TEMPORAL CHANGES IN THE SALINITY AND TEMPERATURE REQUIREMENTS OF TROPICAL MUSSEL LARVAE

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ABSTRACT

Two species of tropical mussels of the genus *Perna* are presently being cultured as food resources. The brown mussel of Venezuela (*Perna perna*) is raft cultured while the Philippine green mussel (*P. viridis* = *Mytilus smaragdinus*) is harvested from submerged bamboo poles. For this study, I imported, quarantined and spawned adults of both species in my laboratory and reared the larvae beyond metamorphosis (24 days) in replicate cultures at 40 different combinations of temperature and salinity. Response surface analyses of data collected at intervals show significant changes in the temperature and salinity optima for growth and survival during the planktonic stages preceding metamorphosis. However, analyses of the data taken only at the termination of the experiment predict quite different optimum conditions. These data amount to a biological summation of the varying temperature and salinity requirements of the larvae throughout the experimental culture. I have developed a generalized model for the growth responses of tropical mussel larvae which is presented as a motion picture study of the changing responses to temperature and salinity.

INTRODUCTION

Much of mariculture research relates to the production of quantities of seed or young of the cultured organism either from wild brood stock or preferably from controlled laboratory spawnings. Efficient production of seed in the laboratory requires detailed knowledge of the effects of many environmental factors including temperature, salinity, oxygen and pH. The literature of the past decade reflects the increasing interest in the simultaneous effects of multiple environmental factors on bivalve larvae (Cain, 1973; Calabrese, 1969; Davis and Calabrese, 1964; Hrs-Brenko, 1974; Hrs-Brenko and Calabrese, 1969; Lough and Gonor, 1973a,b). However, analyses applied to such results have not defined the changes in the optimum conditions required by each stage of the pelagic larvae. Where specific sets of optimum conditions are provided each larval stage, improved growth rates and reduced mortalities may be expected in species where such changes in environmental requirements are significant. The purpose of this laboratory study was to investigate the changing

environmental requirements associated with the various developmental stages of tropical mussel larvae.

For this work, 2 species of tropical mussels (*Perna perna* and *P. viridis*) were selected for their mariculture potential. With world production of cultured mussels exceeding 300,000 metric tons round weight annually, proven technology is available for the culture of such tropical mussels. Growth rates and maximum sizes of these species far exceed those of temperate species. Mussels expend less metabolic energy to form and maintain a heavy shell than do most other bivalves. Mussels are primary consumers and are in a position to feed on vast quantities of readily available phytoplankton. In addition to past efforts to increase production of the South American species (*P. perna*), recent papers have reported an expanding research effort in Southeast Asia directed at *P. viridis* (Rao et al., 1976; Shafee, 1976; Sivalingham, 1977).

MATERIALS AND METHODS

Sexually mature adults of *P. perna* (from Cumana, Venezuela) and *P. viridis* (from Bacoor Bay, Philippines) were imported to Miami between August 1974 and August 1977 and quarantined in the laboratory diagrammed in Figure 1. While adults were maintained for many months on phytoplankton, spawning and experimental procedures were begun immediately upon arrival (less than 40 hours after collection). Spawning was induced in both species by exposure for 10 min to seawater warmed to temperatures of 30-32°C. Release of gametes rarely took more than 30 min. All experimental procedures involved embryos and larvae produced by pooling the eggs and sperm of 12 males and 12 females. Seawater used in all phases of the study was filtered to 5 µm, to one µm in the salinity by temperature factorial experiments. Based on the results of LePennec and Prieur (1972) and LePennec et al. (1973), chloramphenicol (6 mg/liter) was used to reduce bacterial populations in all larval cultures. All embryos were cultured to the "D" stage (fertilization to 24 hours) at conditions estimated to be optimum by preliminary experiments: for *P. perna*, 26°C at 30 ppt and for *P. viridis*, 25°C at 26 ppt.

The effects of salinity and temperature were tested in a factorial design of 8 temperatures (10-14-18-22-24-26-30-34°C) and 5 salinities (14-22-28-34-42 ppt). Temperatures were held to within 0.6°C and salinities within 0.5 ppt of the desired experimental conditions. Variations of pH and oxygen saturation were monitored but were not significant either within or among treatments. After reaching the prodissoconch I stage, larvae were gradually acclimated to the 40 experimental conditions. Into one-liter beakers holding 800 ml of seawater of appropriate temperature and salinity were placed 20,000 larvae (25/ml). Replicate cultures were established at each experimental condition. Aeration was maintained at a low level sufficient to keep both larvae and phytoplankton in suspension. The phytoplankton diet provided was uniform in composition and quantity for all experimental conditions and consisted of 20-25,000 cells/ml (total) of equal parts of *Isochrysis galbana*, *Monochrysis lutheri*, *Tetraselmis suecica* and *Chaetoceros calcitrans*. To reduce the accumulation of metabolic waste products in the cultures, every 48 hours all larvae were gently removed from the old culturewater on a submerged "Nitex" screen (20 µm mesh) and transferred to fresh, aerated seawater of appropriate temperature and salinity containing chloramphenicol

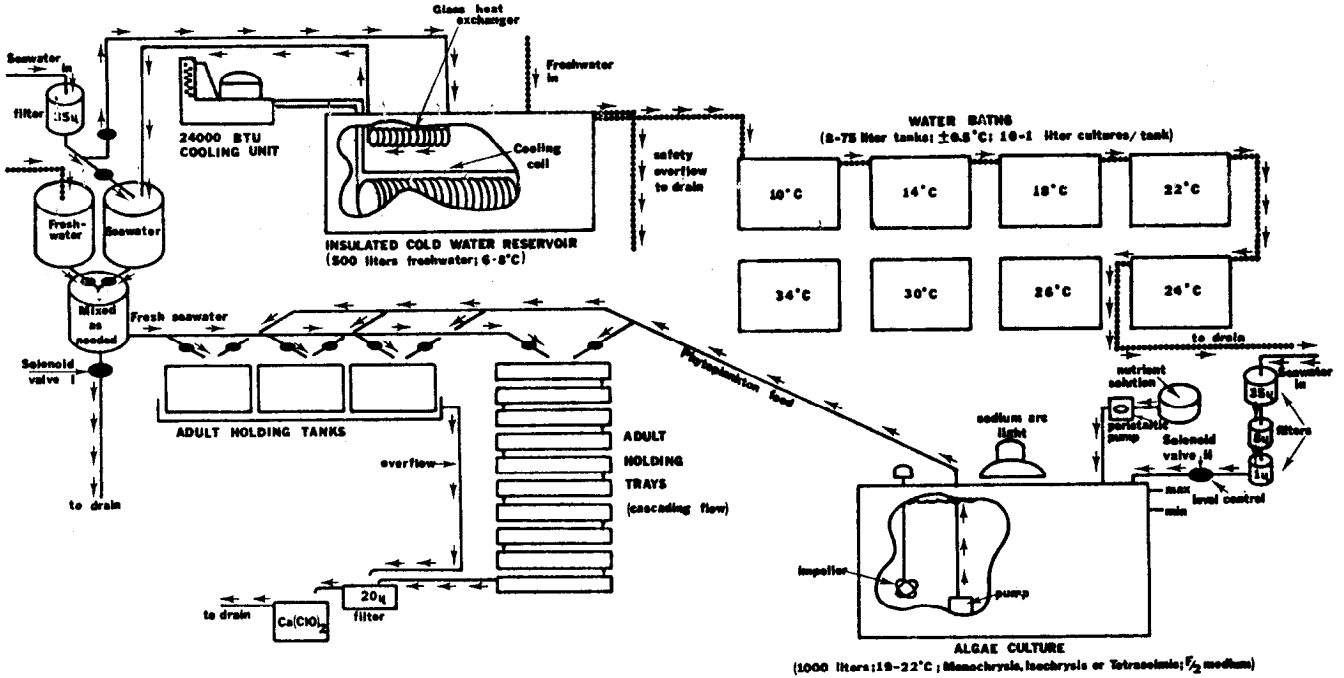


Figure 1. Laboratory schematic diagram.

col and a new dose of the phytoplankton diet. Ten ml quantitative samples of larvae were removed from the uniformly suspended culture prior to this transfer. All samples were preserved in a modified Carriker's solution (Carriker, 1950).

All cultures were maintained and samples collected for 24 days following fertilization. Each preserved sample was examined under a stereoscopic dissecting microscope and all larvae which were living when preserved were counted. Survival data were based on the number of larvae surviving at each sampling time, taking into account the effect of the artificial mortality caused by the sampling procedure. Measurements of shell length and height were taken from 10 larvae in each sample, accurate to 5 µm. Growth data were based on shell length at each sampling time. Experimental error (due to sampling, counting, measuring and inherent biological variation) was estimated by several methods. The total error rate at the 95% confidence level was ±5-12%.

Response surface analyses were applied to data gathered in the multivariate experiments. When data are collected on the effects of 2 or more ratio scale independent variables (e.g., temperature and salinity) on a ratio scale dependent variable (the response), an analysis of variance may be derived from an equation describing the effects. If all possible values for the independent variables are substituted into this equation and the resultant predicted response values plotted on a 3-dimensional graph (factor A by factor B by response), a response "surface" is produced (Alderdice, 1972). The computer program used in the present study, "BOX2," was originally written by Lindsey and Sandnes (1972) for an IBM 1130 but was converted to execute on the University of Miami's UNIVAC 1100/20. This version of BOX2 is on file with the National Oceanographic Data Center and is available for distribution. BOX2 calculates both a linear and nonlinear equation to estimate the factor effects in contrast to most response surface analysis procedures. In general, the nonlinear model fits the data much closer with higher coefficients of determination (R^2) than the symmetrical, linear model. Furthermore, BOX2 calculates the values of the independent variables where the maximum predicted response occurs, calculates and plots goodness of fit tests for each equation parameter and plots contours and 3-dimensional representations of the response surface.

Growth and survival data were calculated as relative proportions or percentages of the maximum value occurring in that data set (sampling time). This was done to provide a basis for comparison with the studies of Cain (1973), Calabrese (1969), Davis and Calabrese (1964), Hrs-Brenko (1974), Hrs-Brenko and Calabrese (1969), Lough (1973) and Lough and Gonor (1973a,b). The replicate with the highest response is defined as a 100% response and all other values are expressed as a percentage of that value. This procedure assumes that the maximum value observed is the maximum response to be obtained under those specific culture conditions. Before analysis, the arcsine transformation was applied to all proportion data. While proportion data range from 0.0 to 1.0, arcsine transformed data range from 0.0 to 90.0; statistical analyses are facilitated by this more normally distributed data (Zar, 1974).

Response surface analyses were carried out on 3 types of information collected from the larval samples.

(1) Analyses were first carried out on the transformed proportion data for shell length and numbers of surviving larvae collected at each sampling time.

(2) Because data were available for each salinity and temperature at several sampling times, growth rates and survival rates were calculated for each experimental condition. For any given factor combination, growth rate calculations were based on the slope of the linear regression line for shell length against sampling time. Growth rates are expressed in units of µm/day. Survival rates as percent survival per day were calculated similarly. Therefore, for each species, a response surface analysis of growth rates and of survival rates was carried out.

(3) Rate data were calculated as arcsine transformed proportions of the maximum rate for any replicate in that data set in a manner similar to that applied earlier to shell length and number of larvae surviving. Because both the relative growth rate and relative survival rate figures were unitless and ranged from 0.0 to 90.0, they were combined and treated as replicates in a single response surface analysis of combined growth and survival for each species.

RESULTS

For both species, temperature affected growth and survival to a greater extent than did salinity. Optimum conditions for growth and survival of both species at each sampling time were calculated and their changes plotted in Figure 2. Many of the changes in predicted optima between sampling times are statistically significant. No trends are apparent in the changes of salinity optima with time for any data set. The absolute value of the changes in temperature optima are plotted against time in Figure 3. As the larvae acquire the ability to metamorphose into juveniles (day 10-12), the magnitude of the change in temperature optima appears to decline. However, only the slopes of the plotted linear regressions for *P. perna* are statistically significant and only at the 85% confidence level. The slopes of temperature changes for the *P. viridis* growth or survival data are not significant. Figure 4 presents the response surface analysis contour and 3-dimensional plots for the growth data collected in the terminal samples (day 24) for both species. Response surfaces predicted from the rate data for each species are presented in Figures 5 and 6. The linear model (R^2 = 0.75) for *P. perna* survival rates is depicted in Figure 5 for comparison with the better fitting, less symmetrical nonlinear model (R^2 = 0.87). For both species, one factor has its greatest adverse effects on rates as the other deviates from the optimum value. For example, at near optimum salinities, the growth and survival rates reflect the larvae's greater tolerance to sub-optimal temperatures. As the limit of tolerance for salinity is approached, the range of tolerated temperatures is reduced. In no case is there a significant interaction between the effects of temperature and salinity for *P. perna* or *P. viridis*. The results of the combined analyses of growth rate and survival rate data of each species shown as contour and response surface plots, and the maximum likelihood ratio (MLR) or goodness of fit plots for the predicted values of the temperature and salinity optima are shown in Figures 7 and 8. The narrower the MLR peak, the better the estimate of the optimum value. The MLR peak for the salinity optimum for *P. viridis* is relatively wide. This indicates a poorer fit to the observed data than for the other parameter estimates.

Table 1 lists the equation form for the nonlinear model along with

the temperature and salinity optima and the predicted maximum response for each data set. Further details of the effects of temperature and salinity on various stages of the larvae of *P. perna* and *P. viridis* including comparisons with other mytilid larvae will be presented elsewhere (Siddall, in preparation).

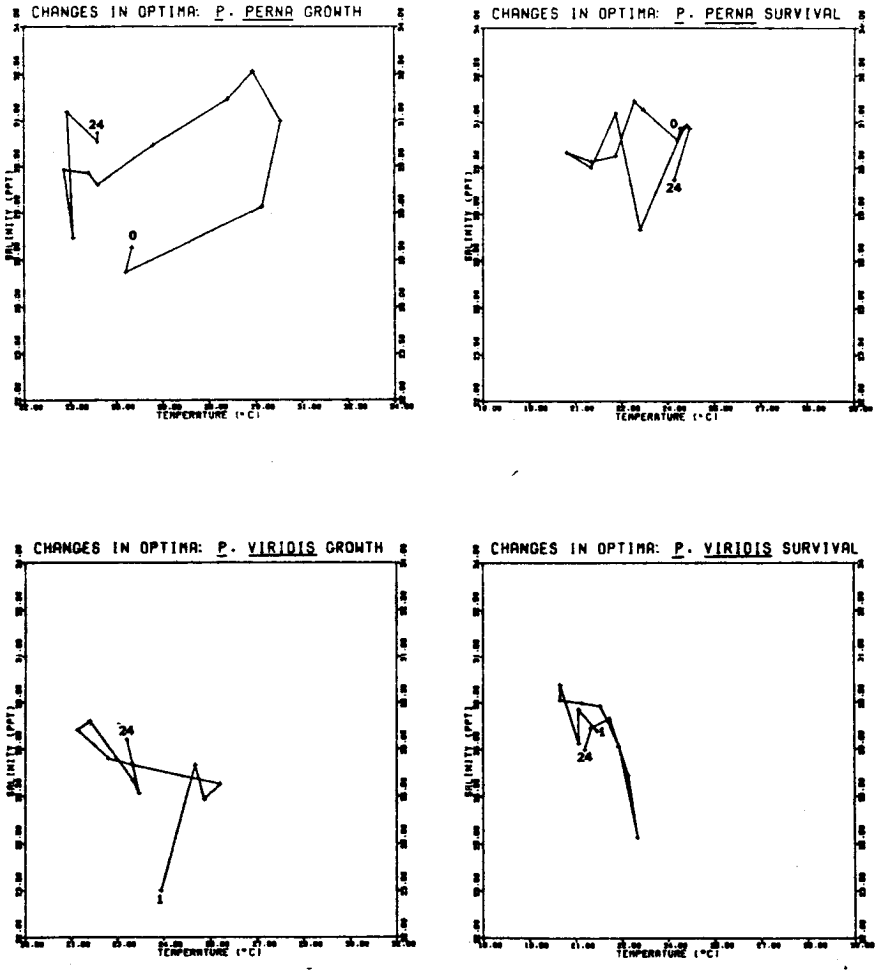


Figure 2. Changes in temperature and salinity optima with time, starting at day 0 for *P. perna*, with day 1 for *P. viridis*, and ending on day 24.

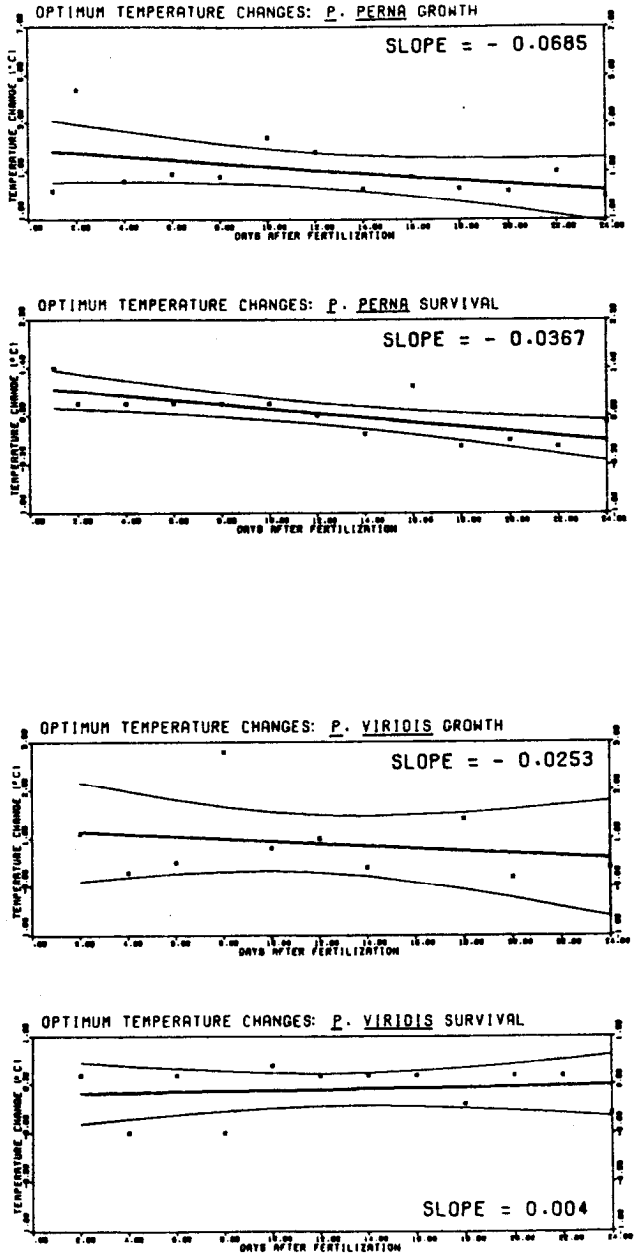
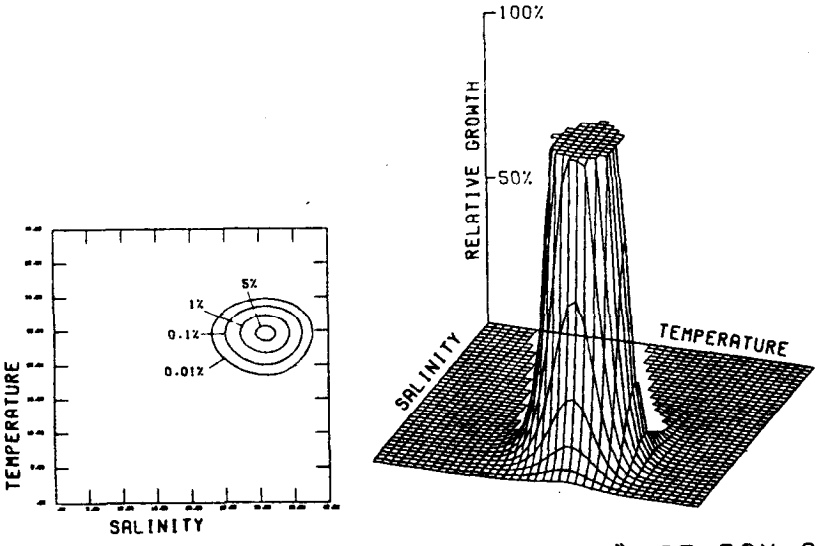
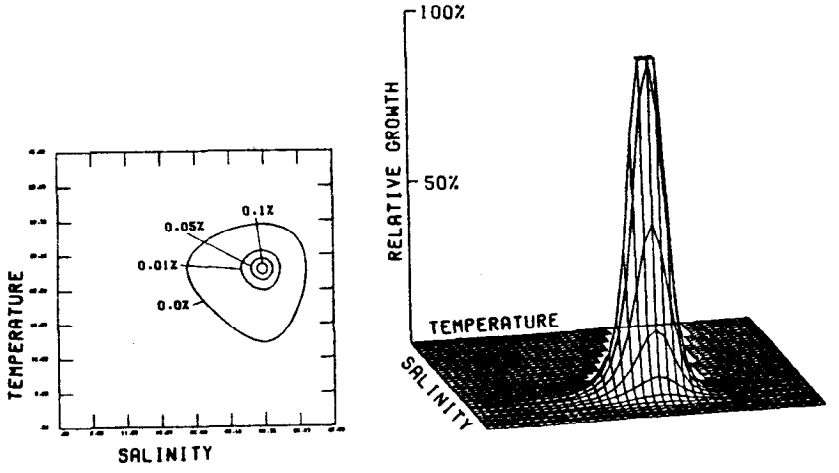


Figure 3. Value of the change in temperature versus sampling time in days. Linear regression line with 95% confidence limits plotted. Only the slopes for *P. perna* are significant at 85% confidence level.

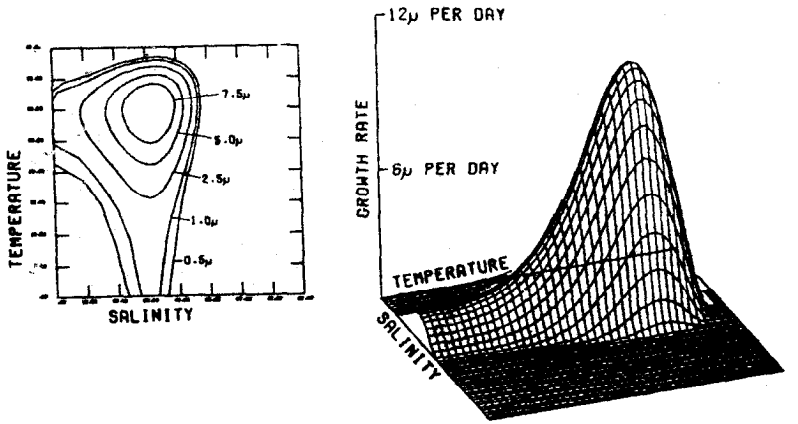


PERNA PERNA GROWTH DATA "SUMMED" AT DAY 24

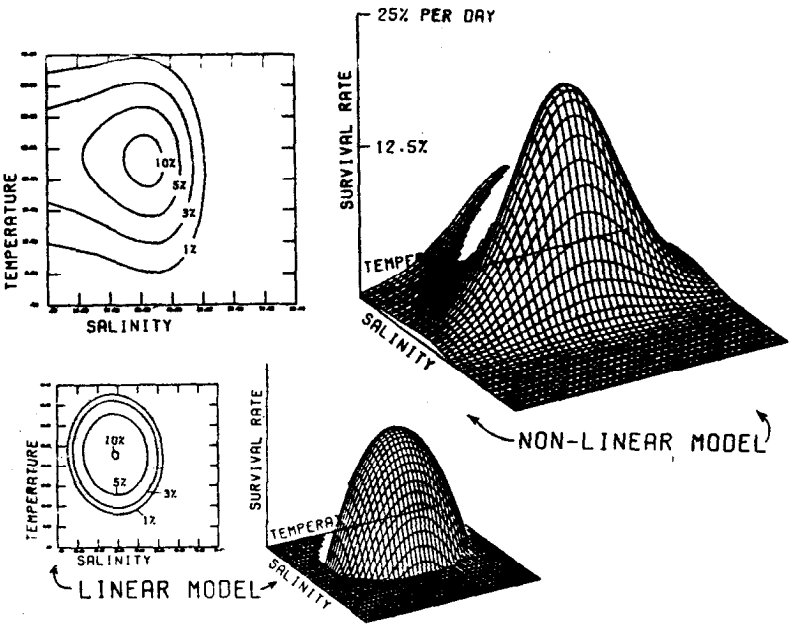


PERNA VIRIDIS GROWTH DATA "SUMMED" AT DAY 24

Figure 4. Response surface analyses from the data taken in the terminal samples (day 24). Demonstrates biological summation of treatment effects.

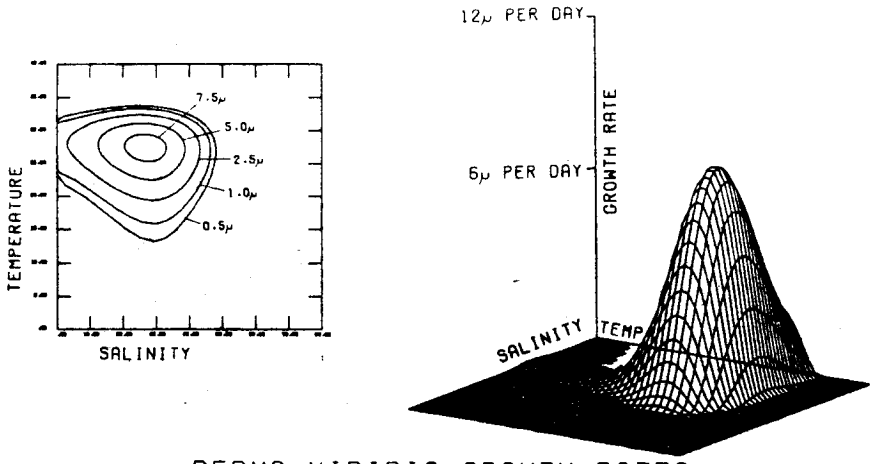


PERNA PERNA GROWTH RATES

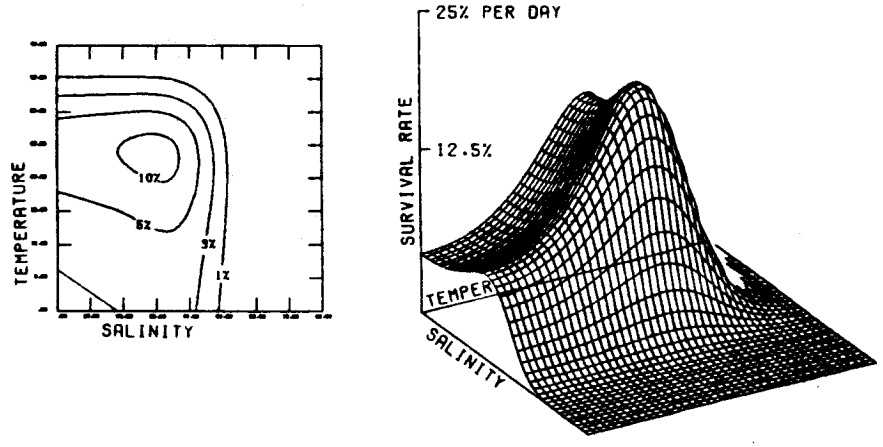


PERNA PERNA SURVIVAL RATES

Figure 5. Response surface analyses for survival and growth rates calculated from the series of samples collected over the 24-day duration of the factorial experiments. Compare the linear and nonlinear models for *P. perna* survival rates.



PERNA VIRIDIS GROWTH RATES



PERNA VIRIDIS SURVIVAL RATES

Figure 6. Response surface analyses for survival and growth rates of *P. viridis*.

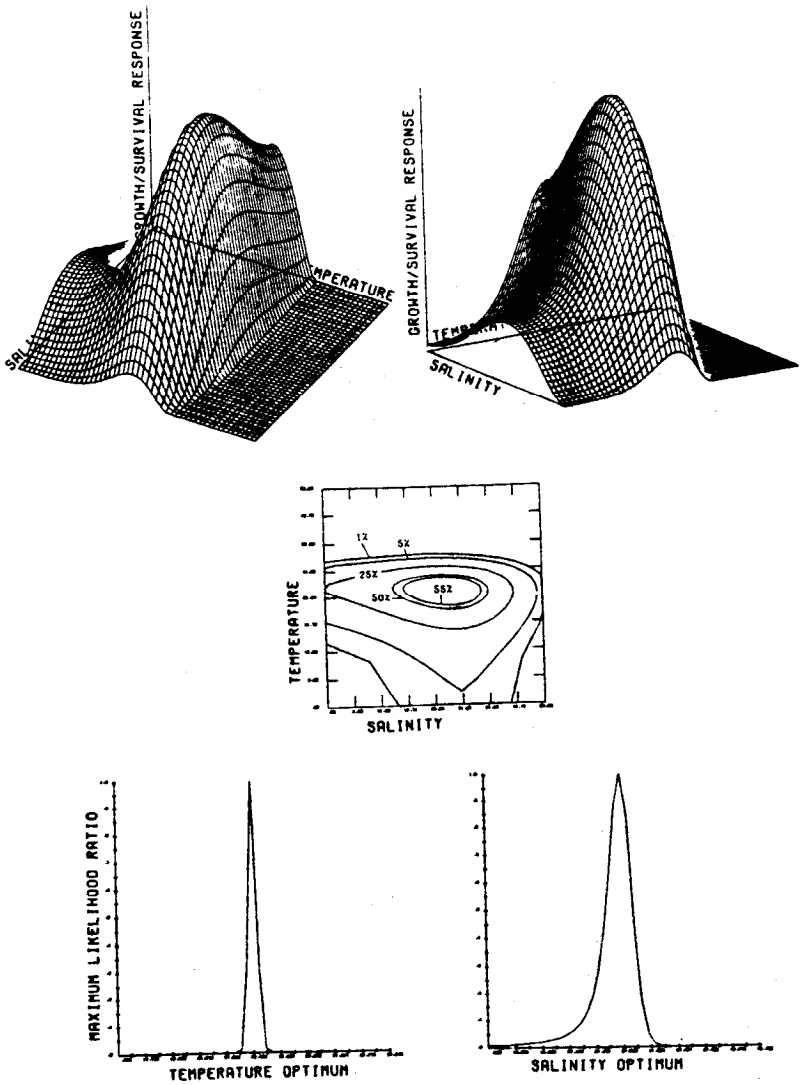


Figure 7. Combined response surface analyses for *P. viridis* survival and growth over 24 days. Maximum likelihood ratio (MLR) plot for the salinity optimum estimate indicates a poorer fit than for the temperature optimum.

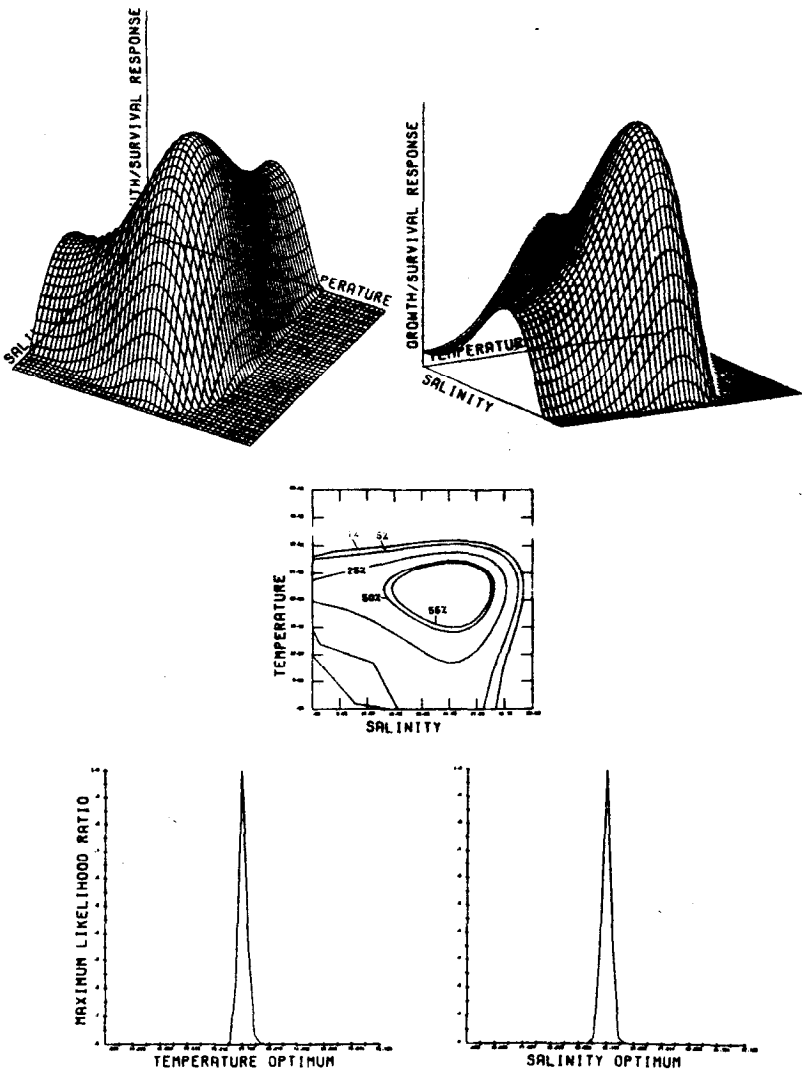


Figure 8. Combined response surface analyses for *P. perna* survival and growth over 24 days.

Table 1. Data from Response Surface Analyses of Each Data Set. Growth rate maxima are expressed as μm per day, survival rate maxima as percent surviving per day. Shell length data are from terminal samples (day 24) only.

NON-LINEAR EQUATION FORM:

$$Y^C = B(0) + B(1)*S^{A1} + B(2)*T^{A2} + B(3)*S^{2A1} + B(4)*T^{2A2} + B(5)*S^{A1}*T^{A2}$$

DATA SET	TEMPERATURE (DEGREES C)	SALINITY (P.P.T.)	PREDICTED MAXIMUM
P. PERNA SHELL LENGTH	24.6	30.9	100.0%
P. VIRIDIS SHELL LENGTH	25.8	33.4	97.8%
P. PERNA GROWTH RATES	30.1	31.8	10.6
P. VIRIDIS GROWTH RATES	27.5	27.1	8.5
P. PERNA SURVIVAL RATES	23.0	31.8	10.8
P. VIRIDIS SURVIVAL RATES	23.6	30.2	10.9
P. PERNA GROWTH & SURVIVAL	27.7	32.1	82.8%
P. VIRIDIS GROWTH & SURVIVAL	26.4	27.9	66.5%

DISCUSSION

Successful laboratory culture of *P. perna* larvae has been reported in the literature only by Velez and Martinez (1967); Tan (1975) was not able to rear *P. viridis* larvae through metamorphosis and the lack of a satisfactory diet appeared to be the major obstacle. Even at predicted temperature and salinity optima, survival rates in the present study do not reach the levels of the highest reported survival rates for *Mytilus edulis* (Hrs-Brenko and Calabrese, 1969; Lough, 1973). Presumably the quality and quantity of the diverse diet (of proven utility; see Walne, 1970) fed the larvae in this study were not major contributors to the reduced survival. Cell counts of live phytoplankton taken immediately before seawater exchanges showed that sufficient material remained to meet the dietary requirements of the larvae at all experimental

conditions. It is reasonable to include the culture procedures, and especially the seawater exchanges every 48 hours, in the unmeasured set of stress factors which may have acted to reduce overall survival rates in these experiments. However, stress derived from culture procedures would act uniformly at all experimental conditions. Errors in the analysis of the factorial experiments would result only if there were interactions between the controlled variables, temperature and salinity, and the uncontrolled mortality factors. Until more extensive factorial experiments are conducted on these undefined mortality factors, survival rates for *P. perna* and *P. viridis* larvae may remain relatively low.

In any case, growth rates of these tropical larvae far exceed those of cold water mussels.

Typically, form, function and physiology are distinctly defined in the ontogeny of the test species. It is reasonable to expect the environmental requirements to reflect the changes associated with critical developmental stages. When analyses of factorial experiments are based on data collected from one set of terminal samples, the resultant conclusions summarize the effects of the experimental treatments over the entire duration of the experiment. When this testing period includes several distinctly different stages in the development of the organism, there may be considerable "biological summation" of many varied responses to the treatments. More frequent collection of data involves more elaborate laboratory effort but may result in a better definition of changing environmental requirements associated with such critical periods as first feeding or metamorphosis. Consider the hypothetical case diagrammed in Figure 9. At developmental stage I, the test species shows a relatively high tolerance of environmental stress. Reaching stage II, the environmental tolerance is reduced. A terminal sample collected at stage III when tolerance is again high will primarily reflect the effects of stress rendered during stage II. Separate analyses of samples collected during each stage would define the temporal changes in environmental responses. Biological summation would be reduced.

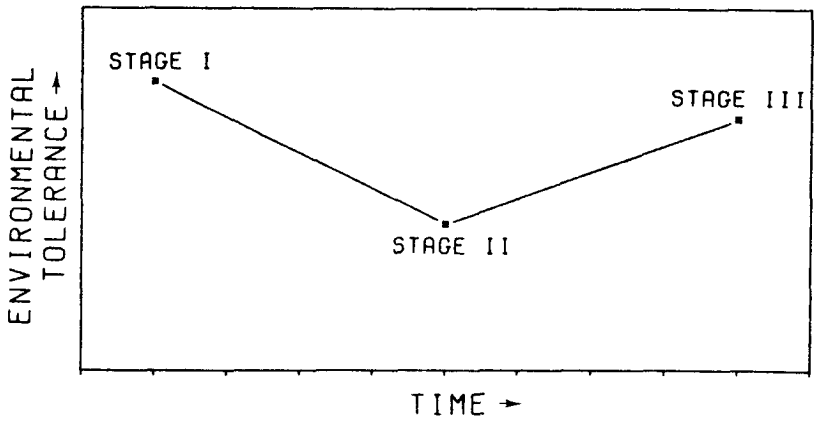


Figure 9. Hypothetical example of changes in environmental tolerance associated with various developmental stages in larval life. Such cases are subject to biological summation when analyses are restricted to single samples taken at only one developmental stage.

When data are collected at several times over the duration of a factorial experiment, environmental responses may be reported as rates. The discrete nature of temporal changes are somewhat smoothed over in the presentation of such response surface analysis. However, there is less loss of information at factor combinations which would be termed lethal by analysis of data collected in the terminal samples. Compare Figures 5 and 6 for growth rate analyses with Figure 4 for terminal sample analysis of growth.

Highest survival rates occurred at significantly cooler temperatures than did highest growth rates for both *P. perna* and *P. viridis*. Though larvae of both species survived significantly longer at lower temperatures, they did not feed effectively below 18°C. Experimental conditions were such that primary settlement of the larvae and subsequent metamorphosis were prevented. For both species, the maximum size attained during this delayed metamorphosis occurred at temperatures below those resulting in highest growth rates. I suggest that at the higher temperatures, and therefore the higher growth rates, the feeding stages are passed more rapidly and the velum is resorbed faster. Both factors reduced the time spent feeding and resulted in smaller maximum sizes after the delayed metamorphosis.

The response to factor treatments of critical interest to the mariculturist is that which maximizes both growth and survival. The combination of growth and survival data as replicates in one single response surface analysis presumes that the outcome, the predicted optima, are based equally on growth and survival. Unless there is a reason to attribute more weight to one response than the other, growth and survival should be treated equally in determining the optimum conditions. The predicted optima for growth and survival of *P. perna* and *P. viridis* larvae are intermediate to the values predicted by separate analysis (with the exception of the salinity optima for *P. perna*). Note that this calculation does not amount to an averaging of separately predicted optima for growth and survival.

From the best fitting nonlinear response surface equations for each species at each sampling time, a descriptive model for the growth of these tropical mussel larvae has been developed. Figure 10 depicts 8 representative response surfaces predicted by the model. The model is based on a second order polynomial fit through time for each salinity and temperature combination. This generalized, 3-dimensional model can be visualized as a motion picture of changing response surfaces from the prodissoconch I stage through metamorphosis and may be used to subjectively compare the temporal changes in environmental requirements of tropical mussel larvae of the genus *Perna*.

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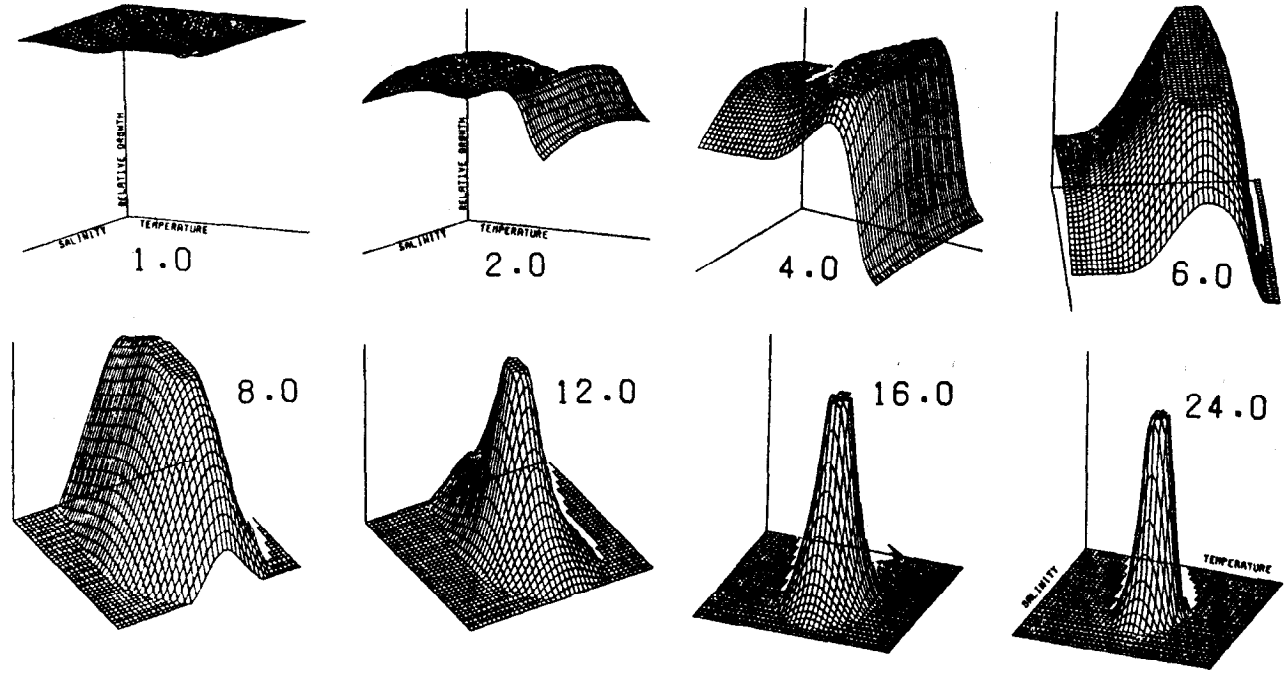

Figure 10. Representative response surfaces from descriptive model of growth of tropical mussel larvae through metamorphosis.

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