival and growth indicating that the higher temperatures and salinities required for optimum growth are significantly different than those conditions estimated for optimum survival. Larval survival and growth estimated by these techniques above the experimental temperature and salinity of 32.5°C and 27.0‰ are questionable. Higher temperature and salinity levels need to be added to the experimental design to more carefully define the response surface.

The combined 10-day survival and growth analysis indicated that they were affected by all of the variables of temperature and salinity, but by salinity more than by temperature. Optimum temperature and salinity conditions (80% contour) for maximizing both larval survival and growth to 12 days was estimated at 21.5° to 33°C and 22 to 31‰.

Mulinia lateralis

Six levels each of temperature and salinity were used to investigate the tolerances of early and late development of this species by Calabrese (1969) in the same manner as used for the other species.

Survival of the early embryos for 2 days under the experimental conditions was affected by all the variables except the interacting effect of temperature and salinity. Maximum survival of the 2-day-old larvae (80% contour) was estimated to occur at temperatures between 18.5° and 24.5°C and salinities between 22 and 28.5‰ (Figure 7).

The analysis of survival after 6 to 8 days of rearing beyond the veliger stage indicated that the linear and quadratic effects of temperature and the interacting effect of temperature and salinity were the more important variables affecting survival. Response surface estimation predicted 80% survival at temperatures between 8.5° and 26.5°C and salinities above 12‰ (Figure 8). A significant difference (1% level) was calculated by the analysis of covariance for the 2- and 6- to 8-day survival polynomials. The veliger larvae showed a much greater tolerance to low temperatures and a wider range of salinities than the early embryos.

Growth of the veliger larvae was most affected by the interacting effect of temperature and salinity, the quadratic effect of salinity, and the linear effect of temperature. Maximum growth (60% contour) was estimated to occur at temperatures between 18° and 38°C and salinities above 16.5‰ (Figure 9). The axis of the growth contours are observed to lie diagonal to the factor axes showing the effect of the temperature-

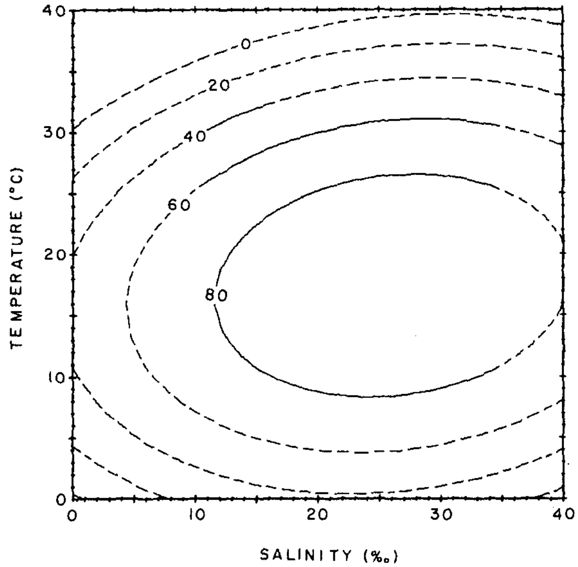

FIGURE 8.—Response surface estimation of percent survival of *Mulinia lateralis* veliger larvae after 6 to 8 days of development at experimental temperature and salinity combinations given in Calabrese (1969).

salinity interaction. There was a significant (1% level) difference between the polynomials estimated for the 6- to 8-day survival and growth indicating that the higher temperatures required

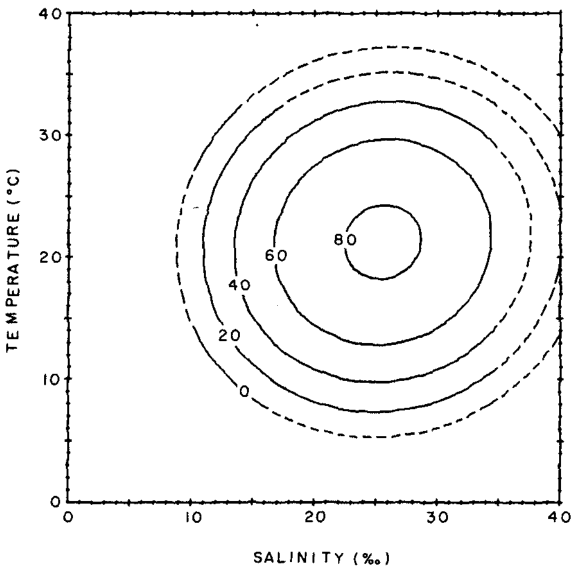

FIGURE 7.—Response surface estimation of percent survival of *Mulinia lateralis* larvae after 2 days of development at experimental temperature and salinity combinations given in Calabrese (1969).

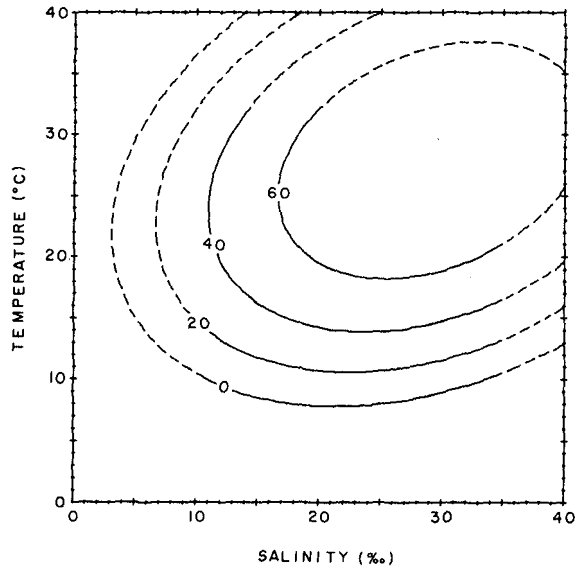

FIGURE 9.—Response surface estimation of percent growth of *Mulinia lateralis* veliger larvae after 6 to 8 days of development at experimental temperature and salinity combinations given in Calabrese (1969).

for optimum growth were significantly different than those required for optimum survival.

Analysis of the combined 6- to 8-day survival and growth indicated that the interacting effect of temperature and salinity and the linear effect of temperature were the more important variables explaining the data, although only 30.4% of the variance was explained by the combined polynomial. Optimum temperature and salinity conditions (80% contour) for maximizing both larval survival and growth to 8 to 10 days of age were predicted between 20° and 26°C and 23 and 32‰.

DISCUSSION

Despite the fact that the adults of the three species studied are euryhaline to varying degrees, their early embryos and larvae have a comparatively narrow salinity range. Early larvae of *Mercenaria mercenaria* appear to be much more tolerant to high temperatures than the other two species, but require essentially oceanic salinities.

The older larvae, having been reared from fertilization to the veliger stage at optimum conditions, now appear to have a generally greater tolerance to both temperature and salinity. The late larvae of *C. virginica* appear to tolerate a higher temperature range than the early larvae while *Mulinia lateralis* late larvae seem to tolerate best temperatures at the lower end of its range. Late larvae of *Mercenaria mercenaria* are able to tolerate low salinities somewhat better than the early larvae, but their temperature range is quite restricted. The observed progressive change in their temperature-salinity tolerance with time approaches the range normally tolerated by the adults. This same progressive change was observed for the larvae of *Adula californiensis* by Lough and Gonor (1973a, b).

The range of temperature-salinity conditions estimated for maximum growth was significantly different from that estimated for maximum survival of the same late stage larvae. Maximum predicted growth occurred at higher temperatures and at somewhat higher salinities than those for maximum survival for all three species studied. All three species showed a significant temperature-salinity interaction effect for growth. Growth, classically, is positively correlated with temperature up to some limit; however, the role of salinity appears to complicate the temperature effect.

The combining of late larval survival and growth to maximize both responses seems intuitively pleasing as one would expect a compromise situation in nature. An organism probably can operate most effectively when it is in a set of environmental conditions which maximize all its biological responses. It has been shown by Lough and Gonor (1973a, b) that temperatures for maximum growth response may be an abnormal stress environment which ultimately results in high mortality. Similarly, low temperatures may be suitable for larval survival but not necessarily highly productive for recruitment and growth to the adult population. Although the optimum temperatures and salinities usually can be estimated from the raw data, the statistical techniques used in this study allow one to define and interpret an organism's response to a matrix of environmental factors and to determine whether the response(s) between stages of development or sampling intervals is significantly different.

INFERENCES

Tolerance studies of various stages or at various times in the life history of an organism are especially important to pollution studies. Different stages of crab larvae have been shown to have different temperature-salinity tolerances of ecological significance (Costlow et al. 1960, 1962, 1966). This study demonstrates that different periods in the life of bivalve larvae also differ in their tolerance to temperature and salinity. The determination of water quality standards based on only one stage in the life of an organism is not realistic. All stages of development are important, particularly when the synergistic effect of a pollutant is studied. Davis and Hidu (1969) found it was necessary to evaluate the effects of pesticides on all stages of clam and oyster larvae as their tolerances are markedly different.

The field of aquaculture also may benefit from these tolerance studies. Based on this study a long-term experimental program should be undertaken to maximize both survival and growth recognizing that different stages of an organism may have different optimum conditions. Possibly, larvae should be reared at one set of conditions from fertilization to veliger stage and then transferred to another set of conditions for the late stages. Juvenile clams may have yet another set of optimum conditions different than those of the late larval stage. The larvae and the adults are two distinct morphological and physiological organisms and occupy distinctly different ecological environments.

Recent work by Costlow and Bookhout (1971) on the cyclic effect of temperatures on the larval development of an estuarine mud crab, *Rhithropanopeus harrisii*, emphasizes the need for more research on the fluctuating environmental variables that normally occur in nature. The possible stimulating or inhibiting effect of fluctuating temperatures on bivalve larval survival and growth in relation to both pollution and aquaculture should be investigated in the future.

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APPENDIX

APPENDIX TABLE 1.—Multiple regression analyses of *Crassostrea virginica* larvae.

Step number	Variable	R^2	F-level	df	Level of significance	Coefficient	T-value	Level of significance
				2-day survival				
1	S	0.661	77.857	(1,40)	1%	32.8617	8.007	1%
2	S^2	.793	24.818	(2,39)	1%	−0.6234	6.706	1%
3	T^2	.795	.356	(3,38)	N.S.	−0.5195	5.715	1%
4	T	.889	31.397	(4,37)	1%	27.7755	5.821	1%
5	$T \times S$	.894	1.875	(5,36)	N.S.	−0.0971	1.369	N.S.
	Constant					−643.9149		
				8-day survival				
1	T	0.426	38.600	(1,52)	1%	10.221	2.992	1%
2	T^2	.493	6.781	(2,51)	1%	−0.2006	3.008	1%
3	$T \times S$	.501	.778	(3,50)	N.S.	0.0996	2.492	5%
4	S^2	.625	1.612	(4,49)	N.S.	−0.1455	3.687	1%
5	S	.643	2.430	(5,48)	N.S.	2.6621	1.559	N.S.
	Constant					−104.389		
				8-day growth				
1	$T \times S$	0.642	93.200	(1,52)	1%	0.2450	6.645	1%
2	S^2	.907	144.539	(2,51)	1%	−0.2329	6.401	1%
3	S	.918	7.139	(3,50)	1%	4.1512	2.636	5%
4	T	.919	.317	(4,49)	N.S.	7.5246	2.389	5%
5	T^2	.927	5.365	(5,48)	1%	−0.1425	2.316	5%
	Constant					−152.2672		
				8-day survival and growth				
1	T	0.431	80.437	(1,106)	1%	8.8727	2.182	5%
2	$T \times S$	.528	21.559	(2,105)	1%	0.1723	3.620	1%
3	S^2	.602	19.110	(3,104)	1%	−0.1892	4.028	1%
4	T^2	.619	4.587	(4,103)	1%	−0.1715	2.160	5%
5	S	.629	2.807	(5,102)	5%	3.4066	1.676	N.S.
	Constant					−128.3283		

APPENDIX TABLE 2.—Multiple regression analyses of *Mercenaria mercenaria* larvae.

Step number	Variable	R^2	F-level	df	Level of significance	Coefficient	t-value	Level of significance
				2-day survival				
1	S^2	0.561	43.513	(1,34)	1%	0.2439	0.611	N.S.
2	$T \times S$	.581	1.555	(2,33)	N.S.	0.3947	2.345	5%
3	T^2	.640	5.223	(3,32)	1%	−0.1219	2.039	N.S.
4	T	.648	.678	(4,31)	N.S.	−2.7229	0.819	N.S.
5	S	.653	.449	(5,30)	N.S.	−12.2188	0.670	N.S.
	Constant					110.3864		
				10-day survival				
1	S	0.488	49.560	(1,52)	1%	15.6884	4.021	1%
2	S^2	.594	13.307	(2,51)	1%	−0.4142	4.570	1%
3	T^2	.609	1.894	(3,50)	N.S.	−0.2630	4.916	1%
4	$T \times S$	.732	22.546	(4,49)	1%	0.2111	3.295	1%
5	T	.769	7.591	(5,48)	1%	7.4766	2.755	1%
	Constant					−201.8315		
				10-day growth				
1	$T \times S$	0.631	88.900	(1,52)	1%	0.2438	4.532	1%
2	T^2	.739	21.109	(2,51)	1%	−0.3305	7.262	1%
3	T	.829	26.270	(3,50)	1%	12.3631	5.363	1%
4	S^2	.841	3.706	(4,49)	5%	−0.3702	4.835	1%
5	S	.885	18.518	(5,48)	1%	14.0885	4.303	1%
	Constant					−288.6339		
				10-day survival and growth				
1	S	0.463	91.215	(1,106)	1%	14.5902	4.532	1%
2	S^2	.535	16.411	(2,105)	1%	−0.3876	5.164	1%
3	$T \times S$	.590	13.881	(3,104)	1%	0.2316	4.378	1%
4	T^2	.685	31.236	(4,103)	1%	−0.2987	6.719	1%
5	T	.736	19.522	(5,102)	1%	9.9556	4.418	1%
	Constant					−243.0117		

APPENDIX TABLE 3.—Multiple regression analyses of *Mulinia lateralis* larvae.

Step number	Variable	R^2	F-level	df	Level of significance	Coefficient	t-value	Level of significance
				2-day survival				
1	S	0.156	6.269	(1,34)	5%	14.4237	4.949	1%
2	S^2	.390	12.705	(2,33)	1%	−0.2942	4.916	1%
3	$T \times S$	.421	1.708	(3,32)	N.S.	0.0284	0.554	N.S.
4	T^2	.478	3.359	(4,31)	5%	−0.3256	5.440	1%
5	T	.709	23.769	(5,30)	1%	13.1265	4.875	1%
	Constant					−240.8807		
				6- to 8-day survival				
1	T^2	0.353	18.540	(1,34)	1%	−0.1976	7.190	1%
2	T	.627	24.193	(2,33)	1%	6.0749	4.914	1%
3	$T \times S$	.716	10.002	(3,32)	1%	0.0307	1.305	N.S.
4	S^2	.724	.898	(4,31)	N.S.	−0.0781	2.843	1%
5	S	.760	7.013	(5,30)	1%	3.5437	2.648	5%
	Constant					−2.8961		
				6- to 8-day growth				
1	$T \times S$	0.498	33.698	(1,34)	1%	0.0993	2.646	5%
2	S^2	.605	8.979	(2,33)	1%	−0.1258	2.867	1%
3	T^2	.641	3.220	(3,32)	5%	−0.2120	4.833	1%
4	T	.765	16.222	(4,31)	1%	8.9352	4.528	1%
5	S	.796	4.584	(5,30)	1%	4.5735	2.141	5%
	Constant					−113.4013		
				6- to 8-day survival and growth				
1	$T \times S$	0.102	7.963	(1,70)	1%	0.0650	1.438	N.S.
2	T^2	.120	1.408	(2,69)	N.S.	−0.2048	3.876	1%
3	T	.262	13.046	(3,68)	1%	7.5051	2.377	1%
4	S^2	.278	1.491	(4,67)	N.S.	−0.1020	1.930	N.S.
5	S	.304	2.489	(5,66)	5%	4.0586	1.578	N.S.
	Constant					−58.1487		