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A response surface investigation of the larval tolerances of three spionid polychaetes to temperature, salinity and food concentration

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A RESPONSE SURFACE INVESTIGATION OF THE LARVAL TOLERANCES OF THREE SPIONID POLYCHAETES TO TEMPERATURE, SALINITY AND FOOD CONCENTRATION

BY

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ABSTRACT

Response surface techniques were used to investigate the tolerances of the planktonic larvae of three spionid polychaetes to a variety of temperature and salinity combinations. Two of the spionids were morphologically very similar members of the genus *Boccardia*, *B. proboscidea* and *B. columbiana* which occupy nearly identical geographic ranges. The other was *Polydora giardi* a common coinhabitant with *B. columbiana*. In addition a third independent parameter, food concentration, was added to the study of *B. columbiana* and *P. giardi*.

The larvae of the two *Boccardia* species were extremely euryhaline, in marked contrast to those of *Polydora giardi* which were confined to an extremely narrow salinity range. They were distinctly separated by their temperature tolerances, however; *B. proboscidea* exhibiting maximum growth and survival at the upper temperature extremes of the design, while *B. columbiana* preferred a moderate to low temperature regime. The larvae of *P. giardi* are extremely eurythermal and are only slightly affected by temperature variations.

It is suggested that the reproductive schedule of the three spionids can be explained, at least in part, in terms of the information generated by the response surfaces. The long duration of the reproductive season of *P. giardi* is a reflection of the larvae's eurythermal nature. The inability of the larvae of *P. giardi* to cope with osmotic stress and the lack of an abundance of food items in the plankton during the winter months are probably of greater consequence in dictating the

cessation of the reproductive season, than the minimum water temperatures that occur at this time. The *Boccardia* larvae are in contrast less subject to the above considerations but are very dependent on the ambient water temperature to promote larval survival and growth.

INTRODUCTION

Boccardia proboscidea Hartman (1940) and *Boccardia columbiana* Berkeley (1927) are two closely related spionid polychaetes occupying nearly identical ranges along the California coast and northwards into British Columbia (Woodwick, 1963). The only disparity in their ranges occurs in southern California where *B. proboscidea* is present as far south as San Diego, California, while *B. columbiana* occurs only as far south as Santa Barbara (Hartman, 1940; Woodwick, 1963).

These spionids are morphologically seperable by only a single characteristic, namely the presence of a conspicuous fascicle of long notosetae on the first setigerous segment of *Boccardia columbiana; Boccardia proboscidea* bears short notosetae on setiger 1. Adult size presents another possible distinguishing characteristic, with *B. columbiana* attaining a maximum size of 15 mm and *B. proboscidea* reaching up to 35 mm in length (Woodwick, 1963).

Boccardia proboscidea inhabits shale and limestone reefs (Hartman, 1940, 1941), soft sandy mud, coralline algae (*Lithophyllum* spp.), gastropod shells inhabited by hermit crabs, and piling material (Woodwick, 1963). *Boccardia columbiana* also occupies most of these habitats and is the dominant organism in open surf regions when the two species are

found together. In bays and estuaries, however, *B. columbiana* is rare and normally does not occur in a sandy or muddy substratum.

Boccardia columbiana and *B. proboscidea* exhibit similar forms of reproduction and early larval morphology, but details have only been published for the latter species (Hartman, 1940, 1941; King, 1976; Woodwick, 1977). Both species deposit their pear-shaped egg capsules in rows within the female's tube; each capsule containing between 35 and 77 eggs. The capsules are individually attached to the wall of the tube by two thin extensions of caspular material. All capsules occur singly and are not connected to one another. Planktotrophic larval development occurs in both species, beginning with the release of the encapsulated larvae at the 3-setiger stage. At this time the alimentary tract is complete and functional; the long larval setae are completely formed. The larvae remain planktonic until they have acquired between 15 and 18 segments; at which time if they are provided with a suitable substratum, they settle and begin their sedentary existance. The planktonic larvae of both species are indistinguishable from one another.

A second type of development has been described by Hartman (1940, 1941) and Woodwick (1977) for *Boccardia proboscidea*. In this type of developmental sequence, not all of the eggs are fertilized and only a few larger individuals develop per capsule to the 12 to 15 setiger stage prior to release. These larvae utilize the remaining eggs as a nutrient source. This type of development was not encountered in this study.

A third spionid polychaete, *Polydora giardi* Mesnil (1896) was also

considered in the present study. A common associate of *Boccardia columbiana* in the coralline algae (*Lithothamniun pacificum* Foslie) at Dillon Beach, California, *P. giardi* provided convient comparative material. The larval development of *P. giardi* has been described recently (Day and Blake, 1979) and with the addition of the larvae of this species to this study, a comparison could be made of the tolerances of the two *Boccardia* species and a close associate, both geographically and taxonomically. Of particular interest was the fact that *P. giardi* was much more abundant in the coralline algae and exhibited a much broader reproductive season, suggesting a greater potential survival of the offspring and less of an effect of temperature on the reproductive season.

Hartman (1941) states that adult *Boccardia proboscidea* are tolerant of a wide range of salinities representing both oceanic and brackish conditions; moreover, the extreme geographical range of both *Boccardia* species suggests a wide latitude of temperature tolerance. Planktonic larvae are even more likely to encounter a variety of temperature and salinity regimes than their sessile adult forms, subject as they are to the vagaries of currents, prevailing winds and tidal flow. Lyster (1965) reports that for the polychaete larvae he studied, adult tolerance to salinity flucuations is mirrored by the larvae of the species. This paper also offers the important observation that salinity stress for polychaete larvae is reduced when they are at some optimum temperature. In other words, the broadest salinity tolerance corresponds to a particular temperature, and will be narrower at any significantly

lower or higher temperature. The timing of reproduction to coincide with this temperature should help to maximize the chance of reproductive success. Conversely, those species with a wider temperature tolerance should also exhibit more latitude in the timing of their reproduction.

Typically two approaches are used to investigate an organism's response to a suite of environmental parameters: (1) the parameters are studied separately in a univariable relationship or (2) they are studied together as a multivariable relationship. Since the univariable approach cannot ascertain possible synergistic effects of the different parameters, a multivariable is preferable. One such multivariable approach is known as "fitting" a response surface to the data, the approach used in this study (see Appendix I).

The present study was undertaken to examine the survival of the larvae of three spionid polychaetes, *Boccardia proboscidea*, *B. columbiana* and *Polydora giardi* exposed to a variety of combinations of temperature and salinity. Of all previous studies employing response surface techniques, only that of Gray (1976) dealt with a larval polychaete. Working with the larvae of *Serpula vermicularis*, he investigated their response to different levels of salinity, temperature and mercury concentration. Gray, however, examined only trochophores up to four days old, leaving a large portion of the larval duration unexamined. In contrast, this study considered the entire larval period from initial release from the egg capsule until the surviving larvae were physically capable of settling and beginning their adult, benthic existence. A third parameter, that of food concentration available to the larvae, was

added to the study of *B. columbiana* and *P. giardi*. This resulted in three independent variables being considered simultaneously. Food concentration was added as a parameter in order to consider the possibility that a well fed organism with greater metabolic reserves might be able to tolerate more stress than an organism receiving only a minimal amount of food. This could be particularly important in determining differential reproductive success for populations reproducing under marginal conditions of temperature or salinity and could be critical for range extension in years with greater than normal planktonic food availability.

The following questions will be explored by this study. What are the physical extremes of temperature and salinity beyond which no survival is possible for the three spionid larvae considered? This is of major importance to the species as a whole because these limits define its greatest possible geographic range, which is further modified by numerous other factors. Within these extremes, how is survival affected by a change in either or both of the parameters? In other words, are both factors of equal importance in determining survival; are they antagonistic or synergistic in their effects upon the larvae's well-being? What role does food availability play in ameliorating the detrimental effects of temperature and salinity extremes? Are taxonomically closely related organisms more alike in their response to similar conditions than more distantly related species? Finally, what is the consequence, with regards to planktonic developmental time and the resultant vulnerability to predators in the plankton, of the particular salinity-temperature regime to which the larvae are subject?

Each of these questions and their ramifications will be explored in this paper.

MATERIALS AND METHODS

Adult *Boccardia proboscidea* were collected from the banks of the small mariculture pond maintained by the Pacific Marine Station. Sediment from the pond was sieved through a 0.5 mm stainless steel sieve and the tubes of the adults were carefully removed and dissected open under a dissecting microscope. Adult tubes containing egg capsules were isolated; the egg capsules were counted, removed to a 9 cm fingerbowl containing filtered sea water and placed in a refrigerated cooler set to maintain a temperature of 15° C (±1° C). The water was changed daily until the encapsulated larvae had reached the 3 setiger stage; at which point, the capsules were opened and the larvae released. This was necessary since Hartman (1941) reported that *B. proboscidea* were unable to effect their own release when the capsules were removed from the tube and the subsequent influence of the adult female's movements. The larvae were counted as they emerged from each capsule and 30 were placed in each of nine 9 cm fingerbowls containing 50 mls of seawater at the desired temperature and salinity combination. Three different salinities and three different temperatures were used corresponding to a 3^2 experimental design (Appendix I). The salinities of 20‰, 30‰, and 40‰ (± 0.5‰) were measured by a hydrometer. Hyposaline levels were produced by dilution with distilled water; hypersaline levels by

evaporation. Refrigerated coolers set at 10° C, 15° C, and 20° C (±1° C) were used to maintain the experimental temperatures. The larvae were fed from a culture of *Dunaliella tertiolecta* Butcher. Every third day the suriviving larvae were counted, transferred to fresh cultures, and fed at a level of 55,000 cells/ml.

The growth experiments were maintained in an identical manner except that the original number of larvae per culture was increased to 40. At three day intervals 5 larvae were removed from each culture and anesthetized in 7.5% MgCL. These larvae were measured with an optical micrometer and the number of setigers was recorded. Subsequently the larvae were discarded.

It was assumed that a second degree polynomial of the form:

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

would approximate the survival and growth data. Survival was expressed in terms of the angular transformation of the per cent survival ($y = \arcsin (\text{per cent})^{\frac{1}{2}}$). Growth was expressed as the number of setigers. Response surface isolpleths were calculated for each 3 day interval.

Adult *Boccardia columbiana* were collected by removing pieces of the coralline algae, *Lithothamnium pacificum* Foslie, form the surfaces of the rocks just north of the beach at Dillon Beach, California. Removing the coralline algae from the rock exposed the galleries bored through the algae by the spionids. Egg capsules were removed when they were found and treated identically as before with *B. proboscidea* until hatching.

Instead of the 3^2 design used for *Boccardia proboscidea* a third

factor, food concentration was added, and this required the use of an orthogonal central composite design with three independent variables (Appendix II).

An artificial seawater preparation Instant Ocean was used because of the large number of cultures required by the design for 2 replicate experiments. Previous studies (Forrester and Alderdice, 1965; Alderdice and Forrester, 1971; Alderdice and Forrester, 1967) had employed a commercial seawater preparation and Sulkin and Minasian (1973) showed only a marginally significant increase in mortality of the xanthid crab, *Rhithropanopeus harrisii*, larvae due to synthetic seawater at salinities below 11‰. In order to insure that no significant difference occured between cultures raised in artificial seawater as opposed to natural seawater, a pair of replicate cultures were raised in artificial and natural seawater at 30‰. The resulting single classification ANOVA indicated no significant difference between the cultures. The salinities were prepared within $\pm$ 0.05‰ as measured by a Knudsen titration. The following levels were prepared in five 22 liter carboys with glass distilled water: 18.85‰, 20‰, 30‰, 40‰, and 42.15‰. Temperatures were as before, with the addition of the two axial point temperatures required by the central composite design 8.925° C and 21.075° C.

The third factor employed was food concentration per ml of culture. Appropriate amounts of a monoculture of *Dunaliella tertiolecta* were calculated with a 1 ml calibrated cell counting chamber according to the method described in *Standard Methods for the Examination of Water and Wastewater* (1971). These amounts were added every three days when the

cultures were changed and the surviving larvae counted. The following levels of the number of cells/ml were used: 325, 10,000, 55,000 100,000, and 109,675.

It was assumed that a second degree polynomial of the form:

$$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + E$$

would adequetely represent the data. Response surface isopleths were calculated at three day intervals with the dependent variable being the angular transfromation of the per cent survival.

Adult *Polydora giardi* were collected in the same locality as *Boccardia columbiana* and often in the same pieces of coralline algae. *P. giardi* unlike the *Boccardia* will hatch by themselves and did not need to be freed from the capsule even in the female's absence. The experimental design and treatment were identical to that of *B. columbiana* with the exception that 50 larvae were used per culture and natural seawater was used. The 20 additional larvae per culture allowed growth measurements to be taken on days 9, 18, and 27. After anesthetization in 7.5% MgCl and counting the number of setigers present in 5 randomly selected larvae, the larvae were discarded.

All cultures were kept in the dark except when counting and culture maintenance was taking place. According to Dean and Mazurkiewicz (1972) total darkness encourages a random dispersel of larvae throughout a standing culture thereby preventing congregation of the larvae which apparently retards growth.

The computer program used for the response surface analysis was

written in FORTRAN IV by the author based on the techniques described by Myers (1971) and is provided in Appendix III.

RESULTS

Boccardia proboscidea: SURVIVAL

3 DAY (Fig. 1): After 3 days exposure to the experimental conditions, a response surface was fit to the per cent survival data associated with the larvae of *B. proboscidea*. This response surface estimated that maximum survival should be 90% within the region of the experimental design. The stipulation that all predictions are valid only within the region of the design is important since the usual dangers of extrapolation beyond this region are critical. The surface was generated by the following polynomial (expressed in terms of the coded variables):

$$y = 60.99 + 4.44\ (T) + 4.13\ (S) + 18.65\ (T^2) + (-23.69)\ (S^2) + 0.91\ (TxS)$$

The coefficient of determination (r^2) = 97%, which means that the polynomial explains 97% of the variance about the mean (Mendenhall, 1968). From the associated ANOVA (Table 1a) it is seen that the fit of the multiple regression is significant at the 5% level but that none of the individual terms alone were significant at this level or above. The surface contains a saddle point as shown by the opposite signs of the eigenvalues (18.65, -23.70) calculated during the canonical analysis. There is approximately equal sensitivity, after 3 days of exposure, to both of the independent variables, as shown by the approximately equal magnitudes of the eigenvalues and the lack of a significant interaction

term (a significant interaction term would indicate a significant rotation of the axes of the design and a corresponding compounding of the variables contribution). The stationary point is within the design region and represents a saddle point, as previously mentioned, occurring at 14.39° C and 30.85‰. The percentage survival increases as one moves along the w_1 axis (closely approximated by the temperature axis) in either direction away from the stationary point, but decreases in either direction along the w_2 axis ≈ salinity. It is particularly interesting to note that high survival is predicted for both very high and very low temperatures but not for the middle range of the design. There is a very slight tilt of the surface towards a coupling of high salinity tolerance with high temperatures.

The predicted maximum of survival (90%) occurs above 19° C and exhibits the broadest salinity range at the design extreme of temperature (20° C) where salinities spanning the 25-38‰ region produce the same response.

6 DAY (Fig. 2): After 6 days of exposure to the experimental conditions a similar surface is obtained, generated by the following equation:

$$y = 57.51 + 16.36\ (T) + 6.09\ (S) + 10.59\ (T^2) + (-24.25)\ (S^2) + 0.86\ (TxS)$$

The surface again contains a stationary point within the experimental region, but slightly lower in temperature than the previous surface (11.11° C, 31.12‰) and is again the saddle point of the design. The eigenvalues associated with this surface are, 10.59 and -24.25; the relative magnitude of the second one, which is associated with the

salinity axis, more than twice as large as the other. This indicates that the surface is elongated along the w_1 axis ($\approx$ temperature axis) and that survival is more affected by a move away from the stationary point along the w_2 axis ($\approx$ salinity axis). Maximum survival for this surface remains at 90%; once again located at the high temperature extremes of the design, above 18.5° C. The region of broadest salinity tolerance again occurs at 20° C where a zone of 26-37‰ produces the maximum survival.

The ANOVA associated with design (Table 1b) indicates that the fit of the response surface is significant at the 5% level. The coefficient of determination is 97%.

9 DAY (Fig. 3): The maximum survival after 9 days of exposure remains at the 90% level. The response surface is generated by the polynomial:

$$y = 54.01 + 27.03\ (T) + 6.29\ (S) + 2.54\ (T^2) + (-25.86)\ (S^2) + 3.92\ (TxS)$$

The associated ANOVA (Table 2a) indicates that the fit of the surface is significant at the 5% level, as is the linear temperature term. The coefficient of determination remains at a high level (r^2 = 96%) but is slightly lower than the values for the previous two surfaces. This indicates an increasing amount of variance is not accounted for by the second order model which is being used to generate the survival isopleths. Probably this indicates that higher order terms are becoming increasingly important.

The response surface remains similar in shape to the previous surfaces; elongated along the temperature axis and contracted along the axis associated with salinity. Moving along the w_2 axis of the surface

results in decreasing survival at the extremes of salinity for all temperature values. The rotation of the axis of the surface towards high salinity and high temperature values is becoming increasingly noticeable, although it remains insignificant. Nevertheless, some interaction between temperature and salinity is beginning to modify the survival of the larvae. The stationary point is located outside of the experimental region, but continues to remain a saddle point for the surface. The region of maximum survival occurs above 18° C, but remains broadest at the design extreme of temperature (20° C). At this temperature, a salinity range of 25-39‰ is spanned by the 90% survival isopleth. This range of salinities is slightly narrower than the previous 6 day values for the same 20° C temperature, suggesting the larvae are becoming more stenoplastic in their response.

15 DAY (Fig. 4): The final response surface calculated for the survival of *B. proboscidea* larvae takes place after 15 days of exposure to the experimental conditions. After 15 days the second order model no longer fits the data with any adequecy. The response surface generated by the polynomial:

$$y = 38.31 + 26.36\ (T) + 4.84\ (S) + (-1.24)\ (T^2) + (-16.07)\ (S^2) + 8.03\ (TxS)$$

continues to reflect the trends of the previous surfaces with a single major exception. The surface no longer contains a saddle point, but rather a simple local maximum is obtained as a stationary point. This is illustrated by the eigenvalues which posess the same sign, if not the same magnitude (-0.22, -17.08). The stationary point however, remains outside of the design region. A simple ellipse replaces the more

complicated shapes of the previous surfaces. The associated ANOVA (Table 2b) once again indicates that the fit and the linear temperature are significant at the 5% level.

Although the surface is now strictly elliptic in nature, the broad trends of the previous surfaces continue to be valid. Maximum survival (80%) continues to occur at the highest temperatures. A temperature of at least 19.5° C is necessary to attain this survival level. Interestingly at 20° C the salinity range is even narrower than that predicted by the 9 day response surface; maximum survival achieved only between 30-38‰. Below 12° C less than 20% survival is achieved; a temperature of at least 15° C is needed to attain at least 50% survival.

Boccardia proboscidea GROWTH

6 DAY (Fig. 5): The second order model exhibits an excellant representation of the growth of the larvae of *B. proboscidea* after 6 days of exposure to the design conditions. The coefficient of determination indicates that 99% of the variation around the mean of the data is adequetly accounted for by the model coefficients. An ANOVA (Table 3a) performed on the regression indicates that the fit of the regression is significant at the 0.1% level, while the linear temperature term is significant at the 1% level and the quadratic temperature term is significant at the 5% level.

The surface has a stationary point well within the confines of the design at 11.86° C and 30.15‰. This stationary point represents a saddle point of the design surface with growth increasing as you move away from the stationary point along the temperature axis; decreasing along

the salinity axis as one moves away from the stationary point. The eigenvalues associated with this stationary point are 0.68 and -0.34.

Since the larvae were introduced into the experimental salinity and temperature combinations at a 3 setiger stage, that being the minimum size at which feeding on phytoplankton can occur and the usual size of release, this was taken as the 0 growth level. After 6 days of exposure to the test conditions the maximum growth of 3 setigers occurred above 19.4° C. No growth is predicted below 11° C. The surface is generated by the following polynomial:

$$y = 3.39 + 0.83\ (T) + 0.16\ (S) + 0.67\ (T^2) + (-0.33)\ (S^2) + 0.25\ (TxS)$$

9 DAY (Fig. 6): The larvae of *B. proboscidea* are able to attain a maximum growth of 5 setigers at 20° C after 9 days of elapsed time. They are able to achieve this size (5 setigers) within a salinity zone covering 30‰ to 38‰. Under these conditions, they have reached approximately 50% of their final settling size after less than a week and a half in the plankton, In contrast, no growth occurs below 10° C at either of the salinity extremes.

The 9 day response surface is calculated by the equation:

$$y = 5.39 + 2.25\ (T) + 0.17\ (S) + 0.42\ (T^2) + (-0.83)\ (S^2) + 0.38\ (TxS)$$

which explains 99% of the variation about the mean. The surface's stationary point is outside of the design limits and represents the saddle point of the design. The associated eigenvalues are 0.44 and -0.86, and as seen, are of opposite sign. Growth increases as the experimental conditions are moved away from the stationary point along the w_1 axis which is approxiametly coplanar with the temperature axis.

Any movement along the w_2 axis away from the stationary point impairs growth.

An ANOVA (Table 3b) performed on the response surface indicates that the fit of the surface to the data is significant at the 1% level. Also significant at this level is the contribution made by the linear temperature term.

12 DAY (Fig. 7): After 12 days of exposure to the various combinations of design parameters, the maximum growth predicted by the response surface fit to this data was 7 setigers, a two setiger increase in 3 days. The response surface was generated from the polynomial:

$$y = 6.67 + 2.92\ (T) + 0.67\ (S) + 0.25\ (T^2) + (-1.50)\ (S^2) + 0.88\ (TxS)$$

and is similar in shape to the previously obtained surfaces. The coefficient of determination is equal to 99%, again indicating an extremely good accounting of the variance about the mean by the model.

The stationary point of the design falls outside of the experimental region and as indicated by the eigenvalues (0.35, -1.60) represents a saddle point. As in the previous surfaces the axes of the surface are approximately coplaner with the design axes. The stationary point represents a temperature minimum and a salinity maximum, indicating that movement away from the stationary point along the temperature axis will increase growth; movement along the salinity axis, however, will decrease growth. The linear temperature term was shown to be significant at the 1% level by an ANOVA (Table 4a).

The maximum growth of 7 setigers was achieved at temperatures in excess of 19.5° C. The broadest salinity spectrum associated with the

maximum growth level continues to be associated with the high temperature extreme of the experimental region. At 20° C this growth isopleth encompasses salinities between 30‰ and 40‰. No growth occurs below 13° C at the salinity extremes of the design.

18 DAY (Fig. 8): The response surface for growth of the larvae subsequent to 18 days of continuous exposure to the experimental conditions was generated by the following equation:

$$y = 8.67 + 3.83\ (T) + 0.33\ (S) + 0.50\ (T^2) + (-3.50)\ (S^2) + 0.75\ (TxS)$$

which explains 95% of the variance about the mean. The shape of the surface remains nearly the same as those of the previous days with a stationary point representing a saddle point outside of the experimental region. The magnitude of the eigenvalues (0.53, -3.53) indicate that the rate of change is becoming more rapid along the w_2 axis ($\approx$ salinity axis) as one moves equal distances away from the stationary point. There is a very slight tilt towards a correlation between high salinities and temperatures, but this remains statistically insignificant as shown by the ANOVA performed on the model equation (Table 4b).

Maximum growth of 11 setigers occurs at 20° C over a salinity range of 30-33‰. After 18 days, no growth is predicted below 12° C at either salinity extreme of the design.

Boccardia columbiana: SURVIVAL

3 DAY (Figs. 9, 10, 11): This response surface represents the first of the central composite designs incorporating 3 independent variables. The three dimensional figures that are generated by the model equations are sectioned at the 1000, 50000 and 100000 cells/ml levels and these

slices are presented as three two dimensional surfaces in the relavent figures.

After 3 days of exposure to the various combinations of temperature, salinity and food concentration, the survival of the larvae of *B. columbiana* is predicted by the following equation:

$$y = 82.17 + (-4.80)\ (T) + (-8.97)\ (S) + (4.57)\ (F) + (-4.91)\ (T^2) + (-4.26)\ (S^2) + (-4.72)\ (F^2) + (-4.80)\ (TxS) + (-3.91)\ (TxF) + (-1.75)\ (SxF)$$

This equation explains 98% of the variation about the mean. The surface is an ellipsoid with a stationary point that is a simple maximum. The stationary point lies outside of the experimental region. Survival decreased along every axis as one moves away from the stationary point. An ANOVA (Table 5a) indicates that the fit of the response surface is significant at the 0.1% level. In addition the linear salinity term is significant at the 0.1% level.

At the level of 1000 cells/ml of *Dunaliella* the maximum predicted survival of the larvae was 80% in a region below 14° C for the entire salinity spectrum. Raising the temperature to 20° C narrowed the range to 20-34‰. Increasing the food concentration available to 50000 cells/ml raised the maximum possible survival to 90% below 13° C for the entire range of salinities. In this case, raising the temperature to 20° C, narrowed the acceptable salinity range to 20-31‰. At 100000 cells/ml, maximum survival remains at the 90% level. The widest salinity tolerance zone occurs at the low extreme of the temperature regime. At 10° C, 90% survival occurs between 20-36‰.

6 DAY (Figs. 12, 13, 14): Subsequent to 6 days of exposure to the test conditions a response surface was generated employing the polynomial:

$y = 70.71 + (-23.41)\ (T) + (-4.23)\ (S) + 6.07\ (F) + (-24.86)\ (T^2) + (-7.59)\ (S^2) + (-3.24)\ (F^2) + (-2.46)\ (TxS) + 4.25\ (TxF) + (-6.64)\ (SxF)$

This equation explains 97% of the variance about the mean. The ANOVA (Table 5b) indicates that not only is the fit of the regression significant at the 0.1% level, the linear food term is significant at the 5% level as well. It is important to notice that the salinity and food interaction term is also significant at the 5% level, indicating a significant rotation of the surface about these axes.

The response surface is an ellipsoid with a stationary point representing a local maximum, outside of the design region. Maximum survival (80%) at the 1000 cells/ml level occurs below 12° C. At this temperature maximum survival can occur between 24‰ and 40‰. Less than 10% survival is predicted for the region above 19.3° C. The predicted maximum survival at the 50000 cells/ml increases to 90%. This isopleth occurs below 12.5° C and spans the salinities between 20‰ and 36‰. Survival does not improve markedly at this level of food concentration, however, at the upper end of the temperature spectrum. Less than 10% survival occurring above 19.5° C in the region spanning 34-40‰. Increasing the food concentration up to 100000 cells/ml expands the 90% survival isopleth to 13° C, but the salinity tolerance is narrowed by 2% spanning the region from 20-34‰. The broadest temperature range occurs at 24‰ and extends from 10.2° C to 16.2° C.

9 DAY (Figs. 15, 16, 17): The 9 day response surface is generated

by the equation:

$$y = 61.00 + (-20.52)\ (T) + 1.38\ (S) + 7.49\ (F) + (-26.45)\ (T^2) + (-3.81)\ (S^2) + (-6.39)\ (F^2) + (-2.00)\ (TxS) + (-5.85)\ (TxF) + (-5.46)\ (SxF)$$

The coefficient of determination is equal to 94%. The shape of the surface remains an ellipsoid with a stationary point that is a local maximum. The stationary point is located at 12.60° C, 26.11‰ and 98747 cells/ml. The temperature terms, both linear and quadratic dominate the remaining regression terms contribution to the fit of the equation. The linear food term is significant, but at the 5% level indicating less of a contribution to the overall regression. (Table 6a). As can be seen from the magnitude of the eigenvalues (-26.95, -2.06, -7.64) the most rapid change in survival occurs along the temperature axis. This is also very apparent in the plots of the response surfaces for this day, which show a small change in temperature is sufficient to raise or lower survival by 10%.

Maximum survival is lowest at the minimum plotted food concentration (1000 cells/ml) attaining a maximum level of only 70% within the design boundaries. At this food concentration the broadest salinity tolerance occurs at 14° C. Survival decreases rapidly as the temperature is increased until less than 10% survival is predicted above temperatures of 19.8° C. An increase in the concentration of food available to the larvae improves the survival potential dramatically. At a concentration of 50000 cells/ml a 90% survival isopleth exists; spanning the salinities of 25‰ to 40‰ at 13° C. The widest temperature span

of this contour occurs at 33‰ where the temperatures bounded by this isopleth range from 11.2° C to 14.5° C. By the time a concentration of 100000 cells/ml is attained, this temperature range has increased to include a 5° C range but the salinity producing this spectrum has fallen to 26‰. The broadest salinity tolerance occurs at 12 C.

15 DAY (Figs. 18, 19, 20): The final surface that I will consider for the larvae of *B. columbiana*, is one fit to the data after 15 days of exposure to the experimental combinations. Subsequent to this time period, the second order model fails to provide an adequate fit to the data. Only 77% of the variance about the mean is explained by the model equation generated for this time period. The equation:

$$y = 32.69 + (-5.58)\ (T) + (-7.98)\ (S) + 10.44\ (F) + (-18.82)\ (T^2) + (-3.81)\ (S^2) + (-3.98)\ (F^2) + 2.99\ (TxS) + (-6.43)\ (TxF) + (-2.99)\ (SxF)$$

however continues to provide a significant fit to the data at the 0.1% level (Table 6b). The quadratic temperature term is also significant at this level, reaffirming the critical role played by the experimental temperature in determining these isopleths of survival. Other significant terms include the linear food contribution, significant at the 1% level and the linear salinity contribution, significant at the 5% level.

The surface remains an ellipsoid with survival decreasing in all directions away from the stationary point, which lies beyond the borders of the experimental design. The level of maximum survival is much lower than the previous surface, reaching only 50% under the most favorable of the design conditions. At a food concentration of 1000

cells/ml the isopleth corresponding to the highest predicted survival is only a 20% contour. Above a temperature of 19 $^{\circ}$C this falls to less than 10%. Raising the available food concentration to 50000 cells/ml, results in only a small gain in predicted survival (30%). This contour encompasses a region which is widest, in respect to salinity, at about 14 $^{\circ}$C. Even at an elevated food concentration of 100000 cells/ml the maximum predicted survival only attains a level of 50%.

Polydora giardi SURVIVAL

3 DAY (Figs. 21, 22, 23): The first response surface generated for the larvae of *P. giardi* contains a saddle point. This saddle point is located outside of the experimental region at the stationary point of the design. The 3 day survival isopleths are generated by the equation (r^2 = 99%):

$$y = 87.49 + (-1.61)\ (T) + (-12.56)\ (S) + (-7.24)\ (F) + (-2.92)\ (T^2)$$
$$(-16.46)\ (S^2) + (-5.66)\ (F^2) + (-4.08)\ (TxS) + 4.60\ (TxF) + (-12.15)\ (SxF)$$

The eigenvalues determined by the canonical analysis of this response surface are -5.83, -19.24 and 0.02; the magnitude of the second eigenvalue suggests that the surface is extremely attenuated along the w_2 axis which is primarily composed of the salinity contribution. This can be seen very clearly in the plot of the surface, survival changing rapidly with a relatively small shift in salinity. The ANOVA (Table 7a) performed on this multiple regression equation indicates that both the linear and quadratic salinity terms are significant at the 0.1% level. In addition to the salinity contribution, the linear food term is significant at the 1% level. The axes of the surface are rotated due to a significant

($p < 0.001$) contribution of the salinity and food interaction term.

The predicted maximum survival of 100% occurs over the entire salinity spectrum below a temperature of 16° C, and even at its narrowest (20° C) it spans a range of 22-36‰, for food concentrations of 1000 cells/ml. Interestingly as food concentration in the cultures is increased, the level of maximum survival decreases to 90% and shifts towards the lower salinity regions. At 100000 cells/ml only the region encompassed by the salinities of 20-28‰ exhibits the maximum level of survival at all design temperatures.

6 DAY (Figs. 24, 25, 26): After 6 days of exposure to the experimental combinations of temperature, salinity and food concentration, the response surface generated from the equation:

$$y = 80.98 + (-0.36)\ (T) + (-21.39)\ (S) + (-4.27)\ (F) + (-10.83)\ (T^2)$$
$$(-28.66)\ (S^2) + (-0.67)\ (F^2) + (-2.08)\ (TxS) + 2.04\ (TxF) + (-6.46)\ (SxF)$$

explained 97% of the variance about the mean and provided a significant fit at the 0.1% level (Table 7b). This response surface is an ellipsoid with a stationary point representing a simple maximum. The stationary point remains outside of the experimental region.

Maximum survival is predicted as 90% at all 3 food concentrations considered. The isopleths delimiting maximum survival at the three food levels are also similar in shape and general location. At 1000 cells/ml the greatest salinity range of this contour occurs at 14.5° C, while at 100000 cells/ml this point has shifted to about 15.5° C with only a slight narrowing of the salinity range. The salinities at this food level range between 20‰ and 31.5‰, instead of between 20‰ and

34.5‰ seen at the 1000 cells/ml concentration.

The significant individual terms of the regression, all of which are significant at the 0.1% level, are linear temperature, linear salinity, and quadratic salinity. A rotation along the salinity and food axes is present, illustrated by a significant interaction term ($.001 < p < .01$).

9 DAY (Figs. 27, 28, 29): The 9 day surface assumes a more complicated geometry, once again containing a stationary point that is a saddle point rather than a simple maximum. This surface is generated by the equation ($r^2 = 98\%$):

$$y = 74.57 + (-1.00)\ (T) + (-25.46)\ (S) + (-1.39)\ (F) + (-7.22)\ (T^2)$$
$$(-33.65)\ (S^2) + 0.54\ (F^2) + (-2.88)\ (TxS) + 1.54\ (TxF) + (-5.08)\ (SxF)$$

The stationary point of the design is located at 14.83° C, 26.48‰ and 40411 cells/ml. Eigenvalues of -7.24, -33.91 and 0.83 indicate that the surface is most sensitive to movement along the axis dominated by the salinity terms. A steeper gradient is exhibited in this direction as predicted by the magnitude of the second eigenvalue. Some rotation along the salinity and food axes is apparent; increasing food concentration resulting in a shift of the center of the maximum survival isopleth to increasingly lower salinities. The ANOVA (Table 8a) indicates that the interaction term is significant at the 5% level. Continuing to dominate the significant individual terms are the salinity contributions, both displaying significance at the 0.1% level. One other individual term is significant, the quadratic temperature term at the 5% level.

The maximum survival predicted at the 9 day extent of the experiment is 90% at all food levels examined. The widest portion of this isopleth,

corresponds to the broadest salinity tolerance which occurs at 15° C at the 1000 cells/ml concentration and shifts only slightly higher (15.5° C) at 100000 cells/ml level. Concurrent with this slight upward shift of the 90% survival isopleth is a small displacement of the salinity range towards lower salinities. At 1000 cells/ml, the range of salinities at the broadest portion of this isopleth includes values from 22‰ to 32‰; which increases to include salinities from 20‰ to 31‰ at the 100000 cells/ml level.

15 DAY (Figs. 30, 31, 32): After 15 days of exposure to the test conditions, the predicted survival of the *P. giardi* larvae remains high. Both the 50000 and 100000 cells/ml plots contain 80% survival isopleths, only the 1000 cells/ml level does not exhibit this high of a survival level, 70% survival being the maximum in this case. These isopleths are centered about a stationary point of 13.78° C, 27.37‰ and 72643 cells/ml, the local maximum of the design. The contours are approximately with the axis corresponding to the individual parameters; a significant interaction term being absent from the ANOVA (Table 8b). The only significant terms at this point in time are the salinity contributions, linear salinity significant at the 1% level and quadratic salinity at the 0.1% level. The equation generating these response surfaces is:

$$y = 64.92 + (-2.91)\ (T) + (-12.85)\ (S) + 7.20\ (F) + (-8.61)\ (T^2)$$
$$(-29.74)\ (S^2) + (-10.3)\ (F^2) + 5.46\ (TxS) + 0.39\ (TxF) + (-3.81)\ (SxF)$$

18 DAY (Figs. 33, 34, 35): The response surfaces generated for th 18 day conditions are almost identical to those of 15 days.

The polynomial generating this surface:

$$y = 66.74 + (-3.77)\ (T) + (-13.19)\ (S) + 4.37\ (F) + (-13.87)\ (T^2)$$
$$(-34.34)\ (S^2) + (-5.56)\ (F^2) + 6.54\ (TxS) + 1.21\ (TxF) + (-2.65)\ (SxF)$$

explains 98% of the variance about the mean.

The isopleths are almost identical to the 15 day situation and are centered around a similar stationary point of 14.15° C, 27.75‰ and 74236 cells/ml. Once again the stationary point represents a local maximum with survival decreasing in all directions on the surface as one moves away from this point. The only difference in the isopleths between this and the previous surfaces is found in the slightly broader contours of the 100000 cells/ml level.

An ANOVA (Table 9a) indicates that the linear salinity, quadratic salinity and quadratic temperature terms are all significant at the 0.1% level. Also, the linear food contribution is significant at the 5% level. Particularly interesting, is the fact that the temperature and salinity interaction term is significant ($0.001 < p < 0.01$) indicating that the surface is rotated along these axes.

27 DAY (Figs. 36, 37, 38): The final response surface calculated for the survival data is that after 27 days of exposure to the experimental design. The stationary point of this surface is similar to the previous 15 and 18 day surfaces; a maximum located at 14.37° C, 28.75‰ and 68483 cells/ml. Predicted survival however has decreased at all 3 food levels with the maximum survival of 70% reached at the 50000 cells/ml concentration. The surface continues as an ellipsoid; canonical analysis indicating that the most rapid change in predicted survival results

from movement along the axis approximately coplaner with the salinity axis.

The 27 day survival isopleths are generated by the equation (r^2 = 90%):

$y = 58.43 + (-2.72)\ (T) + (-7.30)\ (S) + 4.61\ (F) + (-13.14)\ (T^2)$

$(-31.61)\ (S^2) + (-7.54)\ (F^2) + 5.63\ (TxS) + 0.35\ (TxF) + 0.35\ (SxF)$

The ANOVA (Table 9b) indicates that the fit of the equation is good at the 0.1% level, as is the quadratic salinity term which continues to dominate the contribution of the independent terms. Other significant independent terms include the quadratic temperature contribution at the 1% level and the linear salinity contribution at the 5% significance level.

Polydora giardi GROWTH

9 DAY (Figs. 39, 40, 41): Growth in setigers is predicted after 9 days of exposure ot the design conditions by the following polynomial:

$y = 5.92 + 0.05\ (T) + (-0.16)\ (S) + 0.65\ (F) + (-1.26)\ (T^2) + (-1.70)$

$(S^2) + 0.10\ (F^2) + (-0.05)\ (TxS) + 0.0\ (TxF) + (-0.18)\ (SxF)$

This equation explains 96% of the variance around the mean. The canonical analysis produces the eigenvalues -1.25, -1.70 and 0.10 indicating that the surface contains a saddle point. This saddle point occurs at the stationary point of the design which is outside of the experimental region. Any movement along the axis which is approxiametly coplaner to the food axis results in increased growth, conversely, any movement along the other axes reduces the amount of growth predicted by the design.

At 1000 cells/ml the maximum predicted growth after 9 days is 3 setigers which occurs in a region between 25.8‰ and 35‰ at a

temperature of about 15° C. Increasing the food concentration at this stage in time plays little direct role in promoting additional growth with 3 setigers remaining the maximum level of growth for the other two food concentration levels as well. A slightly wider maximum growth isopleth at the elevated food levels is the only observable effect. The 3 setiger isopleth spans a salinity range of 22-37‰ at 15° C and 50000 cells/ml, about a 6‰ increase in salinity tolerance. At 100000 cells/ml this contour is slightly compressed and spans the salinities between 23‰ and 35.5‰. The contours themselves, are approximately circular, reflecting the almost equivalent rate of change along either the temperature or salinity axis.

An ANOVA (Table 10a) indicates that the fit of the regression is significant at the 0.1% level and the important individual contributions are the linear food (1% level), quadratic temperature (1% level) and the quadratic salinity terms (0.1% level).

18 DAY (Figs. 42, 43, 44): The surface generated by the equation (r^2 = 91%):

$$y = 11.72 + 0.37\,(T) + (-0.18)\,(S) + 1.51\,(F) + (-2.01)\,(T^2) + (-5.40)\,(S^2) + (-1.33)\,(F^2) + (-0.07)\,(TxS) + 0.08\,(TxF) + (-0.25)\,(SxF)$$

is an ellipsoid with a stationary point lying within the borders of the experimental design. The stationary point is located at 15.5° C, 29.69‰ and 80786 cells/ml. An ANOVA (Table 10b) indicates that the regression is significant at the 0.1% level as is the quadratic salinity term contribution. The linear food term is significant at the 1% level; the quadratic temperature at the 5% level.

After 18 days of exposure to the test conditions the growth isopleths are becoming more sensitive to the food concentration and the salinity of the water. This is reflected in increased growth at the more elevated food concentrations and a flattening of the contours along the salinity axis. The maximum predicted growth at the 1000 cells/ml level is 9 setigers while this improves to 11 setigers at the 50000 cells/ml level. At the extremes of the design surface the greatest predicted growth is only 5 setigers or in other words, only about 56% of the maximum possible growth. The maximum growth isopleths remain fairly central to the design surface with moderate temperatures and salinities preferred. At 100000 cells/ml, at 15.5° C for instance, the widest salinity range is from 25.8‰ to 34‰; the widest temperature range (12.1° C to 19° C) occurring at 30‰.

27 DAY (Figs. 45,46,47): The final response surface calculated for the growth of the larvae of *Polydora giardi* is generated by the equation (r^2 = 92%):

$$y = 6.67 + 0.23\ (T) + (-0.10)\ (S) + 1.85\ (F) + (-1.09)\ (T^2) + (-3.6)(F^2) + (-8.95)\ (S^2) + 0.14\ (TxS) + (-0.14)\ (TxF) + (-0.14)\ (SxF)$$

The contours produced by this equation are generally similar to the 18 day contours but are even more compressed about the salinity axis; the surface itself remains ellipsoid in shape. The stationary point represents a local maximum of the surface and is located at 15.45° C, 29.93‰ and 66507 cell/ml. Maximum predicted growth is 13 setigers at the 100000 cells/ml level; the isopleth producing this growth includes salinities from 27‰ to 33‰ at 15.5° C. A wide range of temperatures

(11° C to 19.3° C) at 30‰ produce the same level of growth. At the 1000 cells/ml level of food concentration, growth of only 11 setigers is predicted by the response surface; the range of suitable temperatures (13.6° C to 18° C) also being narrower than the 100000 cells/ml level. Growth at the higher salinities is less than 4 setigers which is less than 31% of the possible growth after this time span under more favorable conditions.

An ANOVA (Table 11) performed on the response surface indicates that the fit of the surface is significant at the 0.1% level. The quadratic salinity term is also significant at this level and remains the dominant independent term. Also significant are the linear food term at the 5% level and the quadratic food term at the 1% level.

DISCUSSION

Environments are multidimensional and techniques considering several parameters simultaneously are to be preferred to an examination of a single variable isolated in its effects. The dimensions of the response domain delineated by these parameters should be investigated as completely as possible. These factors will define the limits of an organism's ability to survive and reproduce successfully. Thorson (1950, p. 2) states "But if, on analysing the ecological factors, we consider the limiting values, not the average values, on analyzing an animal population we have to focus our attention upon the most sensitive stages within the life cycle of the animal. These stages-

the weakest link of the chain- will normally be found during the breeding period and larval development, when the requirements of the organisms from the environment are often much more definite than during the other periods of their life cycle." These "limiting values" and their influence upon the "weaklink of the chain", the larval period is the concern of this study.

Previous studies (Dean, 1965; Hatfield, 1965; Simon, 1967, 1968; Blake, 1969; Blake and Woodwick, 1975) have often commented on the approximate duration of the planktonic larval stage of spionid polychaetes. Laboratory studies when employed in these investigations have been analyzed in a univariable manner; temperature being the most frequent independent variable considered. None of these studies, have thus far concerned themselves with mortality induced by altering more than a single variable at a time, or with the intertwined relationship between survival and growth of the organism. Energy devoted to growth must be balanced against energy expended to maintain the organism. Resisting extreme abiotic conditions may upset this balance; the organism is able to survive, but unable to increase in size, this in turn contributing to increased mortality.

The larvae of *Boccardia proboscidea* exhibit the classic effect of temperature on developing larval organisms. Increasing the ambient temperature, accelerates larval growth and improves the chance of survival of the larvae. The maximum growth rate and percentage survival occur at the upper temperature extremes of the experimental design (Figs 1-8). As suggested by Lyster (1965), this

region corresponds to the broadest area of salinity tolerance. Initially, lowered temperature has little apparent effect on the predicted survival of the larvae, but after 15 days of continuous exposure to temperatures less than 12° C, predicted survival is less than 20%. This projected level of survival may not be prohibitively low in order to assure adult population replacement levels being met, if these abiotic factors are the only source of larval mortality. Dorsett (1961), for example, suggests that the survival of approximately 0.3% of the larvae of *Polydora ciliata* is all that is necessary for the maintainence of the adult population. Two other factors may contribute significantly to the mortality experianced by the spionid larvae and these effects must be superimposed on the survival projections. First, all planktonic larvae are subject to some level of predation unlike those individuals in laboratory cultures. Larvae that are already weakened by extreme physical conditions may suffer increased predation, due to their reduced ability to avoid predators, for instance. Lough (1976) suggested this may be the case in his study of the larvae of *Cancer magister*. Secondly, below 12° C growth is also extremely slow; less than 2 setigers being added at all salinities after 12 days of exposure to the test conditions (Fig. 7). This growth represents only 29% of the possible growth at 20° C, the larvae apparently channeling the greatest portion of their energy budget into resisting the environmental extremes and very little into the addition of new body material. While this retardation of the growth rate in itself does not decrease the survival of the larvae, it does

result in an increased amount of time spent in the plankton and, consequently, increases the risk that the organism will be lost to predation before succcessful settlement and metamorphosis can take place.

The results of this study, knowledge of the local timing of egg capsule production in *Boccardia proboscidea* (Personal observations) and information about ambient water conditions in the area over several seasons (Smith et. al., 1971) provides the opportunity to draw together this information and suggest some reasons for the reproductive schedule of *B. proboscidea* that is observed locally.

Surface water temperatures range form 9.8° C to 15.2° C at Tomales Point, the station most clearly reflecting oceanic conditions. Variations in water temperature become more extreme as one moves up the bay, a condition that a larval organism must deal with if it is contained in a water mass undergoing some exchange with water near the head of the bay. In this region surface water temperatures have been recorded from 5°C to 25.5°C. Peak water temperatures for the entire bay occur during the summer and early fall usually from late June until early September. If an organism whose planktonic larval form grows most rapidly at elevated temperatures and whose survival is also markedly increased at these temperatures, it should produce its larvae at such a time that they might take advantage of seasonally higher temperatures. Adult *Boccardia proboscidea* should produce egg capsules in late spring and early summer as water temperatures climb to their seasonal peak and should cease production before late summer in order that the larvae will develop sufficiently prior to the seasonal decline

of temperatures in September. This is exactly what occurs in the area of Tomales Bay; the earliest egg capsules that were found were present in early May and the latest in Mid-July. During this period at all stations the water temperature is above 15° C and is rising. Larvae of *B proboscidea* released during this period can be expected to attain at least 6 setigers of growth within 18 days (Fig. 8) and at least 50% survival (Fig. 4) is predicted if we consider only sources of mortality that occur due to variations in temperature and salinity. Since *B. proboscidea* larvae settle when they have between 15 and 18 setigers, they could complete their development within 36 days. The rising water temperatures will in turn accelerate this growth rate still more. Egg capsules, if they were produced later in the season, would produce larvae that may initially benefit from high water temperatures but must contend with a continual decrease in water temperature as the seasonal temperature cycle falls to its minimum in December and January. This would result in decreased survival, steadily increasing developmental time and, consequently, increased likelihood of death in the plankton.

The response surfaces (Figs. 2-4; 6-8) exhibit a slight tilt towards a combination of high temperatures and salinities suggesting some degree of interaction between these variables, although this correlation is not significant at the 5% level. This suggests that the larvae demonstrate their maximum salinity tolerance in conjunction with their exposure to high temperatures. In central California, low salinity surface waters occur during the winter months when heavy rains occur and reach their maximum values during the relatively dry summers.

In marked contrast to the surfaces generated for *Boccardia proboscidea* with maximum growth and survival at the upper temperature extreme of the design, the surfaces generated for *B. columbiana* exhibit a marked preference for low to moderate temperatures over a wide range of salinities (Figs 9-20). Since these designs also employed the third independent variable of food concentration, the effect of the level of food available to the developing larvae could be ascertained. Temperatures in excess of 19° C invariably lower survival to less than 10% for all surfaces generated after 6 days of exposure, regardless of the amount of food available. Maximum survival occurs after 15 days between 10.8° C and 15.6° C at the highest food level (Fig. 20) and between 13.8° C and 16° C at the lowest (Fig. 18). This information suggests that *B. columbiana* larvae should do equally well in the plankton at all times of the year with the exception of the coldest months under oceanic conditions. Increased summer temperatures and increased salinity in the upper bay will rapidly decrease their level of survival and should result in little if any larvae surviving transit towards the head of the bay. This agrees well with the adult distribution of *B. columbiana* in Tomales Bay since they are only found in regions exposed to strictly oceanic water.

Boccardia columbiana produces egg capsules from April through October with the greatest number of capsules produced in April and May. This agrees well with the information generated from the response surfaces since by late October the water temperature is normally about 11° C and falling, a temperature regime which is rapidly retreating from the

optimum temperature range for survival predicted by the response surface. This optimum temperature isopleth is again entered in February with the seasonal rise in water temperatures. A spring bloom in the phytoplankton numbers which normally occurs in the north Pacific waters (Anderson, 1964) would considerably expand the upper and lower temperature limits of the highest survival isopleth (Fig. 20). Since this bloom occurs later in the season this lack of available food would suggest that the minimum food response surface (Fig 18) is probably more accurate for the late winter. The narrower and considerably higher minimum temperature predicted by this surface is probably the reason no egg capsules are found prior to April.

We can conclude that the survival of larvae of *Boccardia columbiana* is dominated by the individual parameters of temperature and available food (Table 6a,b). This is in marked contrast to the surfaces generated for *B. proboscidea* which prefer the highest seasonally available temperature rather than the more moderate temperature regime preferred by *B. columbiana*. The addition of a third individual parameter, food concentration, provides the supplementary information that the width of the temperature isopleth is expanded with increasing food availability which may be important in allowing the larvae to enter the plankton earlier in the season than strictly predicted by temperature considerations alone.

The larvae of *Polydora giardi* are much more stenohaline than the *Boccardia* sp. previously discussed. The salinity terms dominate all other terms in the calculated regression equation (Table 7,8,9) over the entire experimental period. The ambient water temperature plays

far less of a role in controlling the shape of the survival isopleths; the 50% survival isopleth almost always spans the entire experimental temperature range. A very small shift in salinity, however, drastically decreases the predicted survival of the larvae. Less than 1‰ change in salinity is often sufficient to decrease survival by as much as 10%. The growth rate of *P. giardi* is also very dependent on very small alterations in salinity, at the same time mimicing the survival isopleths in their eurythermal nature. A deviation from the optimum salinity contour of less than 2‰ is sufficient to decrease growth by 1 setiger. In order to decrease the growth rate by a similar amount a temperature change of greater than 10 degrees is required.

Food concentration plays very little role in altering the shape of the survival isopleths. An inspection of the response surfaces concerned with survival (Figs. 21-38) indicates very little shift in the isopleths particularly at the moderate and high levels of food provided to the larvae. From this we can infer that if some minimal value of food availability is met, increased amounts of food do not dramatically improve survival. Thorson (1950 p. 15) states that we need "to distinguish between such quantities of food as 1) starve and kill the larvae, 2) allow the larvae to vegetate for some time though without growth and development, and 3) actually support the growth and development of the larvae." The results of starvation, the death of the larvae, is readily apparent and understandable. Life may continue for some time, as stored metablic reserves are consumed, but death inevitably results. Less evident is the effect of substandard rations

on the developing larvae, where the minimum amount necessary for survival is available but little or no energy is left over for growth and development. In this case, the prolonged time necessary to complete the larval period and the consequential increase in the duration of the planktonic existance give rise to an increased likelihood that the larvae will be lost to predation.

If we compare the maximum survival isopleth to the maximum growth isopleth the overlap defines a much smaller region than either of the isopleths do singly. This is true for every level of food concentration and time of experimental duration. The overlap region defines an area almost immediately central to the design space. An area defined by lower temperatures and salinities is able to support the maximum survival rate but at the cost of a decreased growth rate. Conversely, a region of higher temperatures and salinities is able to support the maximum growth rate, but at the expense of lowered survival.

Polydora giardi egg capsules can be found throughout most of the year with the possible exception of the period from late December through early February. This aggrees well with the eurythermal nature of the larvae. The response surfaces predict that variations in salinity away from values representing nearly oceanic salinity would be a much more crucial concern of the planktonic larvae. Smith et. al. (1971) reports that for the Tomales Bay region the most variable conditions of salinity occur during the winter months between December and February, a period of heavy rains and freshwater runoff. Since this period is also normally prior to a spring bloom of phytoplankton observed

in Pacific ocean coastal waters (Anderson, 1964) the concentration of available food could be expected to be low, further decreasing the overall survival chances of the larvae and reducing the growth rate precipitously at the salinity extremes.

The response surfaces generated for the three species of spionid larvae are extremely dissimilar in shape, which suggests a markedly different approach in coping with the extremes and fluctuations of the physical environment. The two *Boccardia* species have in common their broad tolerance to changes in salinity, in marked contrast to the extremely stenohaline larvae of *Polydora giardi*. Their response to temperature, however, clearly seperates them; elevated temperatures promoting excellant growth and survival in *B. proboscidea*, while *B. columbiana* prefers a more moderate water temperature.

Since these species of *Boccardia* are seperable by only a single morphological character, the presence or absence of a conspicuous fasicle of long notosetae on the first setigerous segement, we may question the validity of seperating these spionids into two distinct species. Woodwick (1963) states that the stage at which these long notosetae develop is unknown. One of the *B. columbiana* larvae in my cultures settled at the 17 setiger stage in a clean glass fingerbowl. This individual remained alive for 5 additional weeks in culture and during that time I was able to make periodic observations of its development. Prior to its death, it added 7 additional setigerous segments for a total of 24, but at no time did the fasicle of long notosetae develop on setiger 1. This suggests that the character used for the morphological seperation of these

two species may be plastic, depending on the substratum of settlement. The response surfaces, however, clearly support the two species concept despite the marginal nature of the character used for the morphological seperation of the two species. The marked preference of *B. proboscidea* larvae for higher water temperatures and the poor survival of *B. columbiana* at these same temperatures is most likely the underlying reason for *B. proboscidea's* greater southern range along the California coast.

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TABLE 1

a) ANOVA - *Boccardia proboscidea* 3 DAY % SURVIVAL

b) ANOVA - *Boccardia proboscidea* 6 DAY % SURVIVAL

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

SOURCE	DF	SS	MS	F
REGRESSION	6	31928.36	5321.39	14.83*
MEAN	1	29885.47		
LINEAR TEMP.	1	118.37	118.37	0.33
LINEAR SAL.	1	102.26	102.26	0.28
QUADRATIC TEMP.	1	692.30	692.30	1.93
QUADRATIC SAL.	1	1126.69	1126.69	3.14
TEMP. * SAL.	1	3.28	3.28	0.01
ERROR	3	1076.50	358.83	
TOTAL	9	33004.86		

SOURCE	DF	SS	MS	F
REGRESSION	6	24311.59	4051.93	16.46*
MEAN	1	21079.12		
LINEAR TEMP.	1	1605.24	1605.24	6.52
LINEAR SAL.	1	222.16	222.16	0.90
QUADRATIC TEMP.	1	222.62	222.62	0.90
QUADRATIC SAL.	1	1179.48	1179.48	4.79
TEMP. * SAL.	1	2.96	2.96	0.01
ERROR	3	738.46	246.15	
TOTAL	9	25050.05		

TABLE 2

a) ANOVA - *Boccardia proboscidea* 9 DAY % SURVIVAL

b) ANOVA - *Boccardia proboscidea* 15 DAY % SURVIVAL

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

SOURCE	DF	SS	MS	F
REGRESSION	6	19351.01	3225.17	11.51
MEAN	1	13316.01		
LINEAR TEMP.	1	4383.18	4383.18	15.64
LINEAR SAL.	1	237.64	237.64	0.85
QUADRATIC TEMP.	1	12.59	12.59	0.04
QUADRATIC SAL.	1	1340.12	1340.12	4.78
TEMP. * SAL.	1	61.47	61.47	0.22
ERROR	3	840.93	280.31	
TOTAL	9	20191.93		

SOURCE	DF	SS	MS	F
REGRESSION	6	11538.53	1923.09	12.37*
MEAN	1	6449.91		
LINEAR TEMP.	1	4169.62	4169.62	26.82*
LINEAR SAL.	1	140.46	140.46	0.90
QUADRATIC TEMP.	1	3.15	3.15	0.02
QUADRATIC SAL.	1	517.46	517.46	3.33
TEMP. * SAL.	1	257.92	257.92	1.66
ERROR	3	466.46	155.49	
TOTAL	9	12004.99		

TABLE 3

a) ANOVA - *Boccardia proboscidea* 6 DAY GROWTH SETIGERS

b) ANOVA - *Boccardia proboscidea* 9 DAY GROWTH SETIGERS

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

SOURCE	DF	SS	MS	F
REGRESSION	6	123.06	20.51	316.43***
MEAN	1	117.36		
LINEAR TEMP.	1	4.17	4.17	64.29**
LINEAR SAL.	1	0.17	0.17	2.57
QUADRATIC TEMP.	1	0.88	0.88	13.60*
QUADRATIC SAL.	1	0.23	0.23	3.48
TEMP. * SAL.	1	0.25	0.25	3.86
ERROR	3	0.19	0.06	
TOTAL	9	123.25		

SOURCE	DF	SS	MS	F
REGRESSION	6	267.95	44.66	86.51**
MEAN	1	235.10		
LINEAR TEMP.	1	30.38	30.38	58.84**
LINEAR SAL.	1	0.17	0.17	0.32
QUADRATIC TEMP.	1	0.34	0.34	0.66
QUADRATIC SAL.	1	1.40	1.40	2.72
TEMP. * SAL.	1	0.56	0.56	1.09
ERROR	3	1.55	0.52	
TOTAL	9	269.50		

TABLE 4

a) ANOVA - *Boccardia proboscidea* 12 DAY GROWTH SETIGERS

b) ANOVA - *Boccardia proboscidea* 18 DAY GROWTH SETIGERS

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

SOURCE	DF	SS	MS	F
REGRESSION	6	367.65	61.27	59.22**
MEAN	1	306.23		
LINEAR TEMP.	1	51.04	51.04	49.33**
LINEAR SAL.	1	2.67	2.67	2.58
QUADRATIC TEMP.	1	0.12	0.12	0.12
QUADRATIC SAL.	1	4.53	4.53	4.37
TEMP. * SAL.	1	3.06	3.06	2.96
ERROR	3	3.10	1.03	
TOTAL	9	370.75		

SOURCE	DF	SS	MS	F
REGRESSION	6	516.08	86.01	9.08
MEAN	1	399.94		
LINEAR TEMP.	1	88.17	88.17	9.31
LINEAR SAL.	1	0.67	0.67	0.07
QUADRATIC TEMP.	1	0.49	0.49	0.05
QUADRATIC SAL.	1	24.57	24.57	2.59
TEMP. * SAL.	1	2.25	2.25	0.24
ERROR	3	28.42	9.47	
TOTAL	9	544.50		

TABLE 5

a) ANOVA - *Boccardia columbiana* 3 DAY % SURVIVAL

b) ANOVA - *Boccardia columbiana* 6 DAY % SURVIVAL

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

SOURCE	DF	SS	MS	F
REGRESSION	10	159553.40	15955.34	101.33***
MEAN	1	155601.69		
LINEAR TEMP.	1	505.47	505.47	3.21
LINEAR SAL.	1	1761.98	1761.98	11.19**
LINEAR FOOD	1	457.63	457.63	2.91
QUADRATIC TEMP.	1	210.99	210.99	1.34
QUADRATIC SAL.	1	159.15	159.15	1.01
QUADRATIC FOOD	1	194.75	194.75	1.24
TEMP. * SAL.	1	368.16	368.16	2.34
TEMP. * FOOD	1	244.53	244.53	1.55
SAL. * FOOD	1	49.04	49.04	0.31
ERROR	20	3149.21	157.46	
TOTAL	30	162702.60		

SOURCE	DF	SS	MS	F
REGRESSION	10	80203.24	8020.32	65.78***
MEAN	1	59922.40		
:INEAR TEMP.	1	12005.32	12005.32	98.46***
LINEAR SAL.	1	391.98	391.98	3.21
LINEAR FOOD	1	807.53	807.53	6.62**
QUADRATIC TEMP.	1	5394.32	5394.32	44.24***
QUADRATIC SAL.	1	498.45	498.45	4.09
QUADRATIC FOOD	1	92.08	92.08	0.76
TEMP. * SAL.	1	96.48	96.48	0.79
TEMP. * FOOD	1	288.58	288.58	2.37
SAL. * FOOD	1	706.10	706.10	5.79*
ERROR	20	2438.51	121.93	
TOTAL	30	82641.75		

TABLE 6

a) ANOVA - *Boccardia columbiana* 9 DAY % SURVIVAL

b) ANOVA - *Boccardia columbiana* 15 DAY % SURVIVAL

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

SOURCE	DF	SS	MS	F
REGRESSION	10	53348.89	5334.89	29.46***
MEAN	1	35176.99		
LINEAR TEMP.	1	9225.75	9225.75	50.94***
LINEAR SAL.	1	41.84	41.84	0.23
LINEAR FOOD	1	1229.77	1229.77	6.79*
QUADRATIC TEMP.	1	6102.60	6102.60	33.69***
QUADRATIC SAL.	1	127.22	127.22	0.70
QUADRATIC FOOD	1	357.37	357.37	1.97
TEMP. * SAL.	1	64.16	64.16	0.35
TEMP. * FOOD	1	546.86	546.86	3.02
SAL. * FOOD	1	476.33	476.33	2.63
ERROR	20	3622.31	181.12	
TOTAL	30	56971.20		

SOURCE	DF	SS	MS	F
REGRESSION	10	14041.01	1404.10	6.83***
MEAN	1	5276.54		
LINEAR TEMP.	1	681.45	681.45	3.31
LINEAR SAL.	1	1394.54	1394.54	6.78*
LINEAR FOOD	1	2385.64	2385.64	11.60**
QUADRATIC TEMP.	1	3089.55	3089.55	15.02***
QUADRATIC SAL.	1	127.21	127.21	0.62
QUADRATIC FOOD	1	138.97	138.97	0.68
TEMP. * SAL.	1	142.86	142.86	0.69
TEMP. * FOOD	1	661.39	661.39	3.22
SAL. * FOOD	1	142.86	142.86	0.69
ERROR	20	4112.95	205.65	
TOTAL	30	18153.96		

TABLE 7

a) ANOVA - *Polydora giardi* 3 DAY % SURVIVAL

b) ANOVA - *Polydora giardi* 6 DAY % SURVIVAL

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

SOURCE	DF	SS	MS	F
REGRESSION	10	153986.95	15398.70	146.62***
MEAN	1	143644.67		
LINEAR TEMP.	1	56.44	56.44	0.54
LINEAR SAL.	1	3453.45	3453.45	32.88***
LINEAR FOOD	1	1146.91	1146.91	10.92**
QUADRATIC TEMP.	1	75.08	75.08	0.71
QUADRATIC SAL.	1	2365.37	2365.37	22.52***
QUADRATIC FOOD	1	280.73	280.73	2.67
TEMP. * SAL.	1	265.93	265.93	2.53
TEMP. * FOOD	1	338.10	338.10	3.22
SAL. * FOOD	1	2360.26	2360.26	22.47***
ERROR	20	2100.45	105.02	
TOTAL	30	156087.40		

SOURCE	DF	SS	MS	F
REGRESSION	10	99463.64	9946.36	72.46***
MEAN	1	80038.08		
LINEAR TEMP.	1	2.89	2.89	0.02
LINEAR SAL.	1	10021.71	10021.71	73.01***
LINEAR FOOD	1	399.78	399.78	2.91
QUADRATIC TEMP.	1	1025.83	1025.83	7.47*
QUADRATIC SAL.	1	7168.78	7168.78	52.23***
QUADRATIC FOOD	1	4.03	4.03	0.02
TEMP. * SAL.	1	69.01	69.01	0.50
TEMP. * FOOD	1	66.46	66.46	0.48
SAL. * FOOD	1	667.06	667.06	4.86*
ERROR	20	2745.28	137.26	
TOTAL	30	102208.91		

TABLE 8

a) ANOVA - *Polydora giardi* 9 DAY % SURVIVAL

b) ANOVA - *Polydora giardi* 15 DAY % SURVIVAL

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

SOURCE	DF	SS	MS	F
REGRESSION	10	86281.14	8628.11	124.31***
MEAN	1	61091.75		
LINEAR TEMP.	1	21.74	21.74	0.31
LINEAR SAL.	1	14204.17	14204.17	204.65***
LINEAR FOOD	1	42.16	42.16	0.61
QUADRATIC TEMP.	1	456.01	456.01	6.57*
QUADRATIC SAL.	1	9879.24	9879.24	142.33***
QUADRATIC FOOD	1	2.43	2.43	0.04
TEMP. * SAL.	1	132.99	132.99	1.92
TEMP. * FOOD	1	38.04	38.04	0.55
SAL. * FOOD	1	412.60	412.60	5.95*
ERROR	20	1388.13	69.41	
TOTAL	30	87669.32		

SOURCE	DF	SS	MS	F
REGRESSION	10	40840.80	4084.08	14.29***
MEAN	1	25892.61		
LINEAR TEMP.	1	185.57	185.57	0.65
LINEAR SAL.	1	3615.36	3615.36	12.65**
LINEAR FOOD	1	1134.33	1134.33	3.97
QUADRATIC TEMP.	1	648.25	648.25	2.67
QUADRATIC SAL.	1	7719.51	7719.51	27.01***
QUADRATIC FOOD	1	933.65	933.65	3.27
TEMP. * SAL.	1	477.42	477.42	1.67
TEMP. * FOOD	1	2.45	2.45	0.01
SAL. * FOOD	1	231.65	231.65	0.81
ERROR	20	5715.32	285.77	
TOTAL	30	46556.12		

TABLE 9

a) ANOVA - *Polydora giardi* 18 DAY % SURVIVAL

b) ANOVA - *Polydora giardi* 27 DAY % SURVIVAL

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

SOURCE	DF	SS	MS	F
REGRESSION	10	40248.09	4024.81	51.68***
MEAN	1	22644.03		
LINEAR TEMP.	1	311.58	311.58	4.00
LINEAR SAL.	1	3813.39	3813.39	48.96***
LINEAR FOD	1	417.92	417.92	5.37*
QUADRATIC TEMP.	1	1682.16	1682.16	21.60***
QUADRATIC SAL.	1	10287.87	10287.87	132.09***
QUADRATIC FOOD	1	271.73	271.73	3.49
TEMP. * SAL.	1	683.43	683.43	8.78**
TEMP. * FOOD	1	23.35	23.35	0.30
SAL. * FOOD	1	112.63	112.63	1.45
ERROR	20	1557.67	77.88	
TOTAL	30	41805.76		

SOURCE	DF	SS	MS	F
REGRESSION	10	25334.98	2533.50	17.13***
MEAN	1	12307.12		
LINEAR TEMP.	1	161.70	161.70	1.09
LINEAR SAL.	1	1166.59	1166.59	7.89*
LINEAR FOOD	1	465.09	465.09	3.15
QUADRATIC TEMP.	1	1508.24	1508.24	10.20**
QUADRATIC SAL.	1	8717.22	8717.22	58.95***
QUADRATIC FOOD	1	498.23	498.23	3.37
TEMP. * SAL.	1	506.93	506.93	3.43
TEMP. * FOOD	1	1.93	1.93	0.01
SAL. * FOOD	1	1.93	1.93	0.01
ERROR	20	2957.63	147.88	
TOTAL	30	28292.61		

TABLE 10

a) ANOVA - *Polydora giardi* 9 DAY GROWTH SETIGERS

b) ANOVA - *Polydora giardi* 18 DAY GROWTH SETIGERS

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

SOURCE	DF	SS	MS	F
REGRESSION	10	491.72	49.17	49.87***
MEAN	1	442.36		
LINEAR TEMP.	1	0.05	0.05	0.05
LINEAR SAL.	1	0.60	0.60	0.60
LINEAR FOOD	1	9.23	9.23	9.36**
QUADRATIC TEMP.	1	13.77	13.77	13.96**
QUADRATIC SAL.	1	25.10	25.10	25.46***
QUADRATIC FOOD	1	0.08	0.08	0.09
TEMP. * SAL.	1	0.04	0.04	0.04
TEMP. * FOOD	1	0.00	0.00	0.00
SAL. * FOOD	1	0.49	0.49	0.50
ERROR	20	19.72	0.99	
TOTAL	30	511.44		

SOURCE	DF	SS	MS	F
REGRESSION	10	1213.68	121.37	20.28***
MEAN	1	853.31		
LINEAR TEMP.	1	2.92	2.92	0.49
LINEAR SAL.	1	0.73	0.73	0.12
LINEAR FOOD	1	50.20	50.20	8.39**
QUADRATIC TEMP.	1	35.38	35.38	5.91*
QUADRATIC SAL.	1	254.36	254.36	42.51***
QUADRATIC FOOD	1	15.59	15.59	2.61
TEMP. * SAL.	1	0.09	0.09	0.02
TEMP. * FOOD	1	0.09	0.09	0.02
SAL. * FOOD	1	1.00	1.00	0.17
ERROR	20	119.68	5.98	
TOTAL	30	1333.36		

TABLE 11

a) ANOVA - *Polydora giardi* 27 DAY GROWTH SETIGERS

* $0.01 < p < 0.05$

** $0.001 < p < 0.01$

*** $p < 0.001$

SOURCE	DF	SS	MS	F
REGRESSION	10	2234.08	223.41	23.40***
MEAN	1	1333.30		
LINEAR TEMP.	1	1.18	1.18	0.12
LINEAR SAL.	1	0.22	0.22	0.02
LINEAR FOOD	1	75.23	75.23	7.88*
QUADRATIC TEMP.	1	10.53	10.53	1.10
QUADRATIC SAL.	1	699.37	699.37	73.25***
QUADRATIC FOOD	1	113.33	113.33	11.87**
TEMP. * SAL.	1	0.30	0.30	0.03
TEMP. * FOOD	1	0.30	0.30	0.03
SAL. * FOOD	1	0.30	0.30	0.03
ERROR	20	190.96	9.55	
TOTAL	30	2425.04		

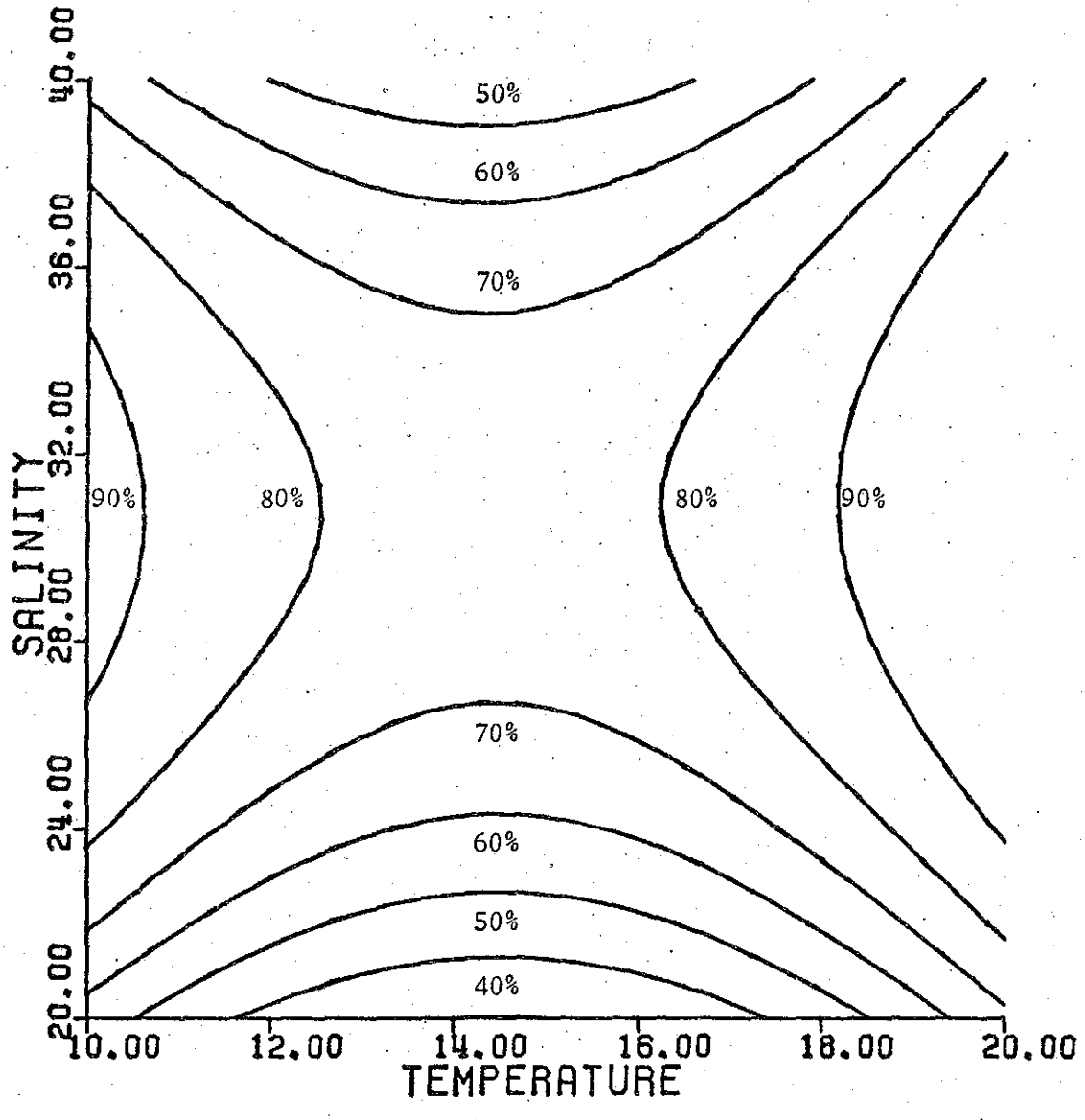

FIGURE 1

BOCCARDIA PROBOSCIDEA

3 DAY SURVIVAL ISOPLETHS

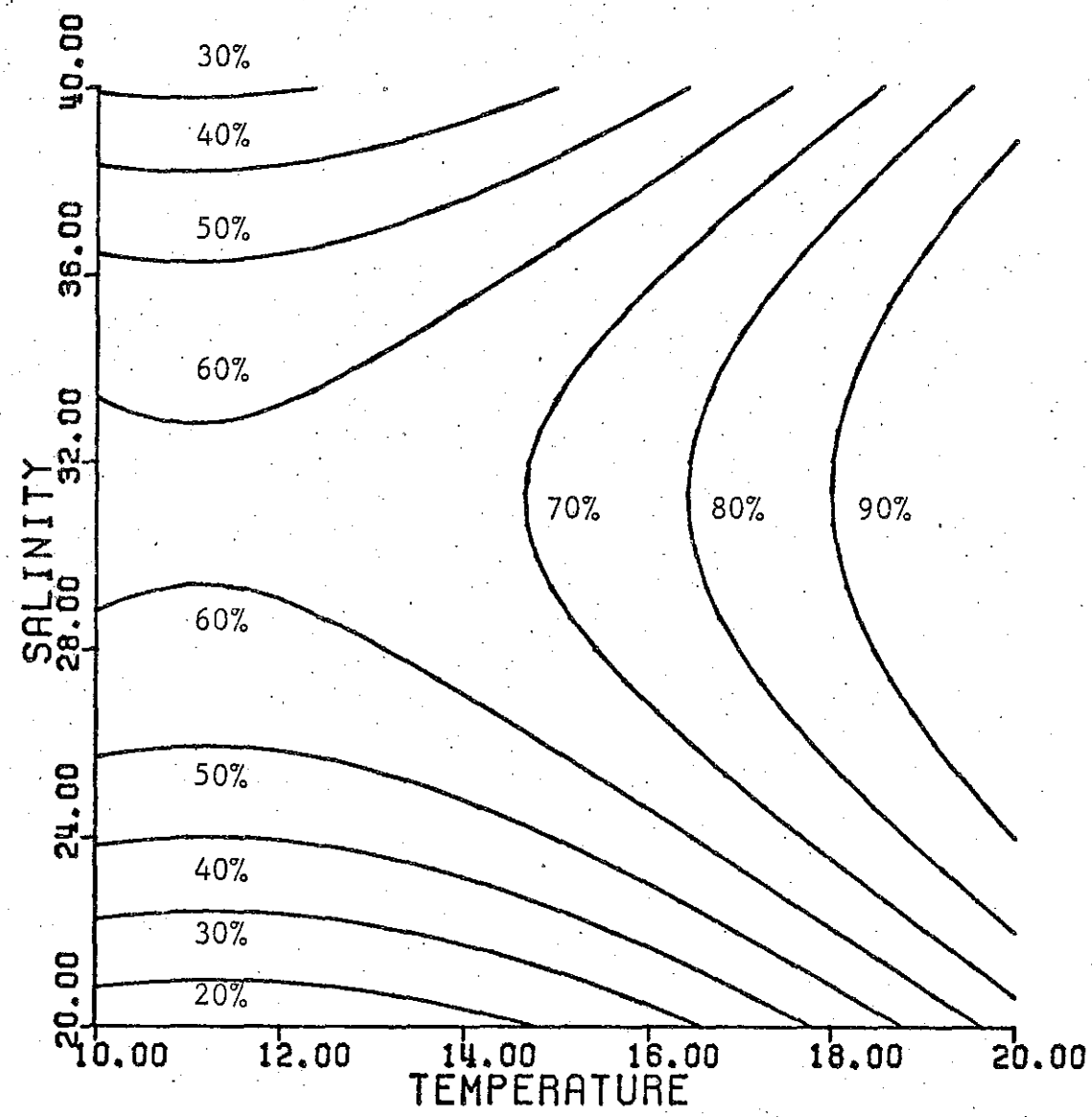

FIGURE 2

BØCCARDIA PRØBØSCIDEA

6 DAY SURVIVAL ISØPLETHS

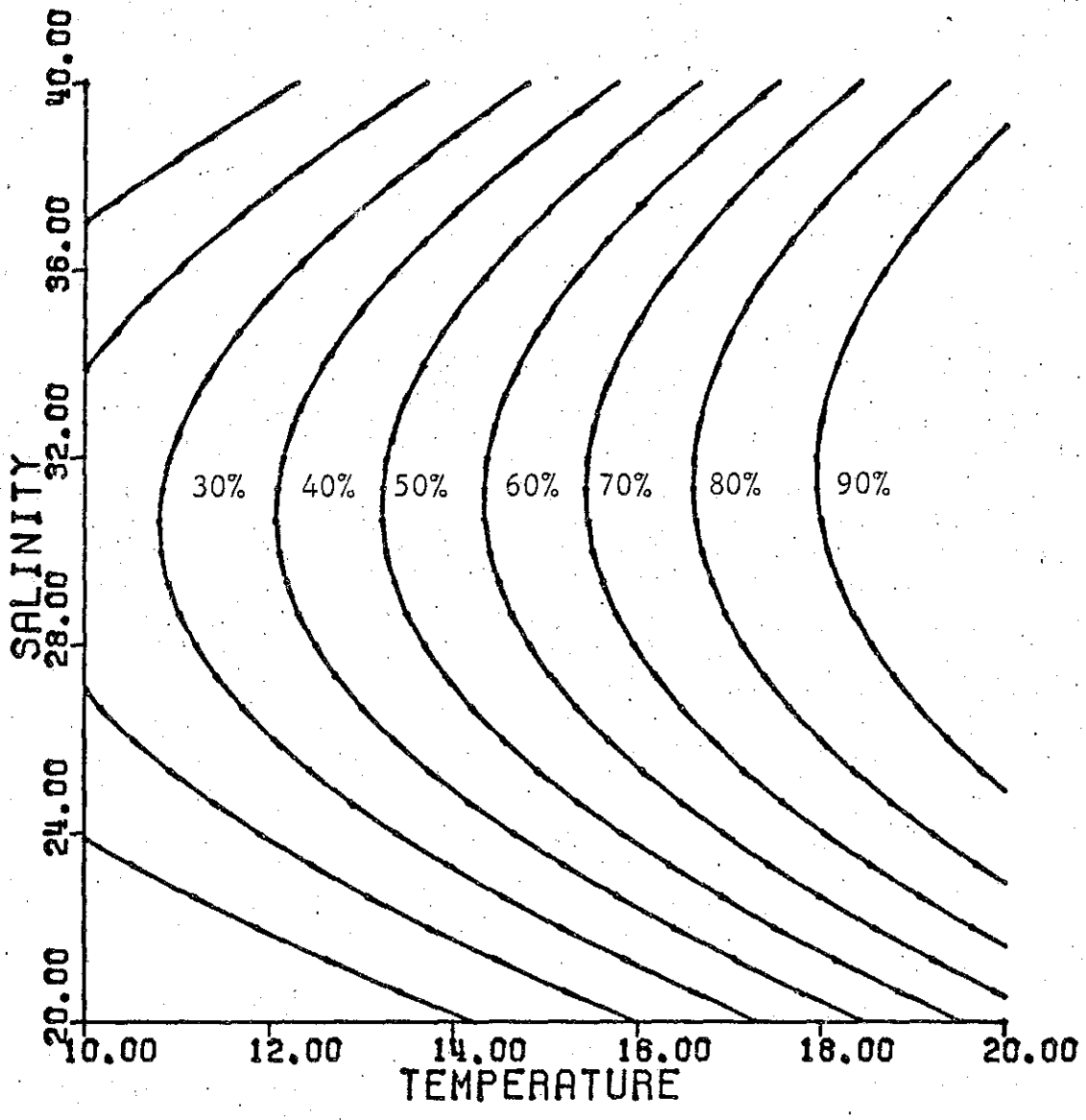

FIGURE 3

BOCCARDIA PROBOSCIDEA

9 DAY SURVIVAL ISOPLETHS

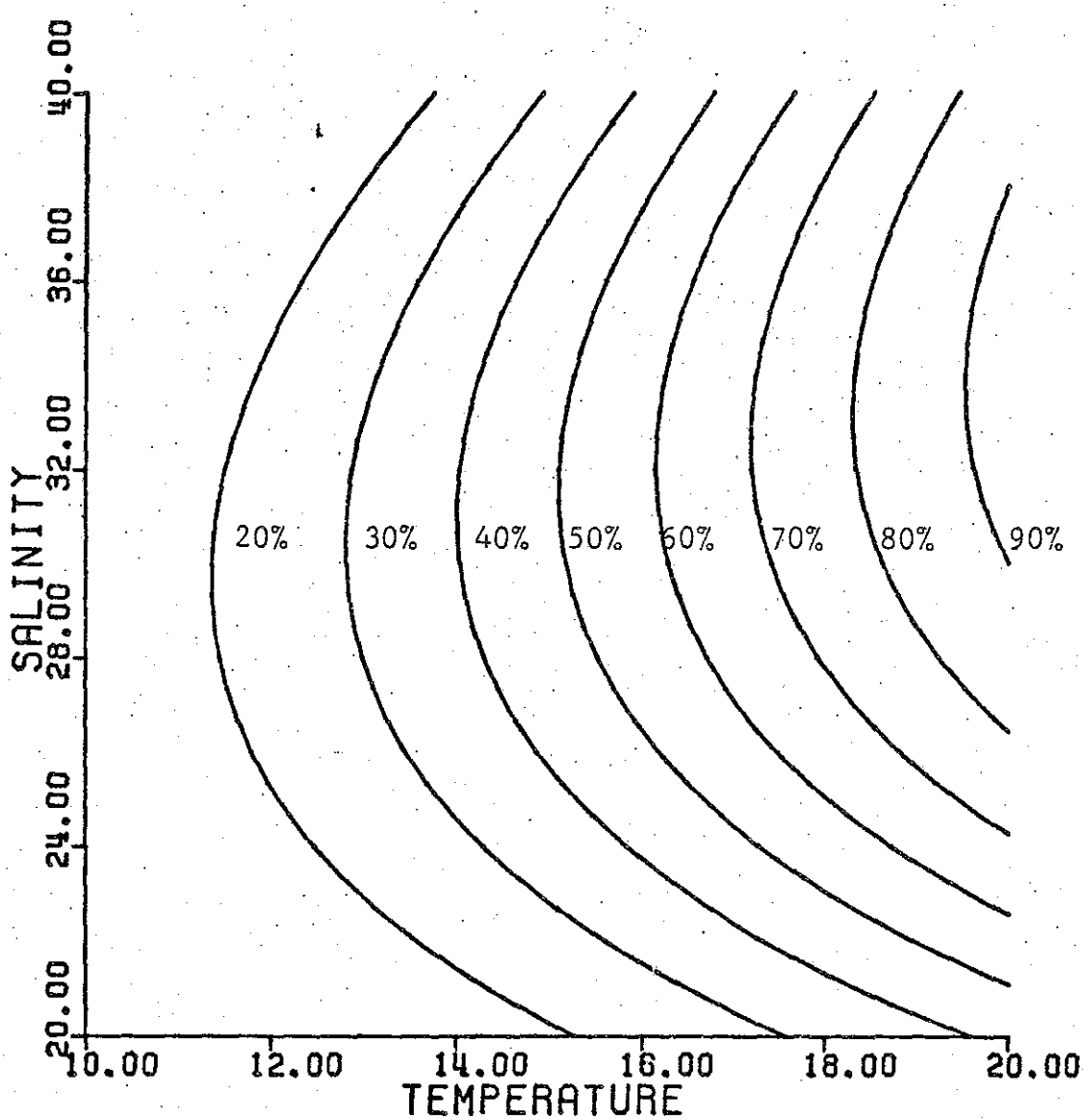

FIGURE 4

BOCCARDIA PROBOSCIDEA

15 DAY SURVIVAL ISOPLETHS

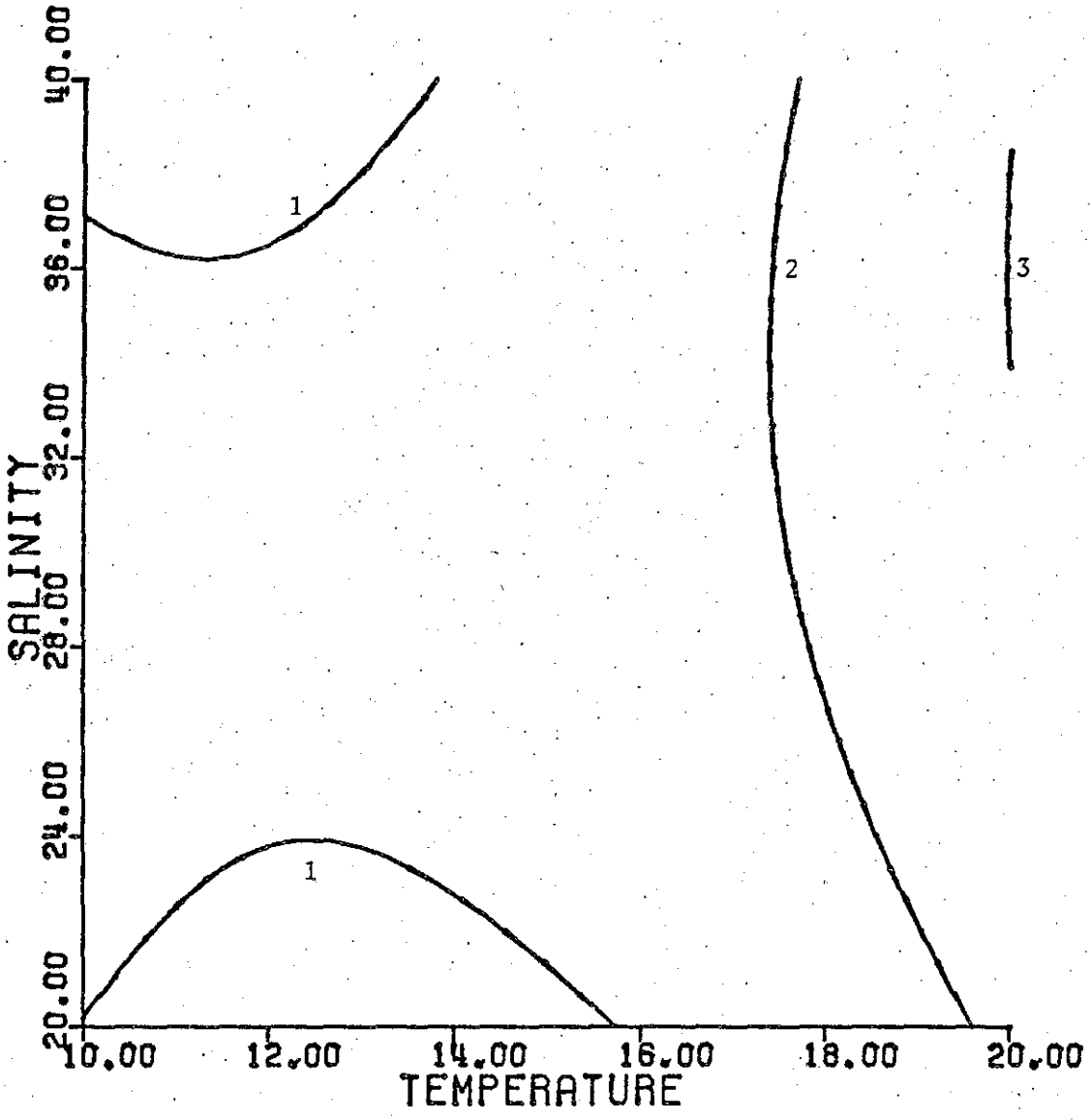

FIGURE 5

BOCCARDIA PROBOSCIDEA

6 DAY GROWTH (SETIGERS)

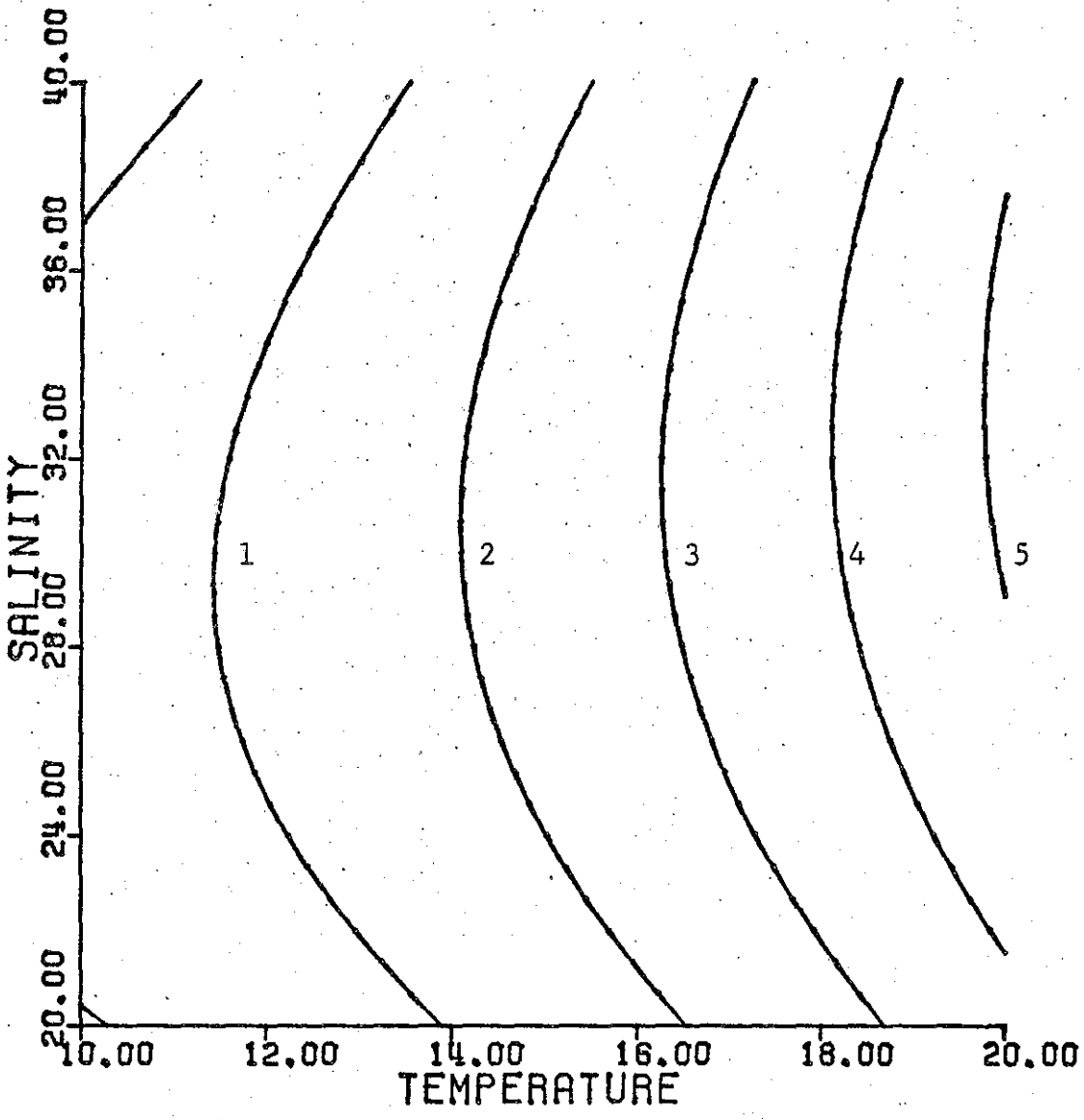

FIGURE 6

BOCCARDIA PROBOSCIDEA

9 DAY GROWTH (SETIGERS)

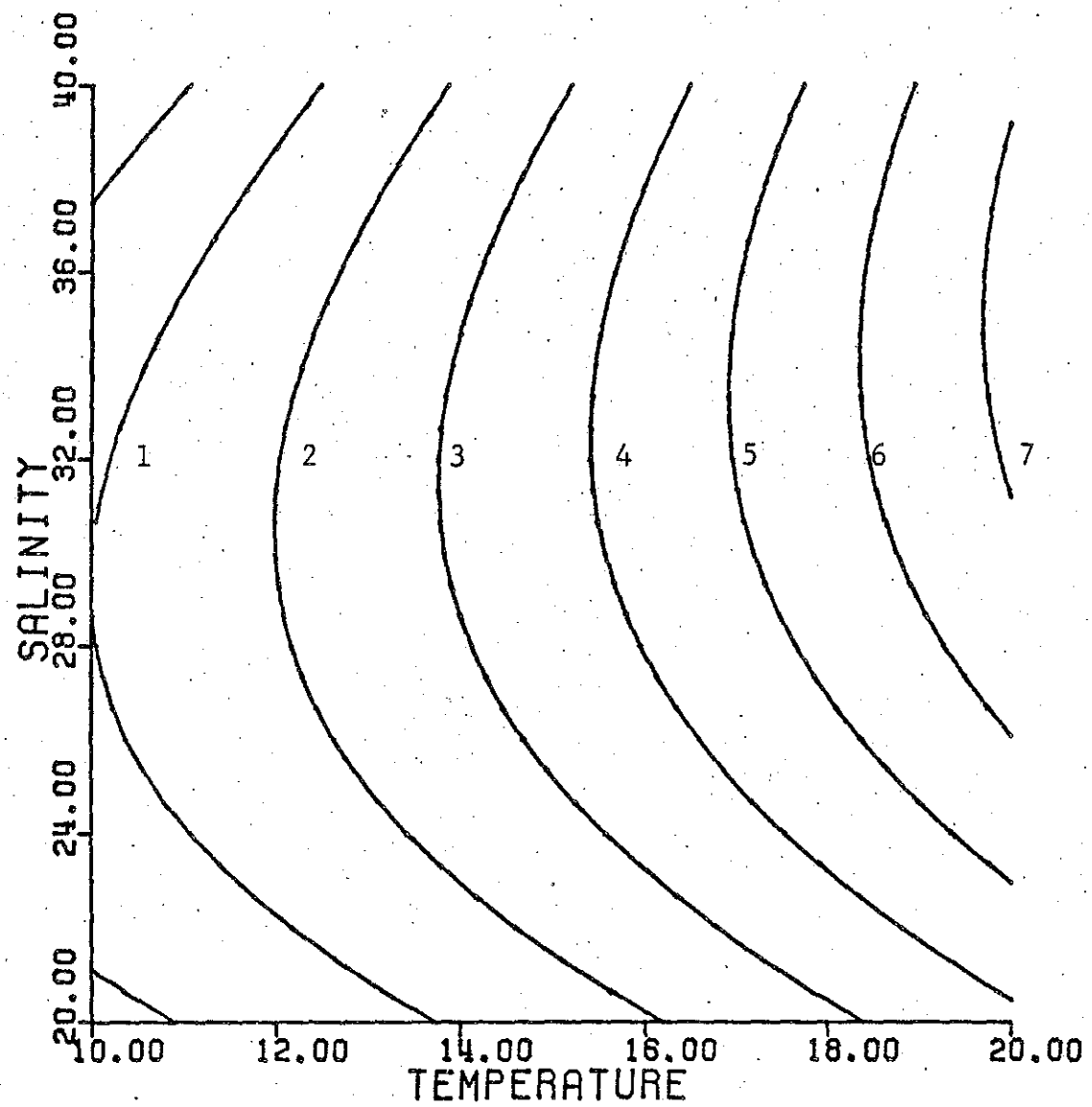

FIGURE 7

BØCCARDIA PRØBØSCIDEA

12 DAY GRØWTH (SETIGERS)

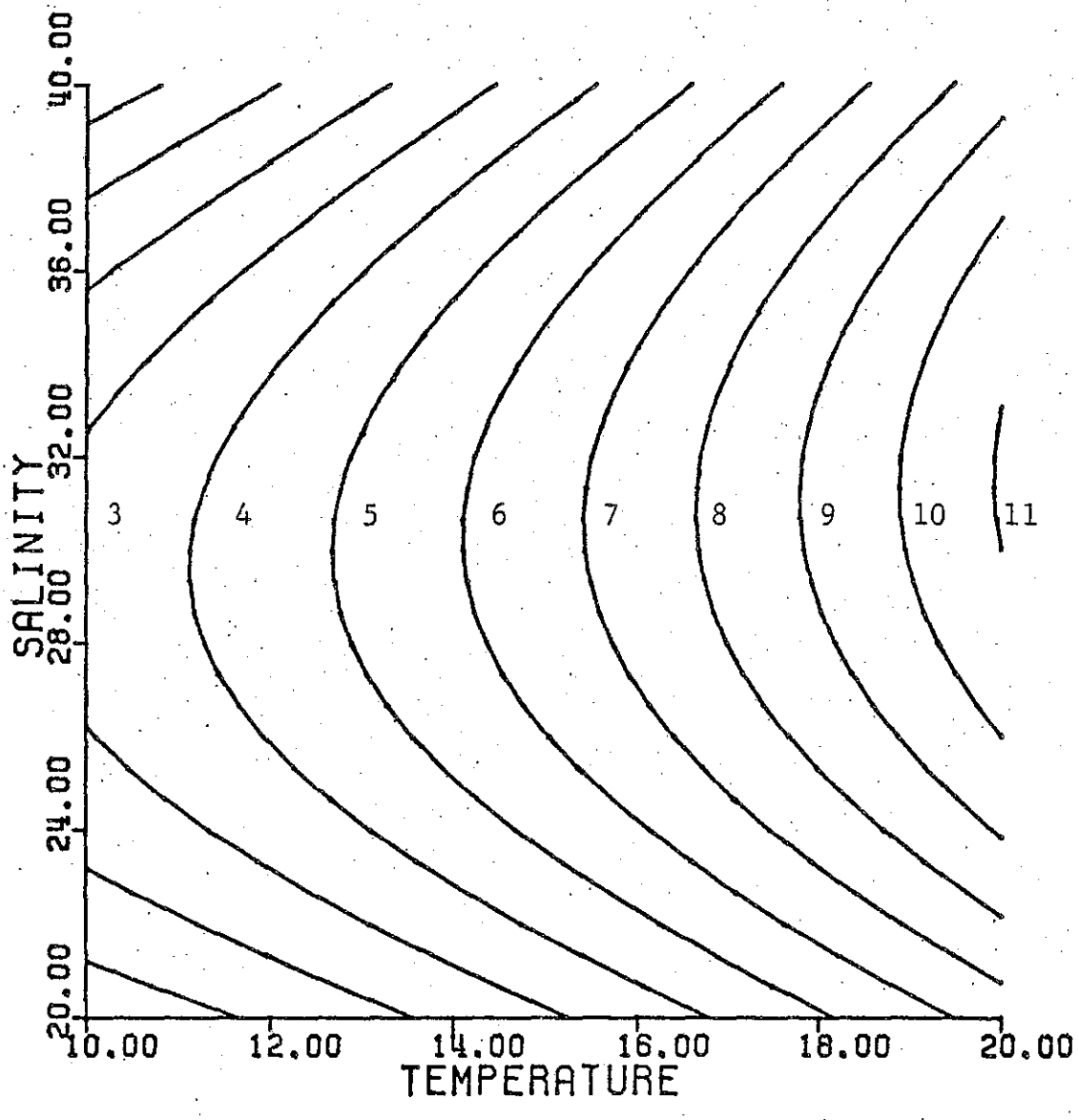

FIGURE 8

BOCCARDIA PROBOSCIDEA

18 DAY GROWTH (SETIGERS)

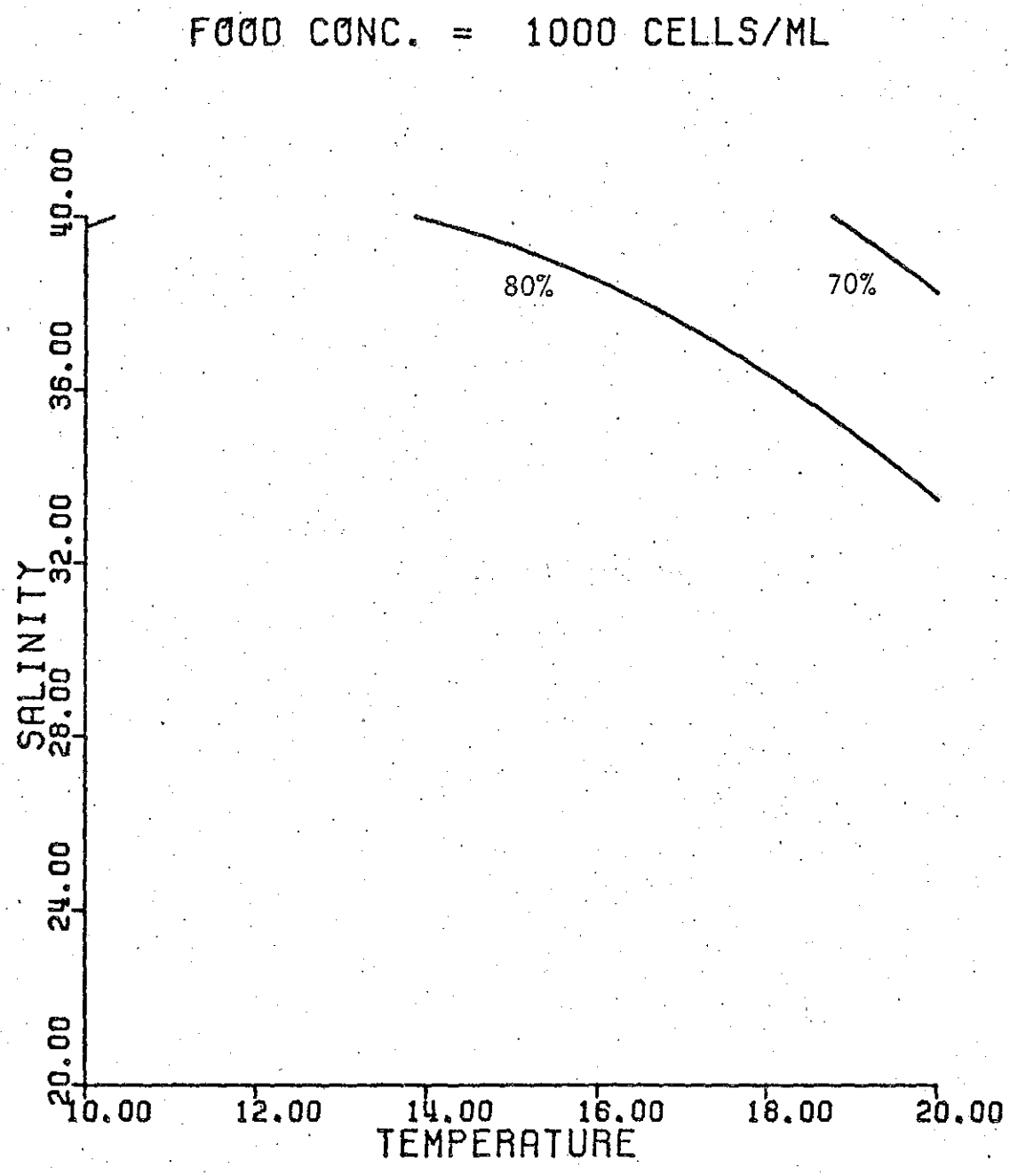

FIGURE 9

BOCCARDIA COLUMBIANA

3 DAY SURVIVAL ISOPLETHS

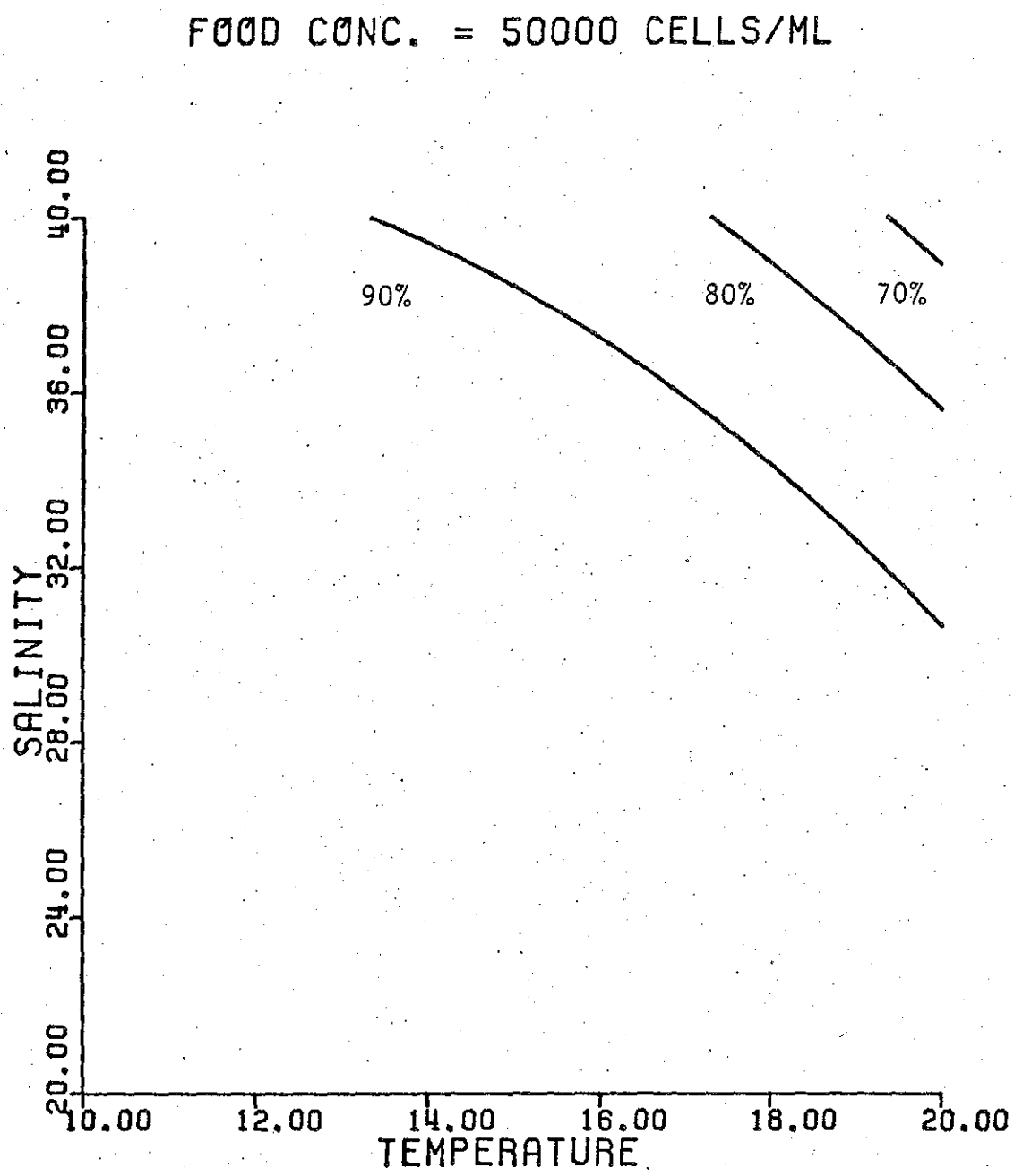

FIGURE 10

BOCCARDIA COLUMBIANA

3 DAY SURVIVAL ISOPLETHS

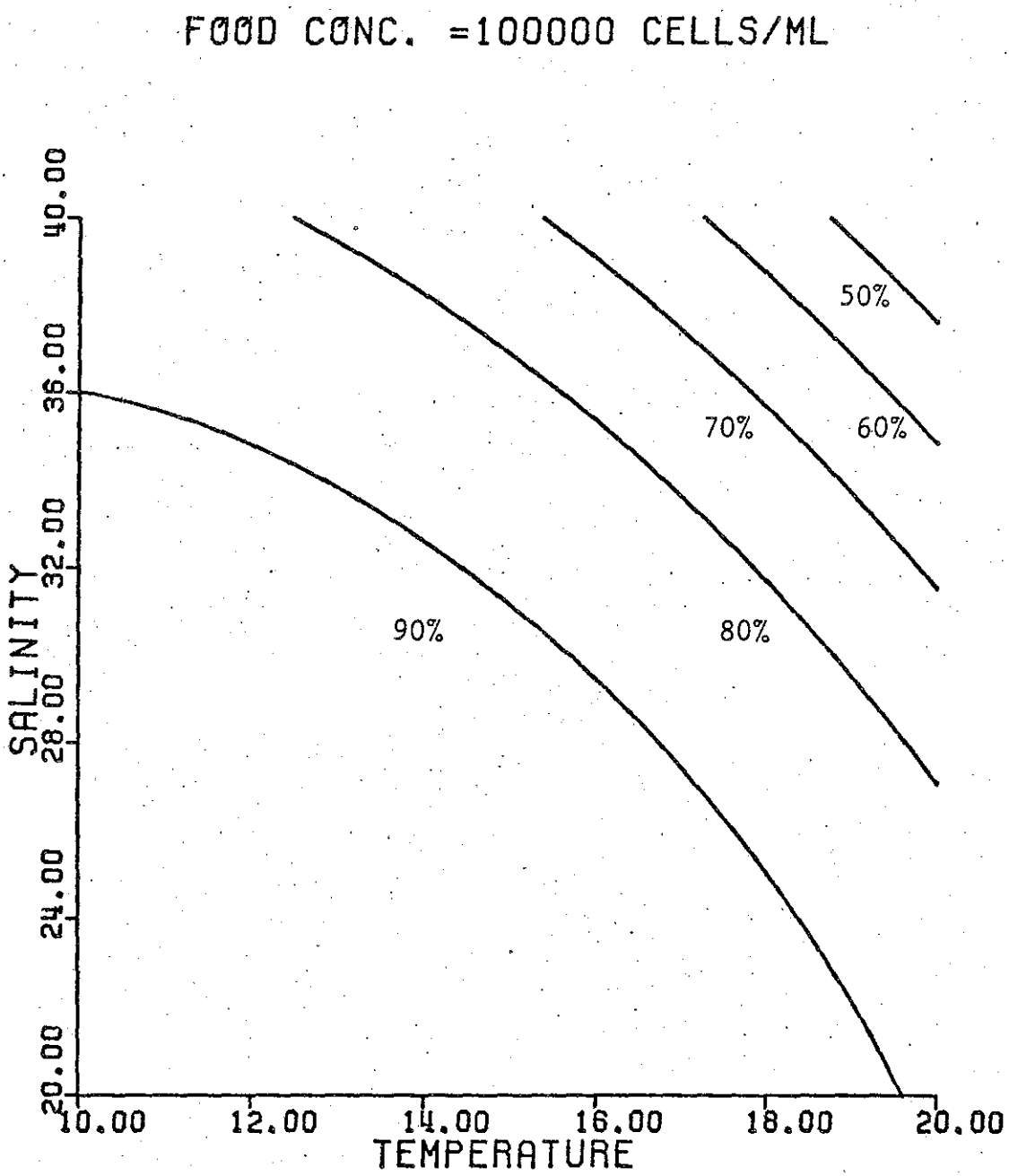

FIGURE 11

BØCCARDIA CØLUMBIANA

3 DAY SURVIVAL ISØPLETHS

FØØD CØNC. = 1000 CELLS/ML

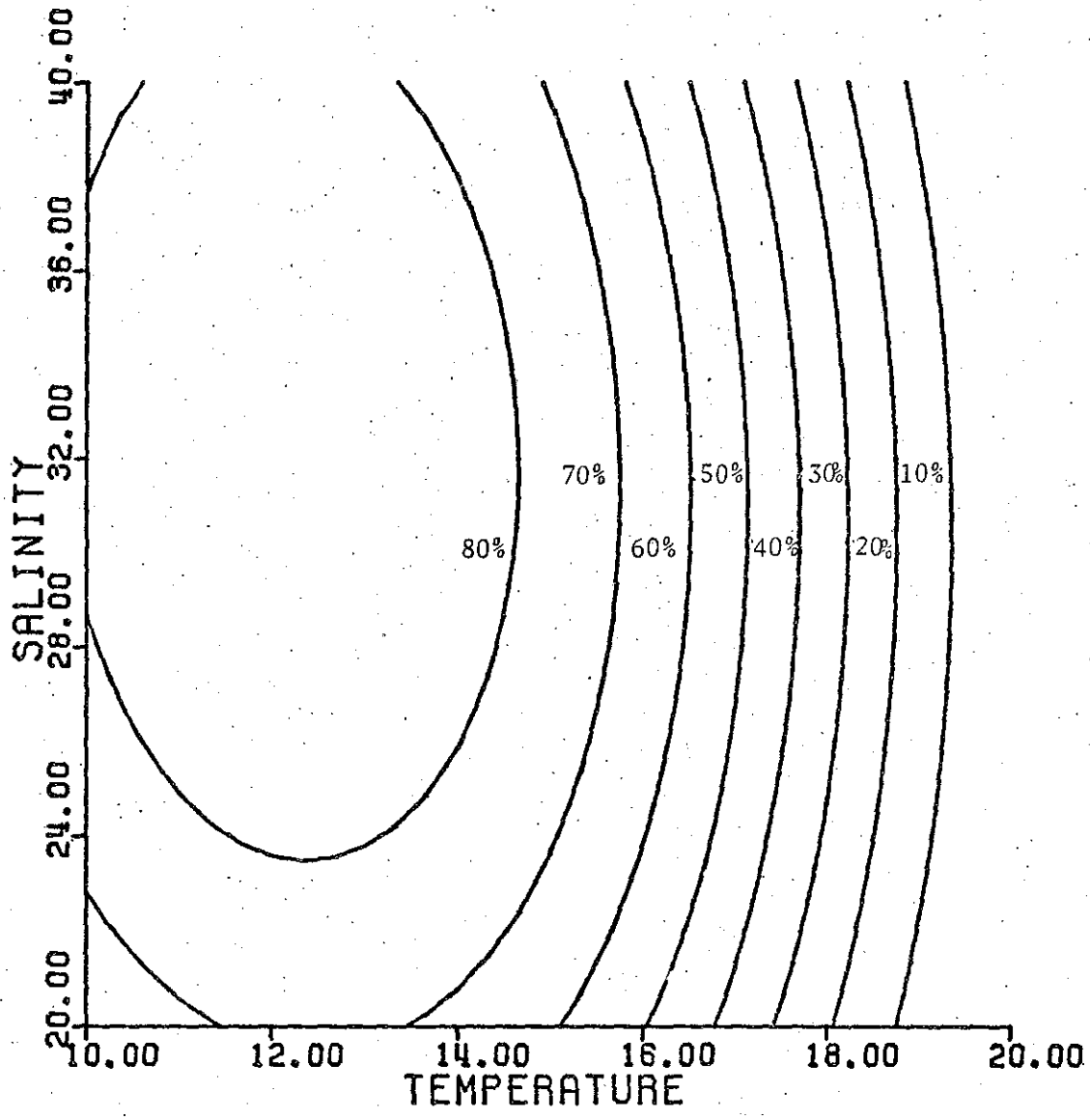

FIGURE 12

BØCCARDIA CØLUMBIANA

6 DAY SURVIVAL ISØPLETHS

FØØD CØNC. = 50000 CELLS/ML

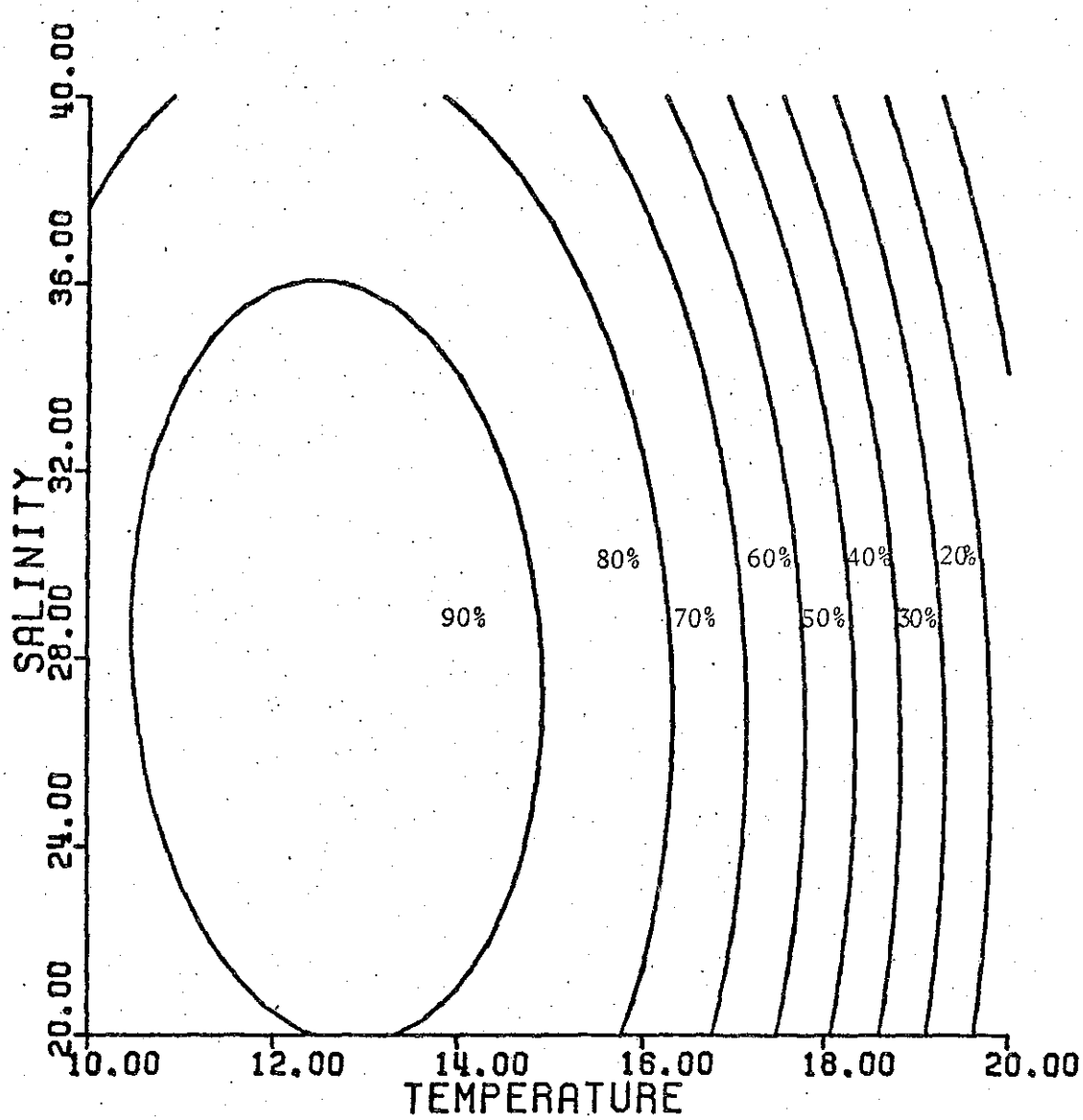

FIGURE 13

BØCCARDIA CØLUMBIANA

6 DAY SURVIVAL ISØPLETHS

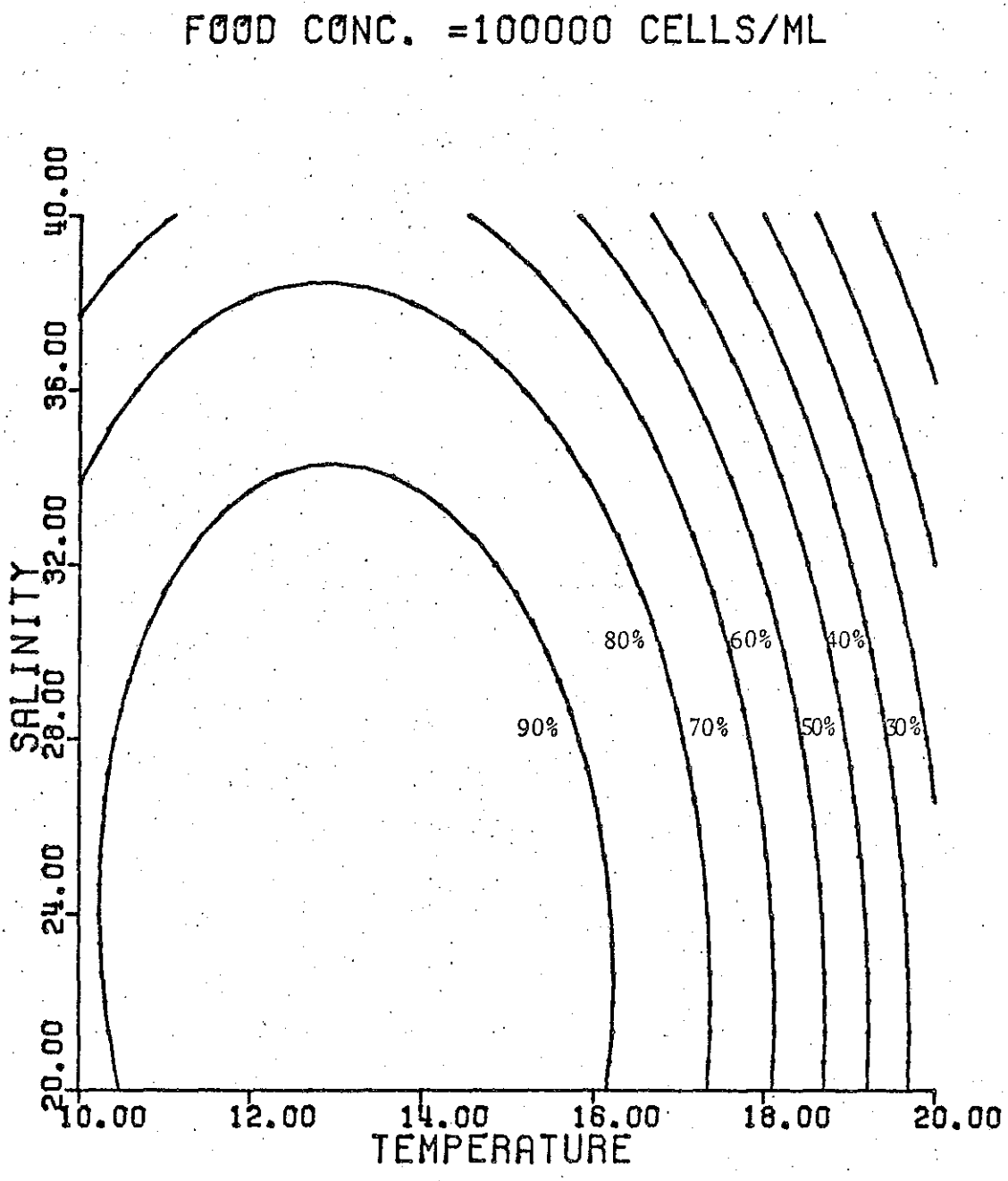

FIGURE 14

BOCCARDIA COLUMBIANA

6 DAY SURVIVAL ISOPLETHS

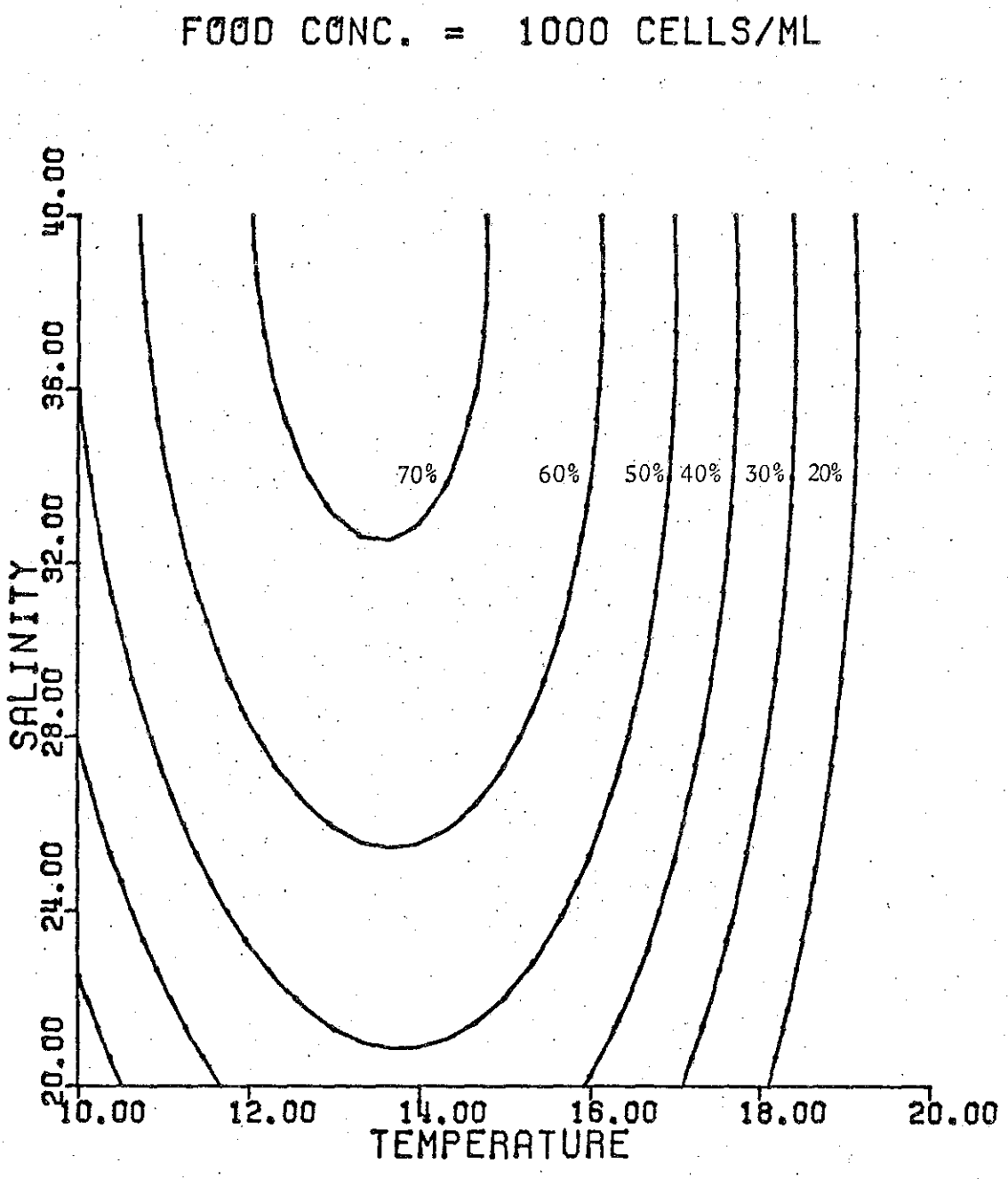

FIGURE 15

BOCCARDIA COLUMBIANA

9 DAY SURVIVAL ISOPLETHS

FOOD CONC. = 50000 CELLS/ML

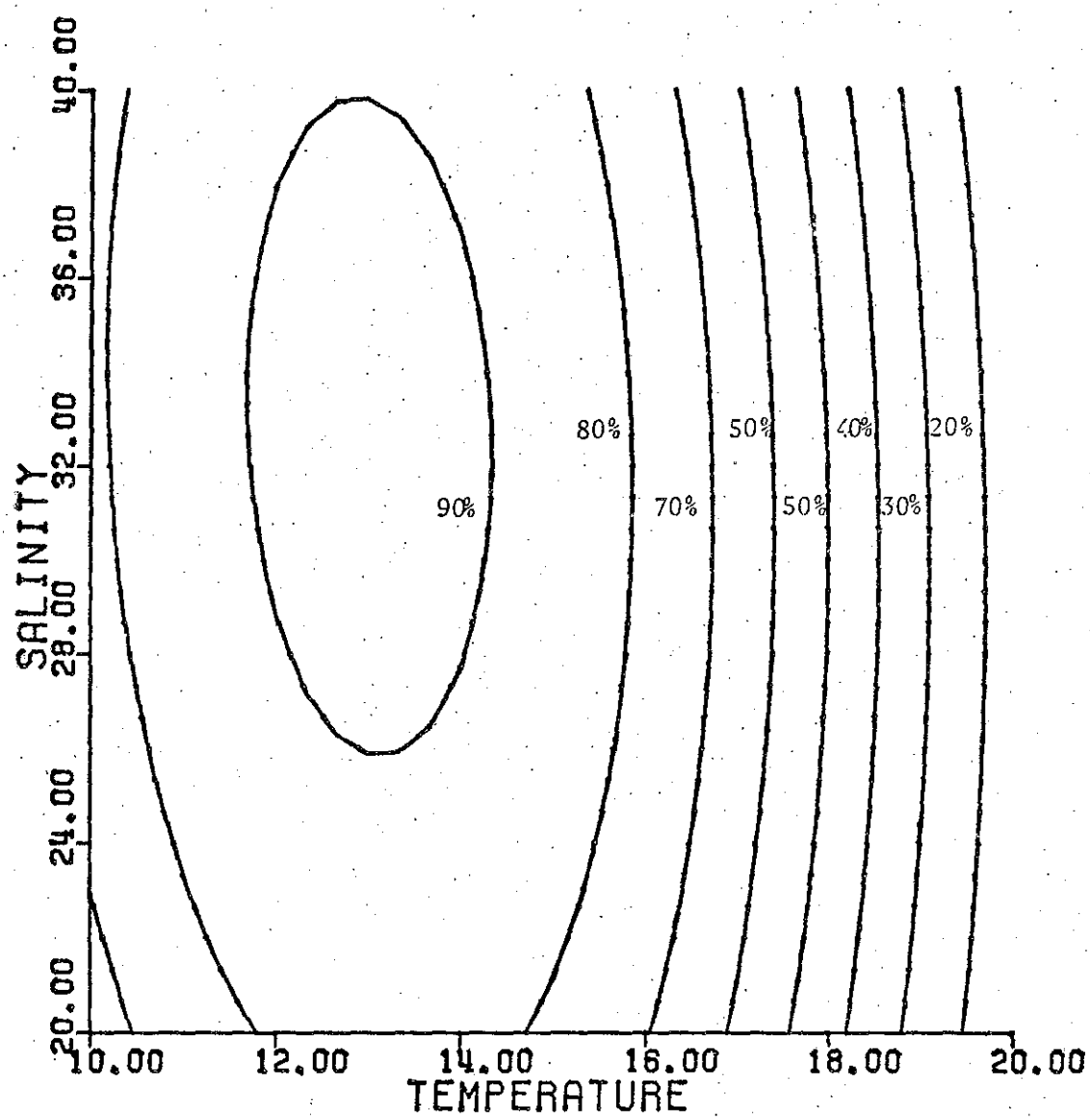

FIGURE 16

BOCCARDIA COLUMBIANA

9 DAY SURVIVAL ISOPLETHS

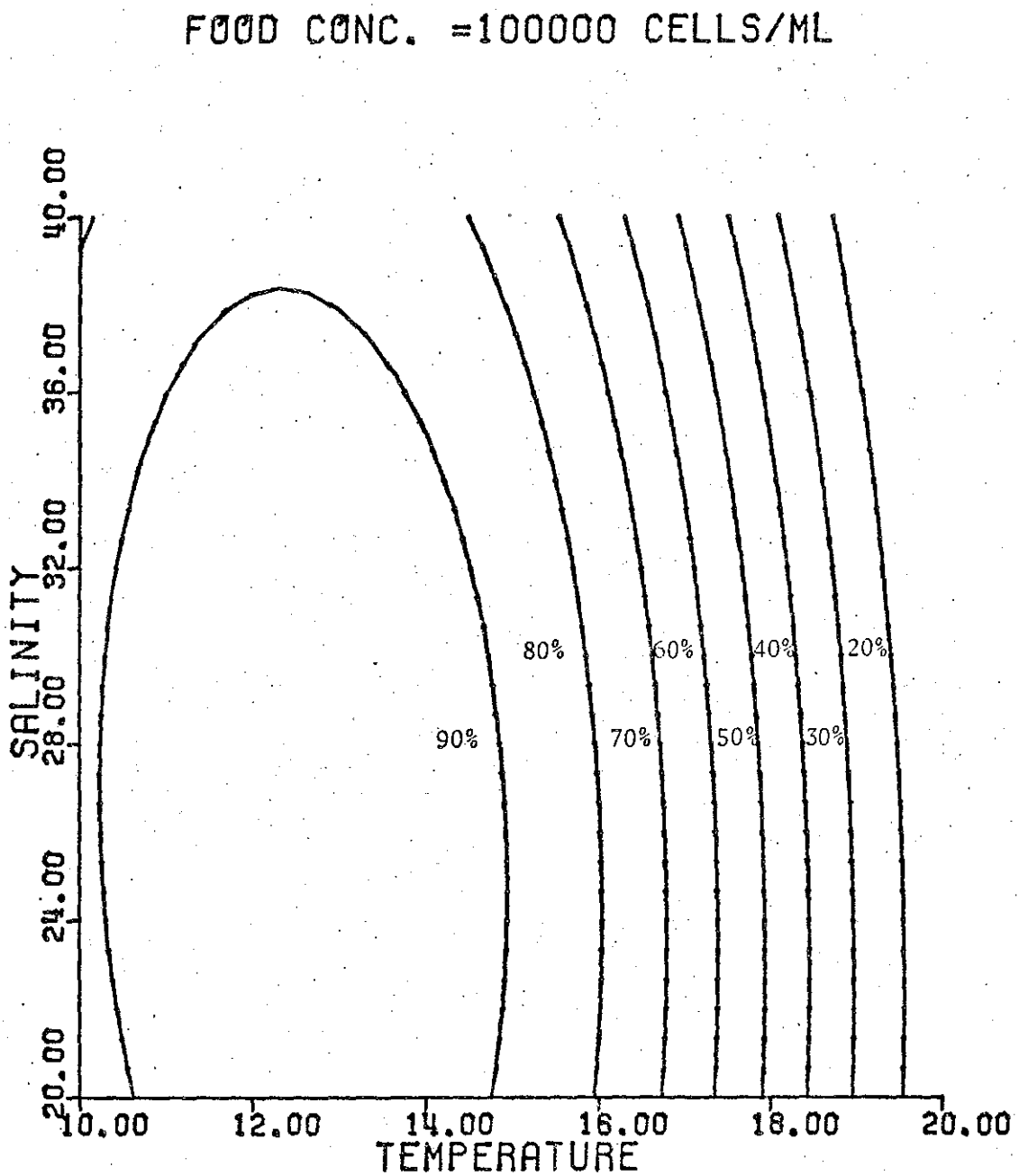

FIGURE 17

BOCCARDIA COLUMBIANA

9 DAY SURVIVAL ISOPLETHS

FØØD CØNC. = 1000 CELLS/ML

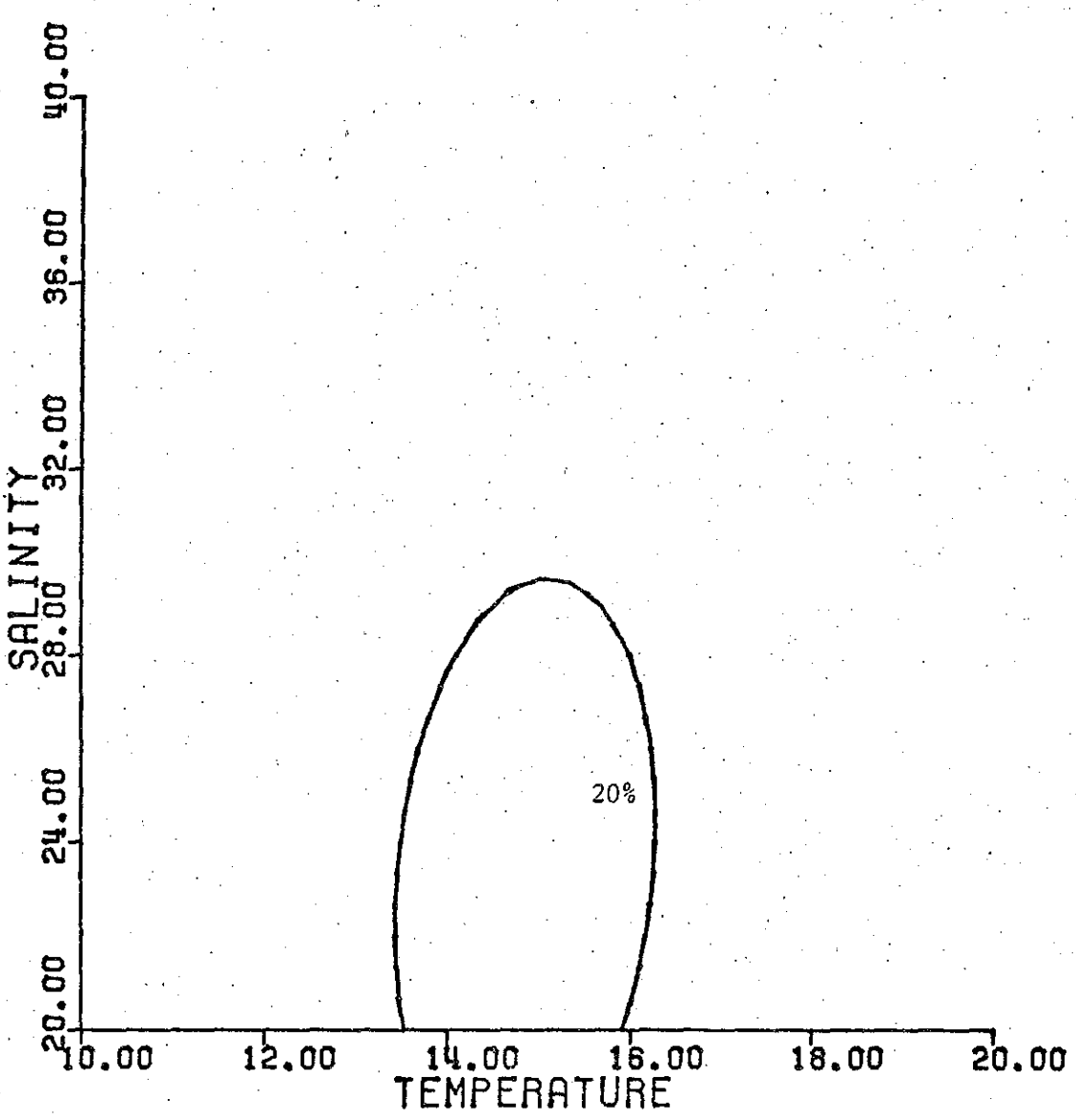

FIGURE 18

BØCCARDIA CØLUMBIANA

15 DAY SURVIVAL ISØPLETHS

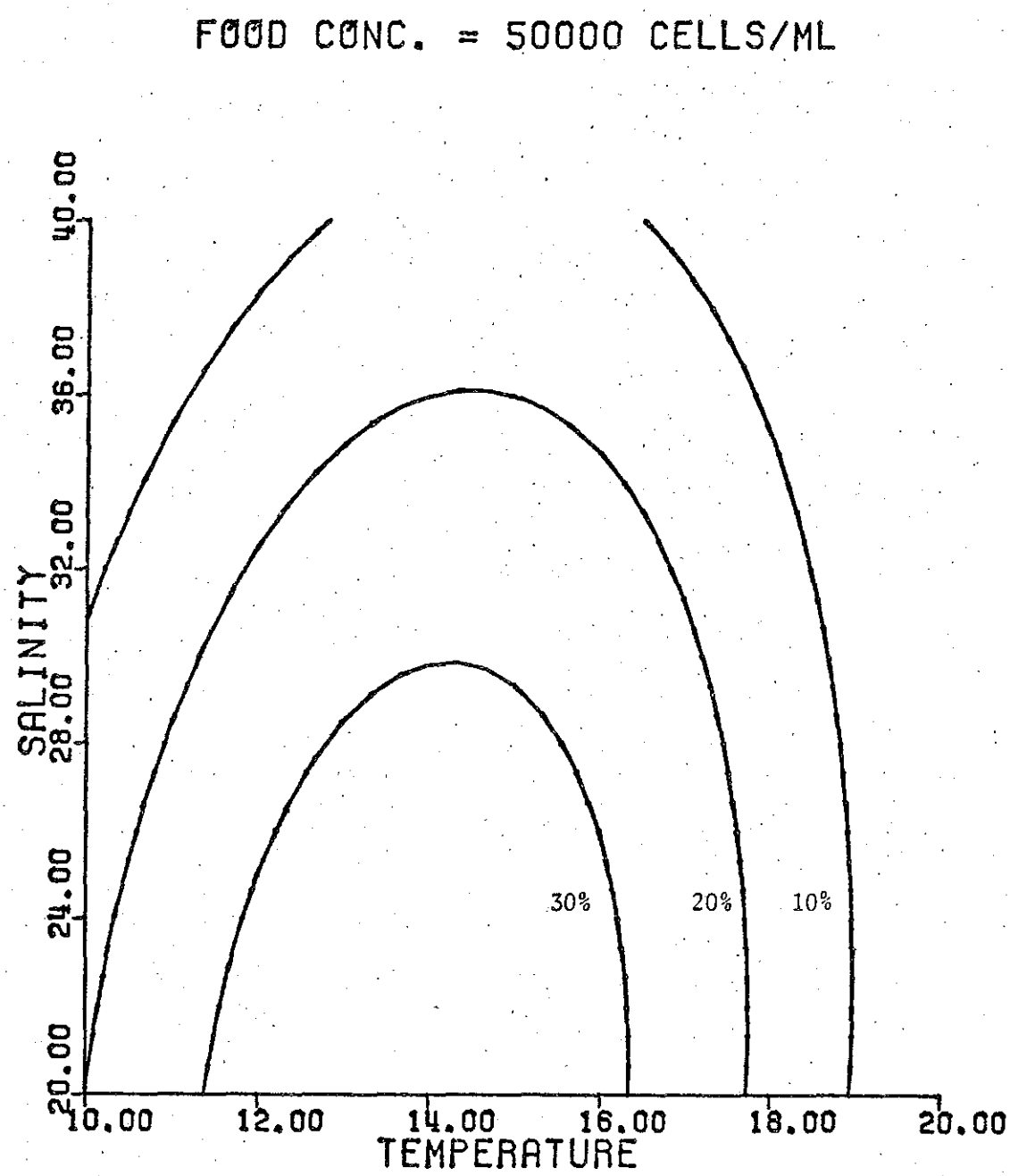

FIGURE 19

BØCCARDIA CØLUMBIANA

15 DAY SURVIVAL ISØPLETHS

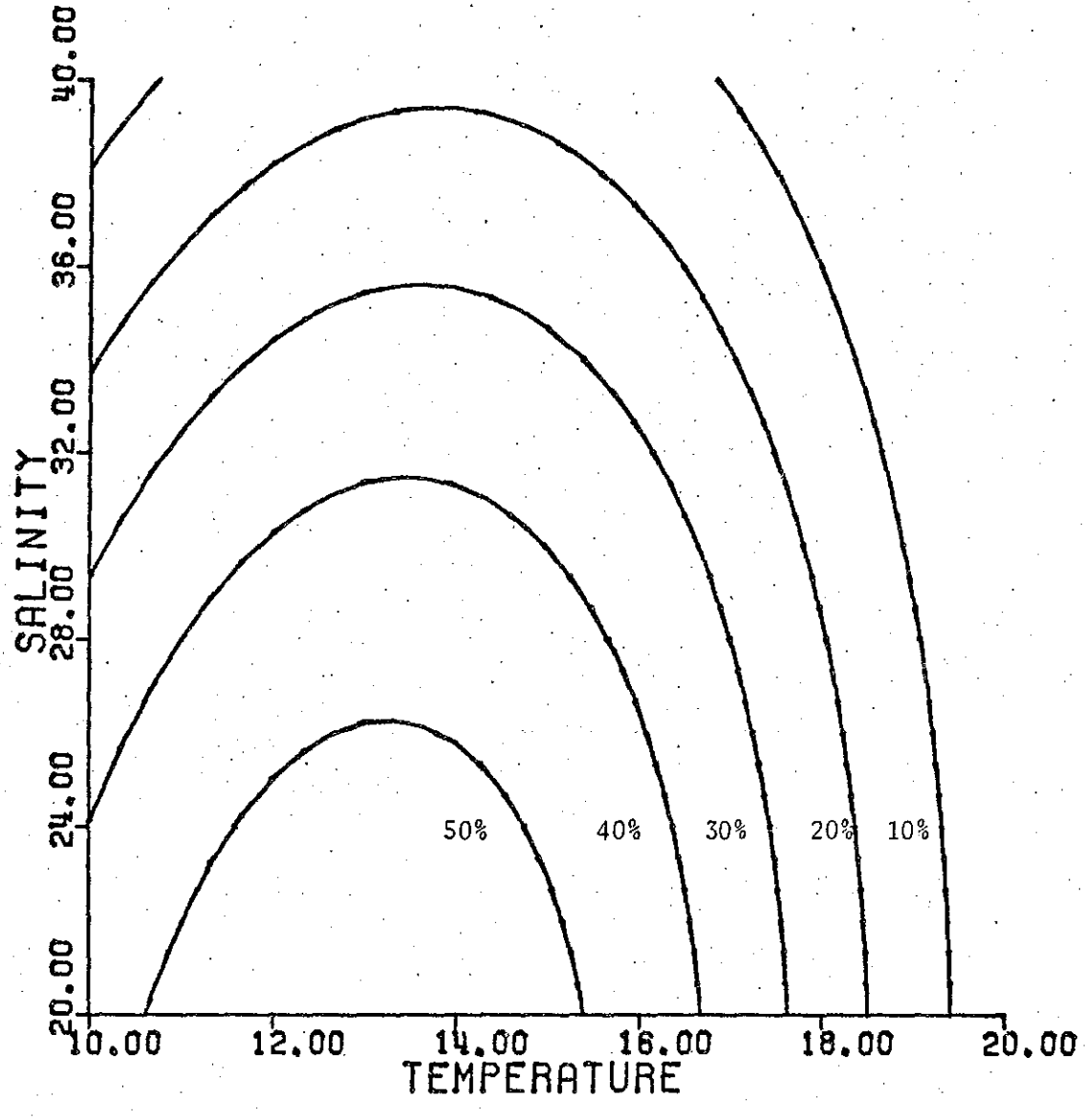

FIGURE 20

BØCCARDIA CØLUMBIANA

15 DAY SURVIVAL ISØPLETHS

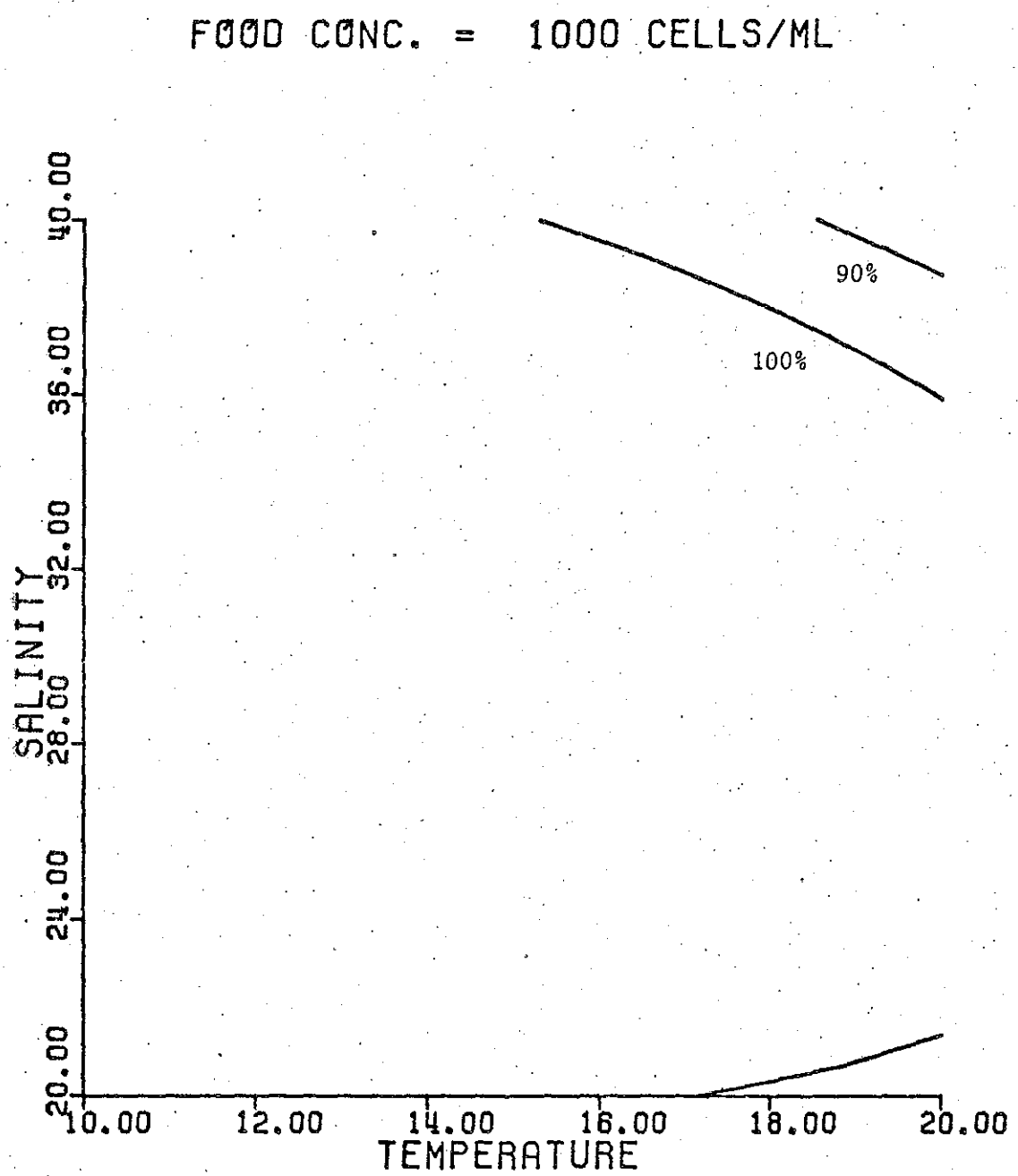

FIGURE 21

POLYDORA GIARDI

3 DAY SURVIVAL ISOPLETHS

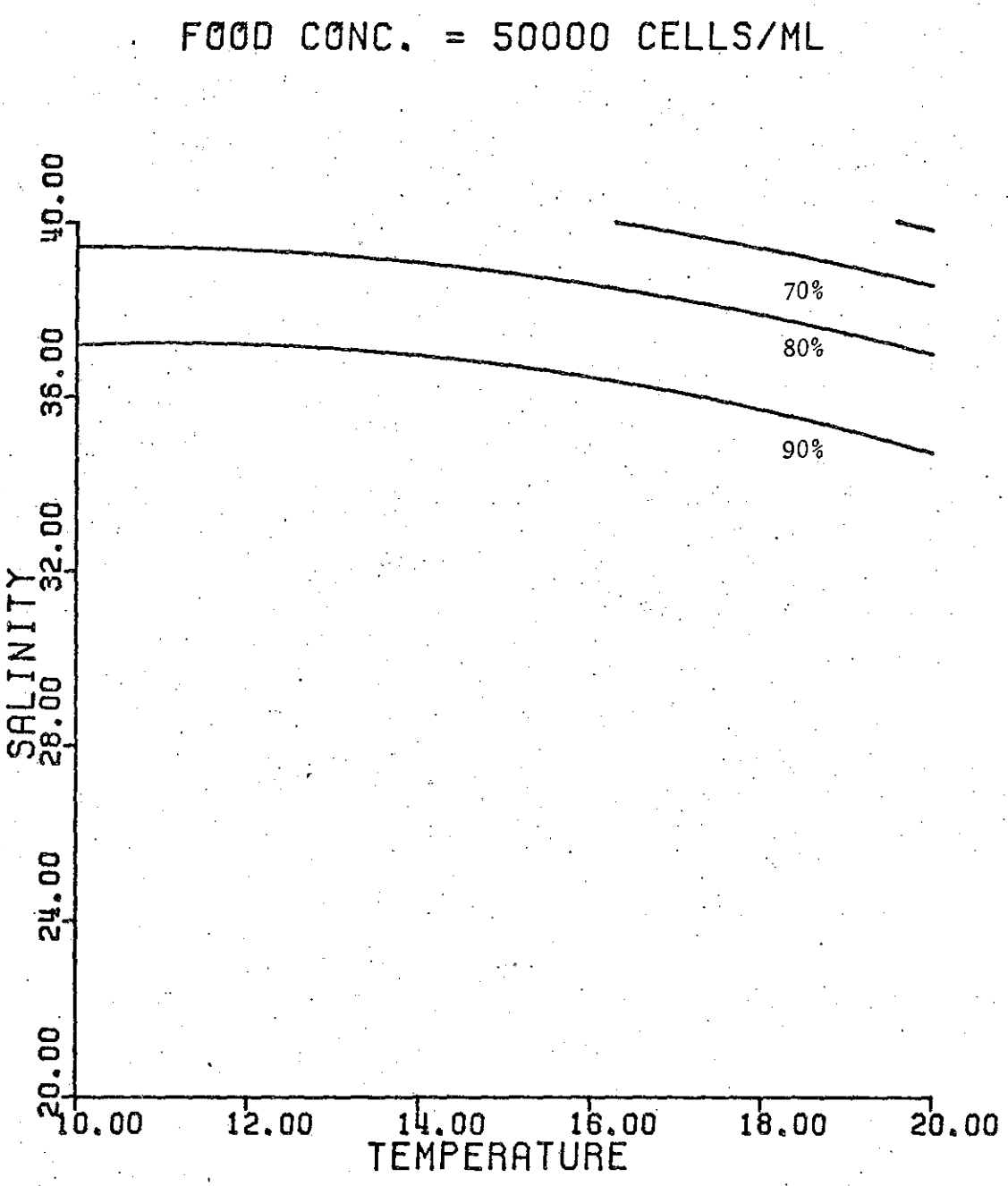

FIGURE 22

POLYDORA GIARDI

3 DAY SURVIVAL ISOPLETHS

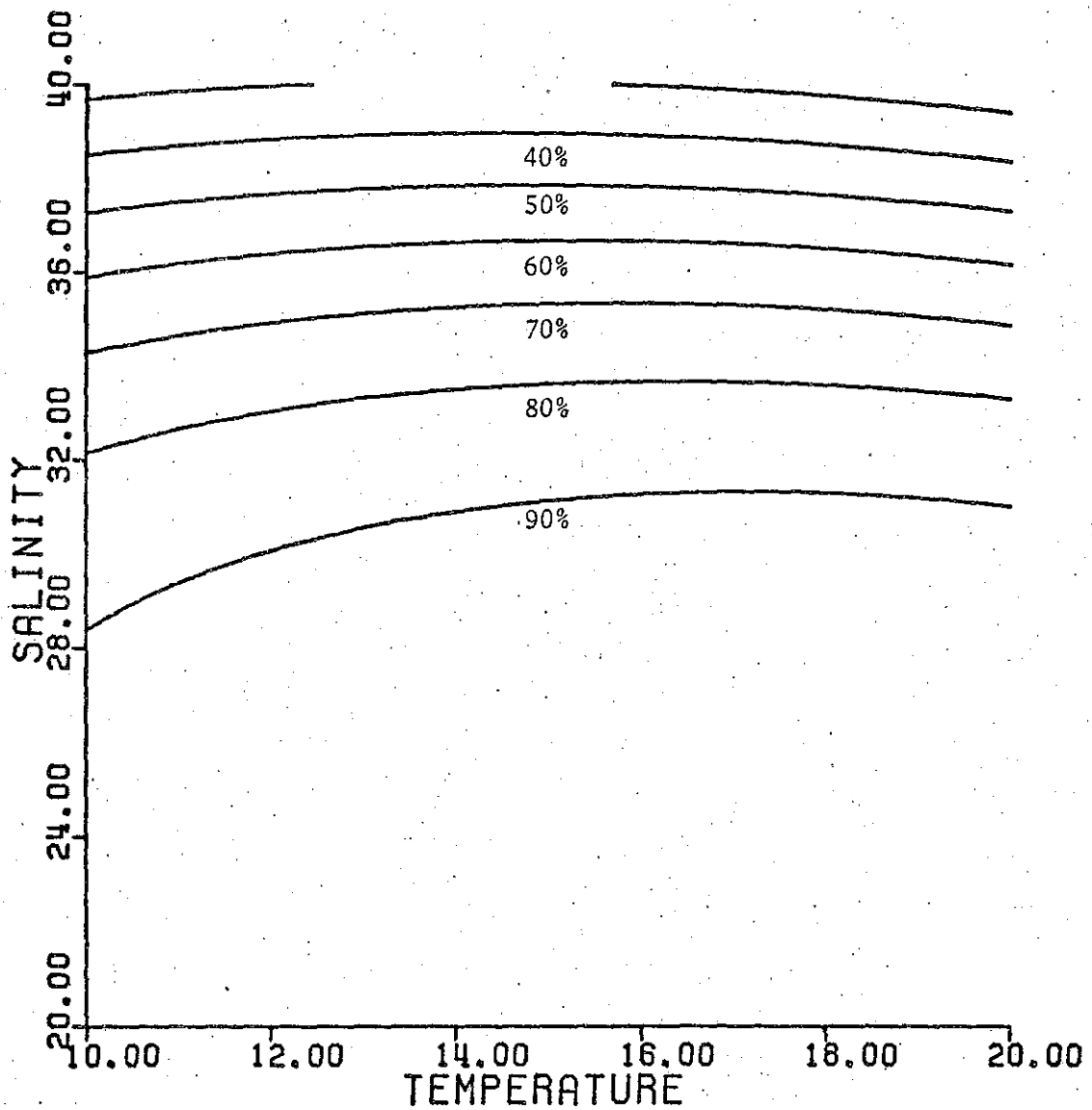

FIGURE 23

PØLYDØRA GIARDI

3 DAY SURVIVAL ISØPLETHS

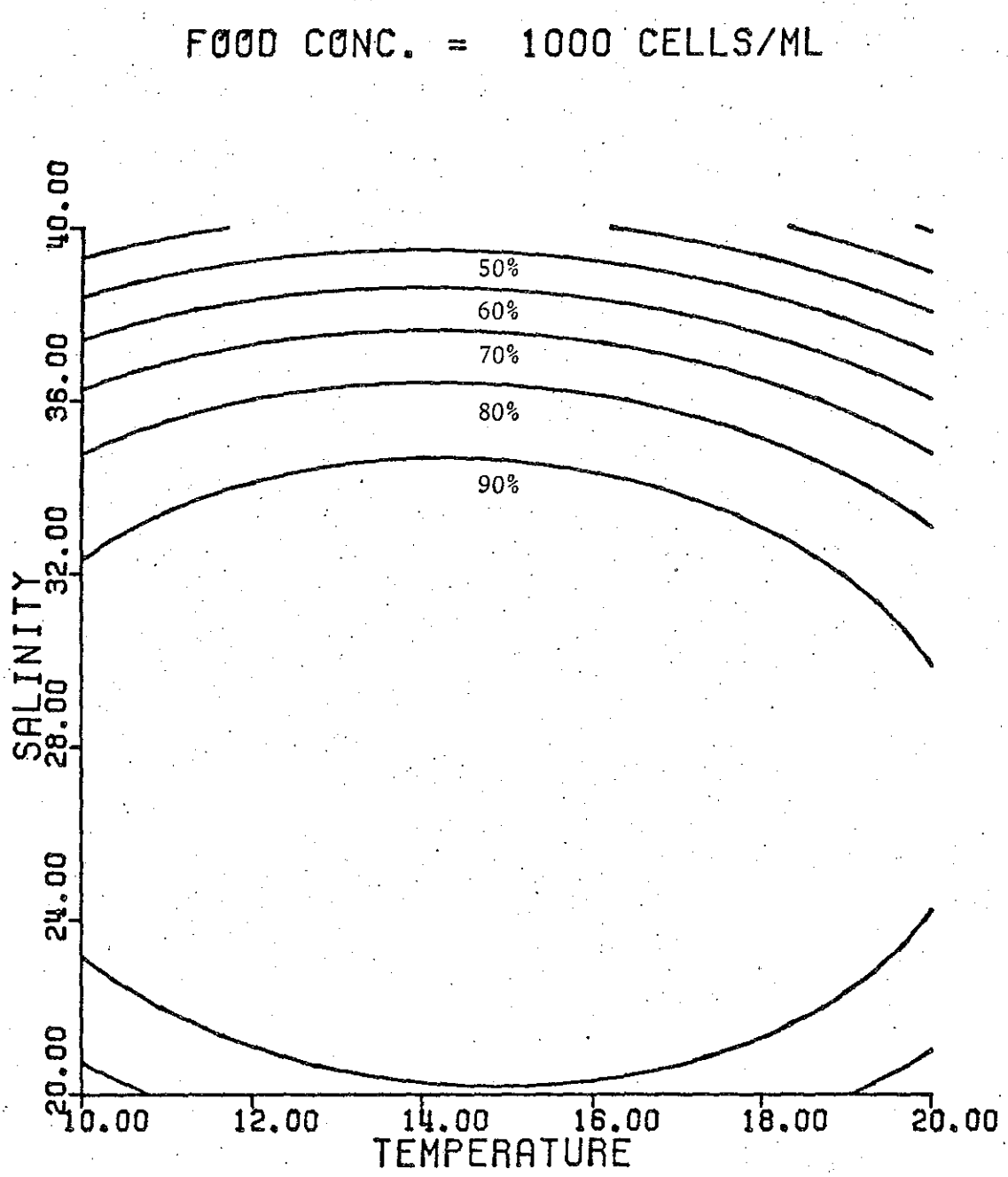

FIGURE 24

POLYDORA GIARDI

6 DAY SURVIVAL ISOPLETHS

FØØD CØNC. = 50000 CELLS/ML

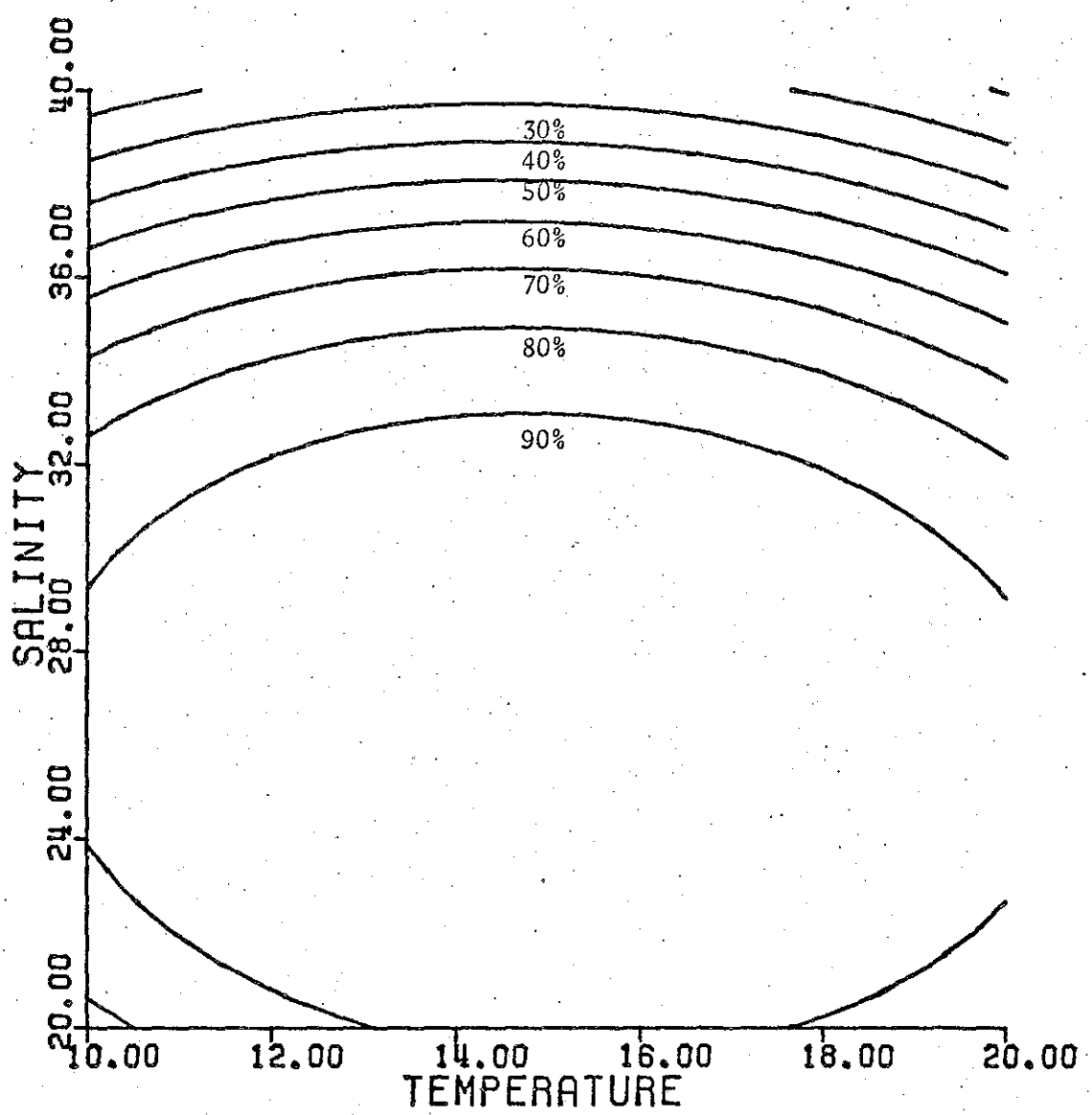

FIGURE 25

PØLYDØRA GIARDI

6 DAY SURVIVAL ISØPLETHS

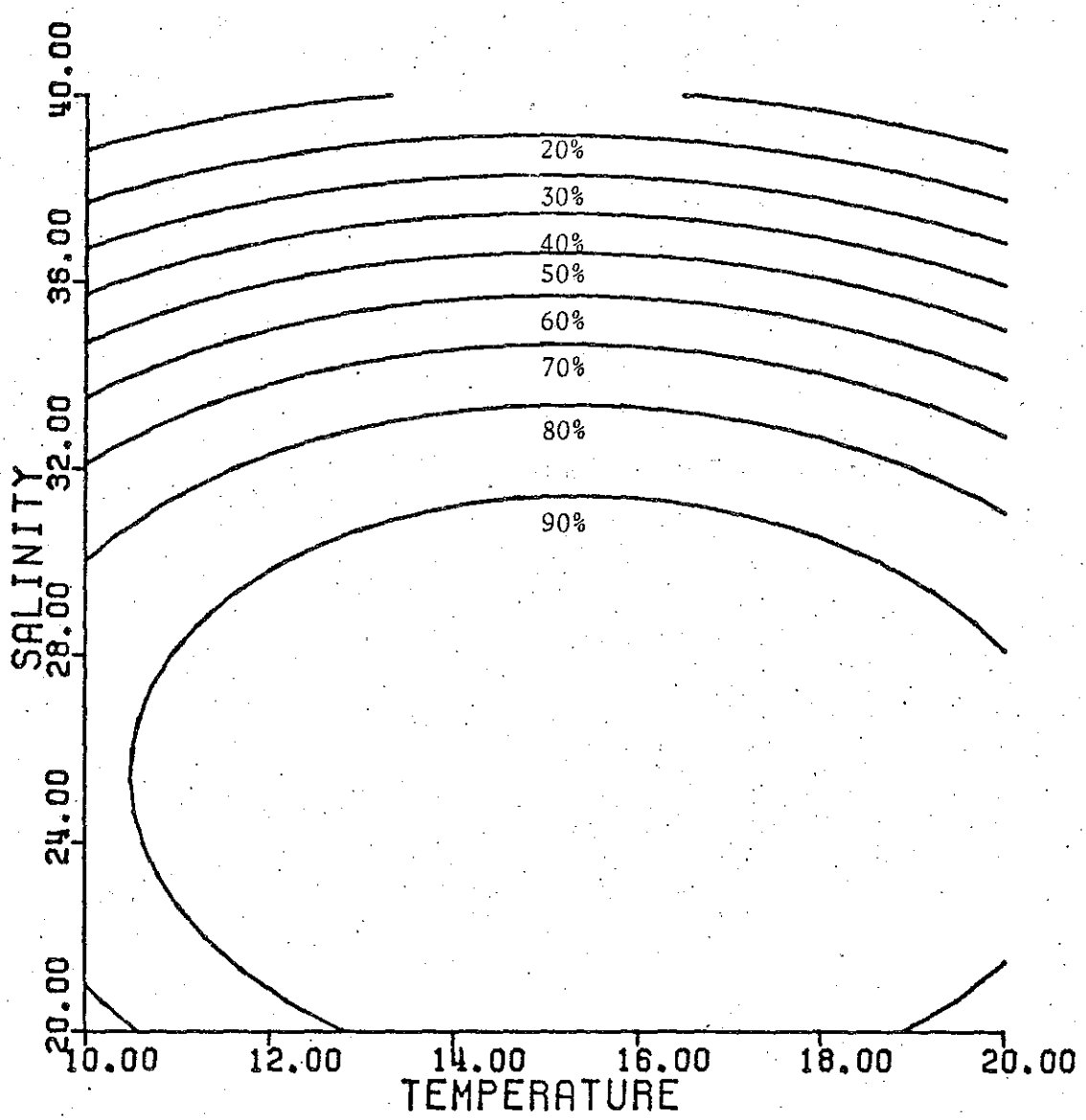

FIGURE 26

PØLYDØRA GIARDI

6 DAY SURVIVAL ISØPLETHS

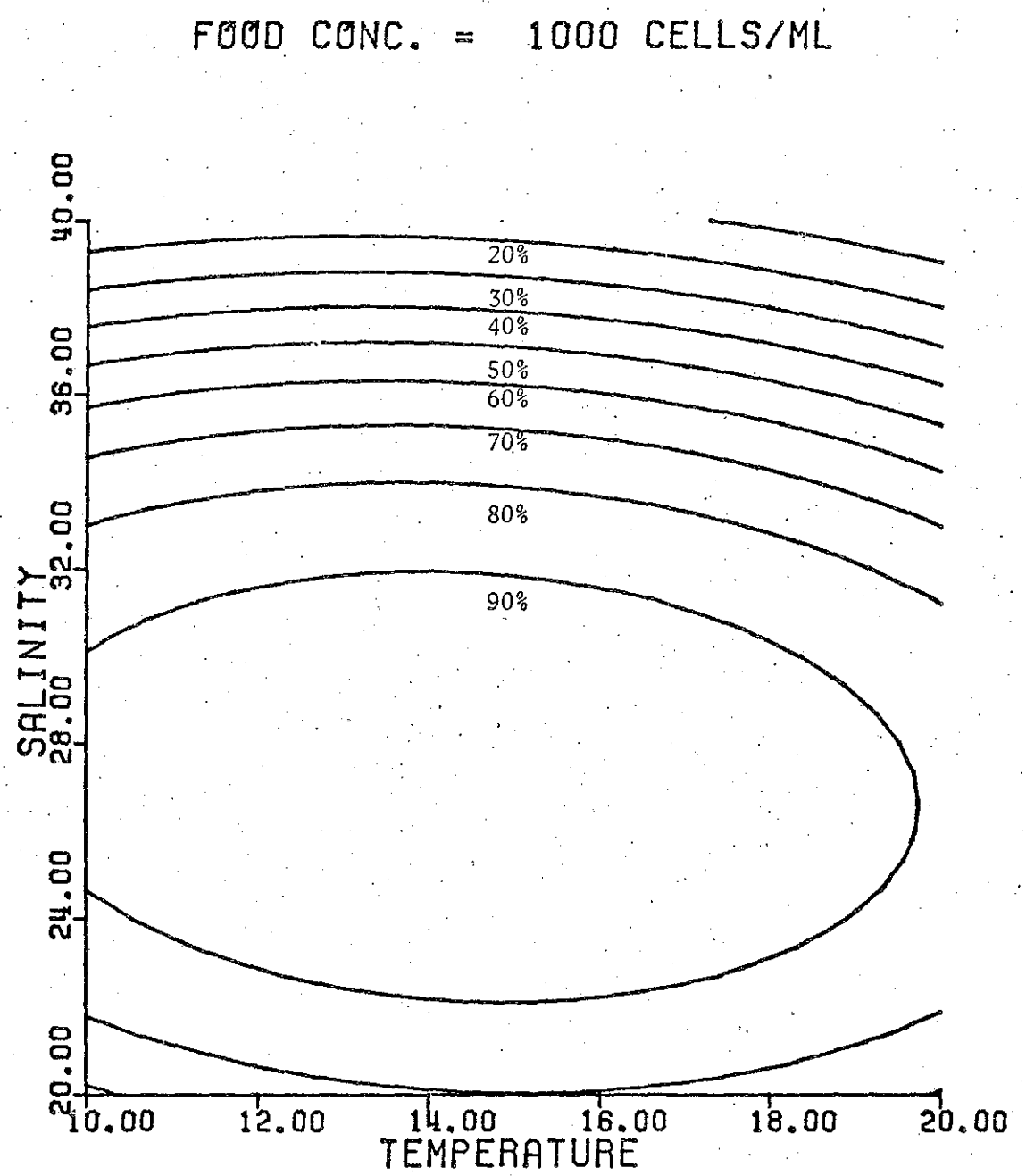

FIGURE 27

POLYDORA GIARDI

9 DAY SURVIVAL ISOPLETHS

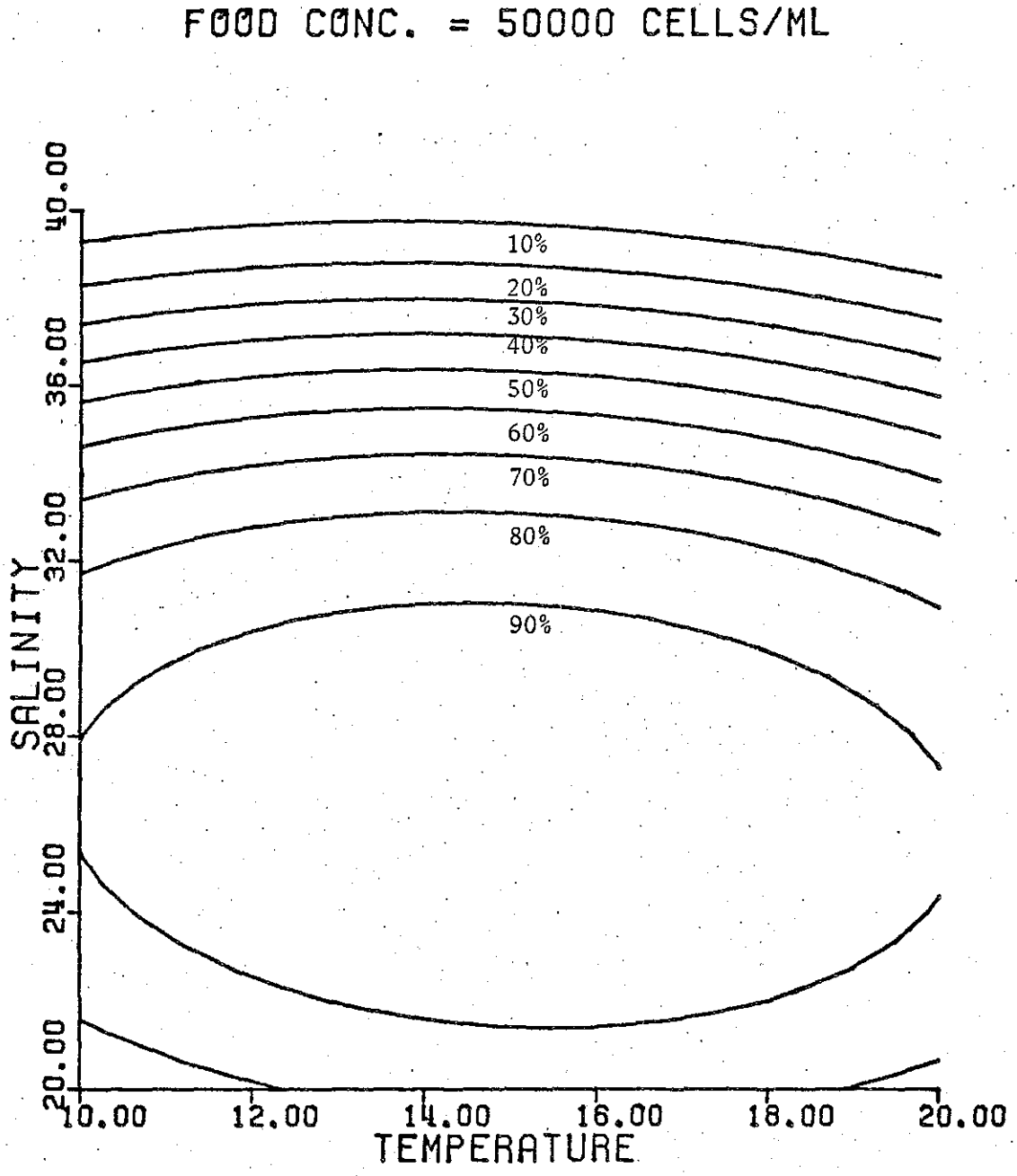

FIGURE 28

POLYDORA GIARDI

9 DAY SURVIVAL ISOPLETHS

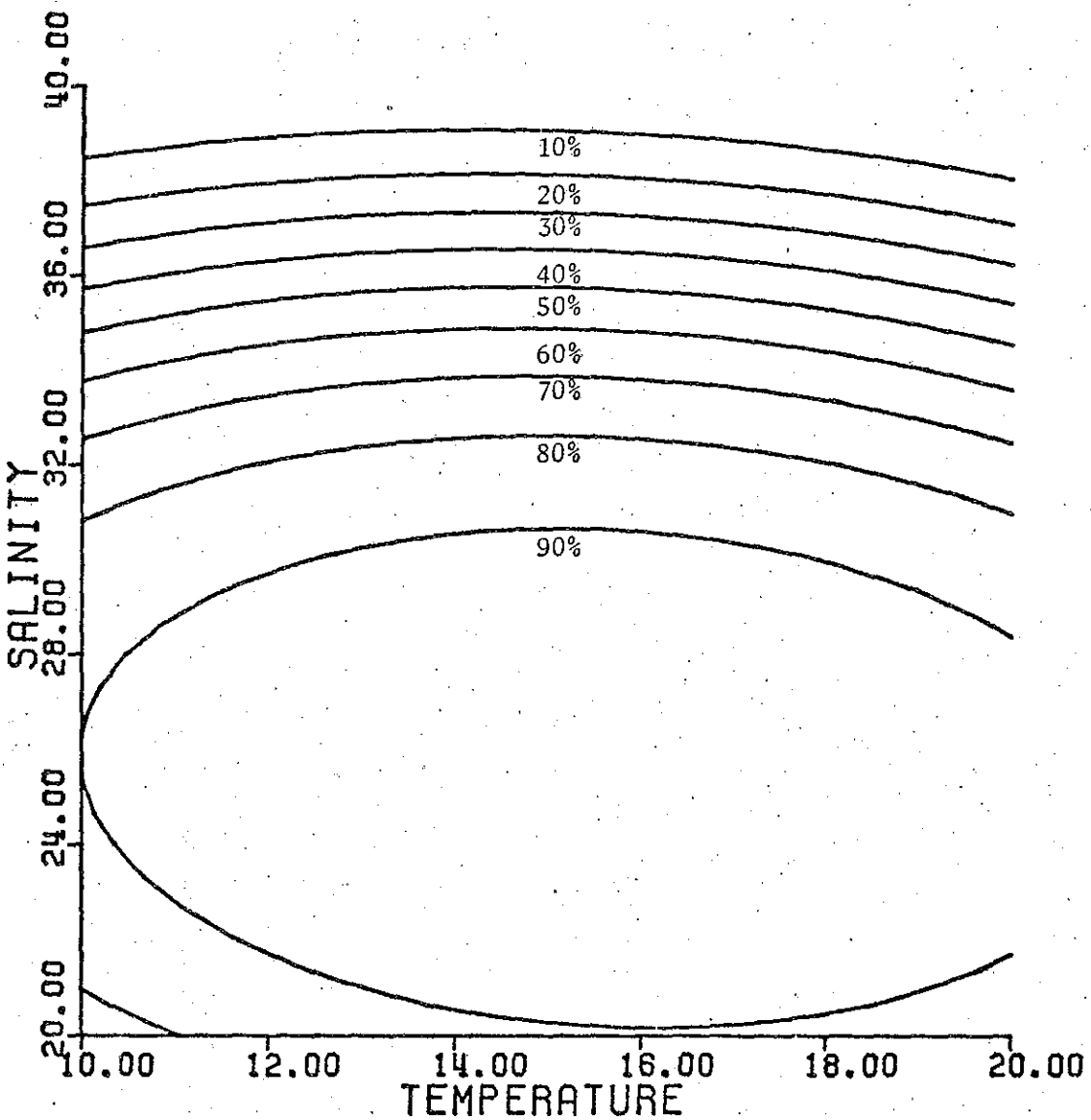

FIGURE 29

PØLYDØRA GIARDI

9 DAY SURVIVAL ISØPLETHS

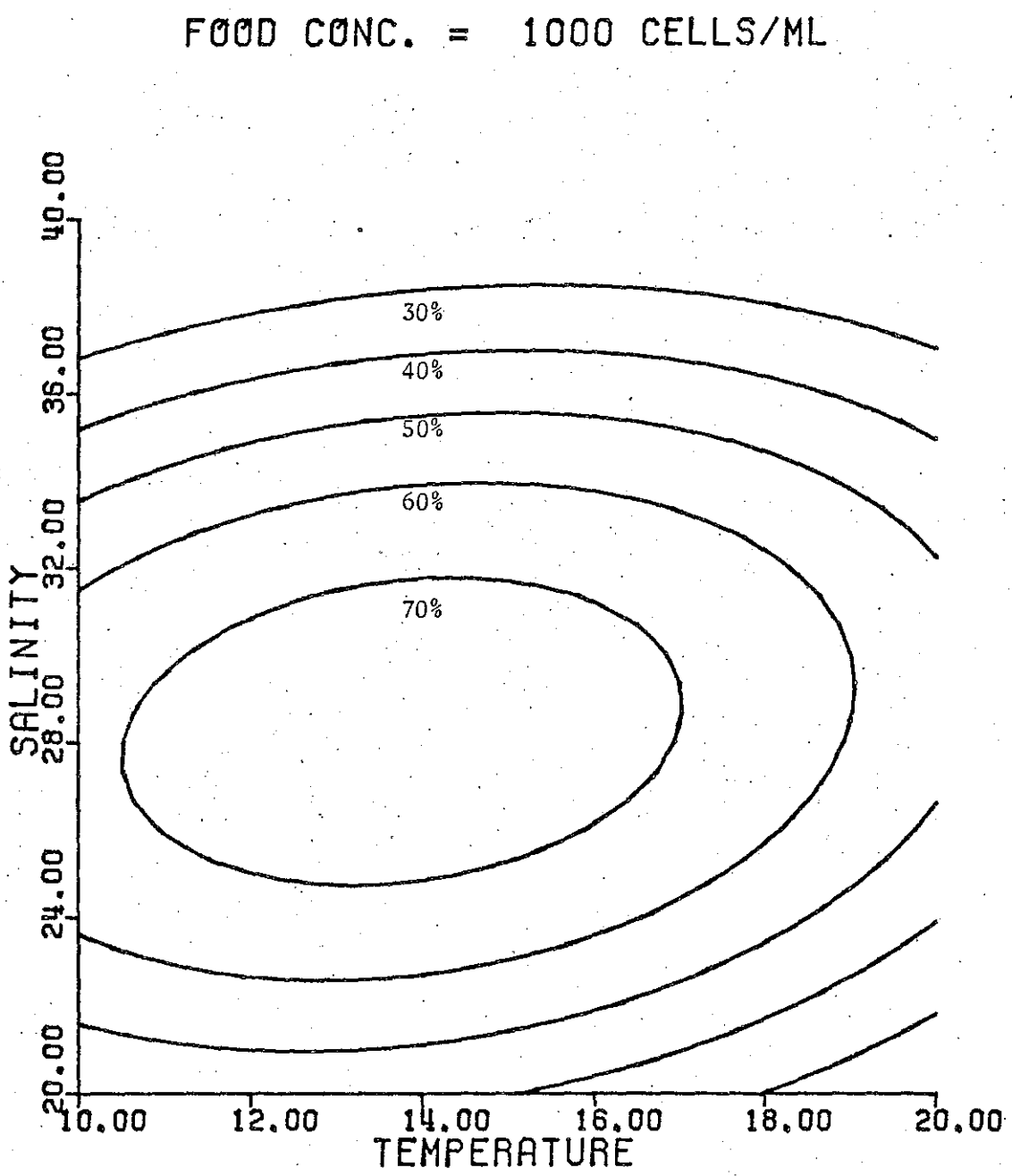

FIGURE 30

POLYDORA GIARDI

15 DAY SURVIVAL ISOPLETHS

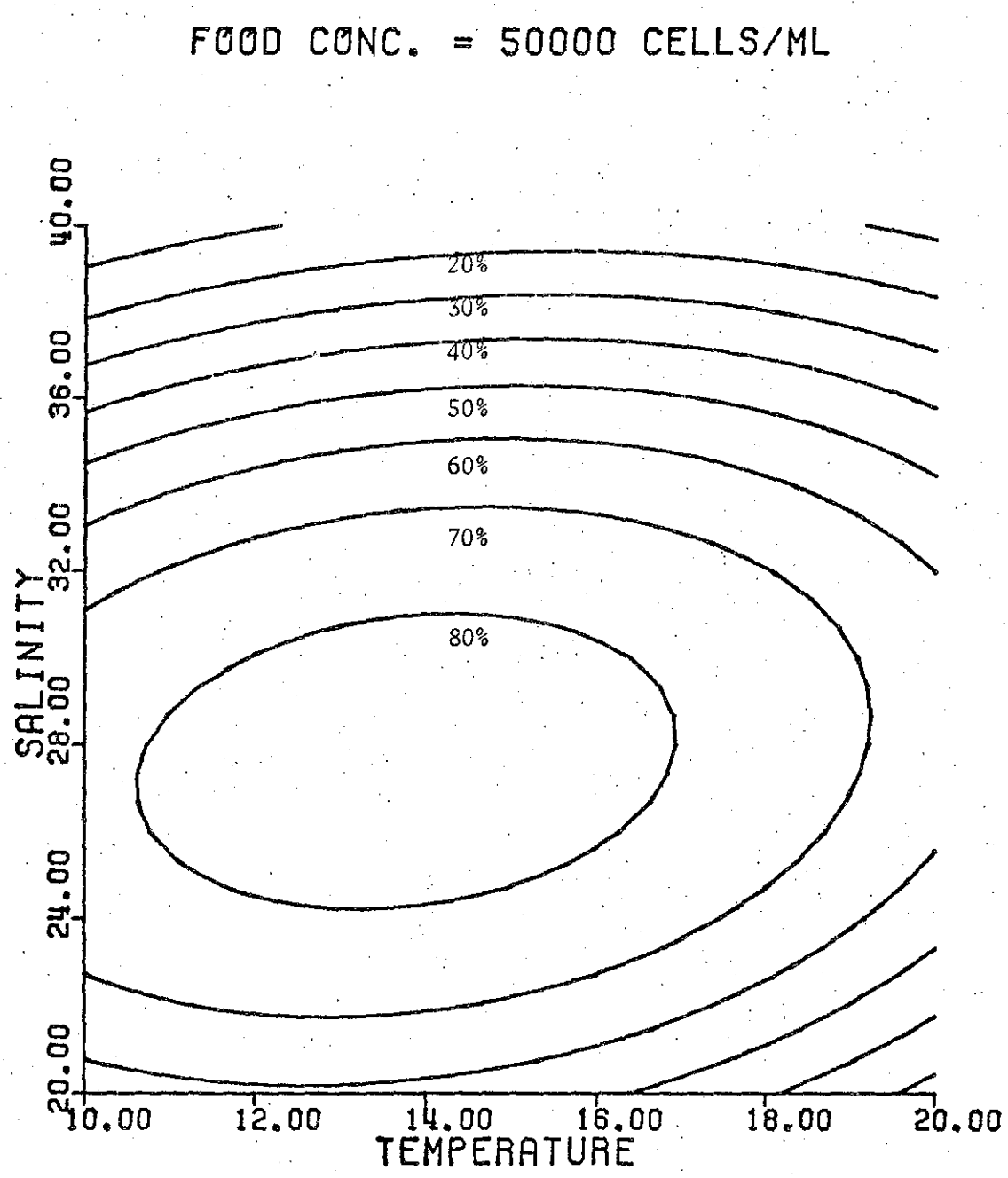

FIGURE 31

POLYDORA GIARDI

15 DAY SURVIVAL ISOPLETHS

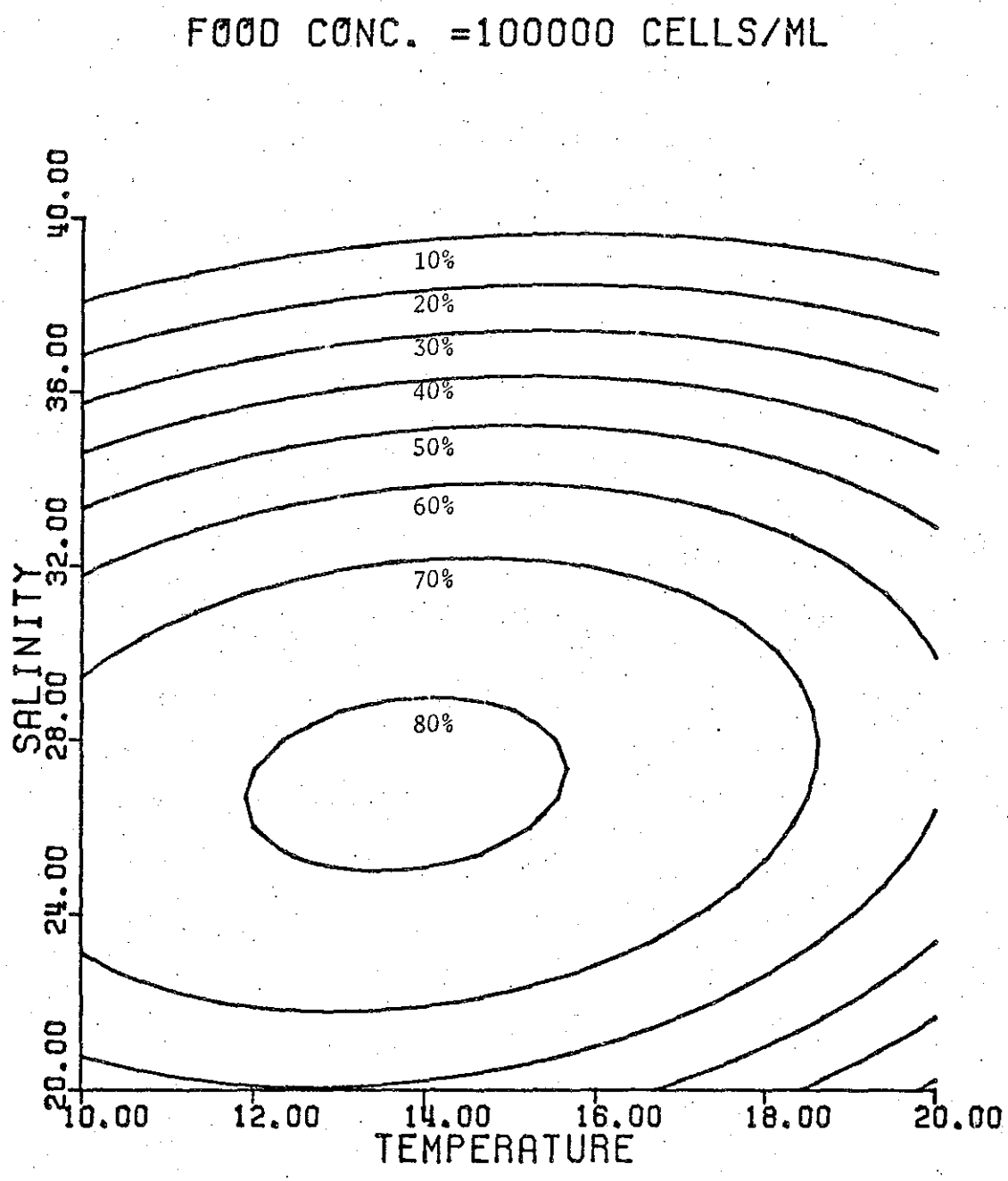

FIGURE 32

POLYDORA GIARDI

15 DAY SURVIVAL ISOPLETHS

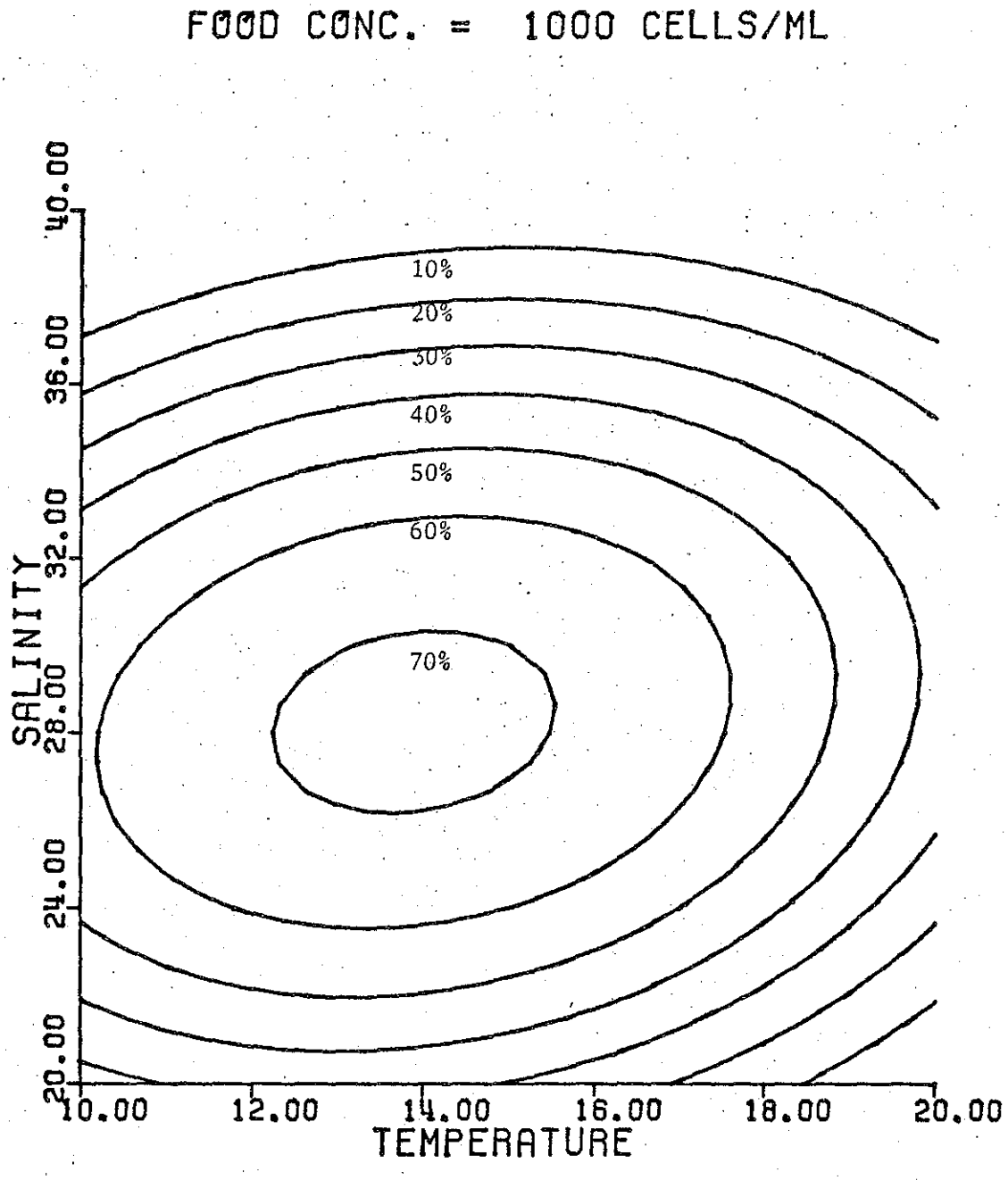

FIGURE 33

POLYDORA GIARDI

18 DAY SURVIVAL ISOPLETHS

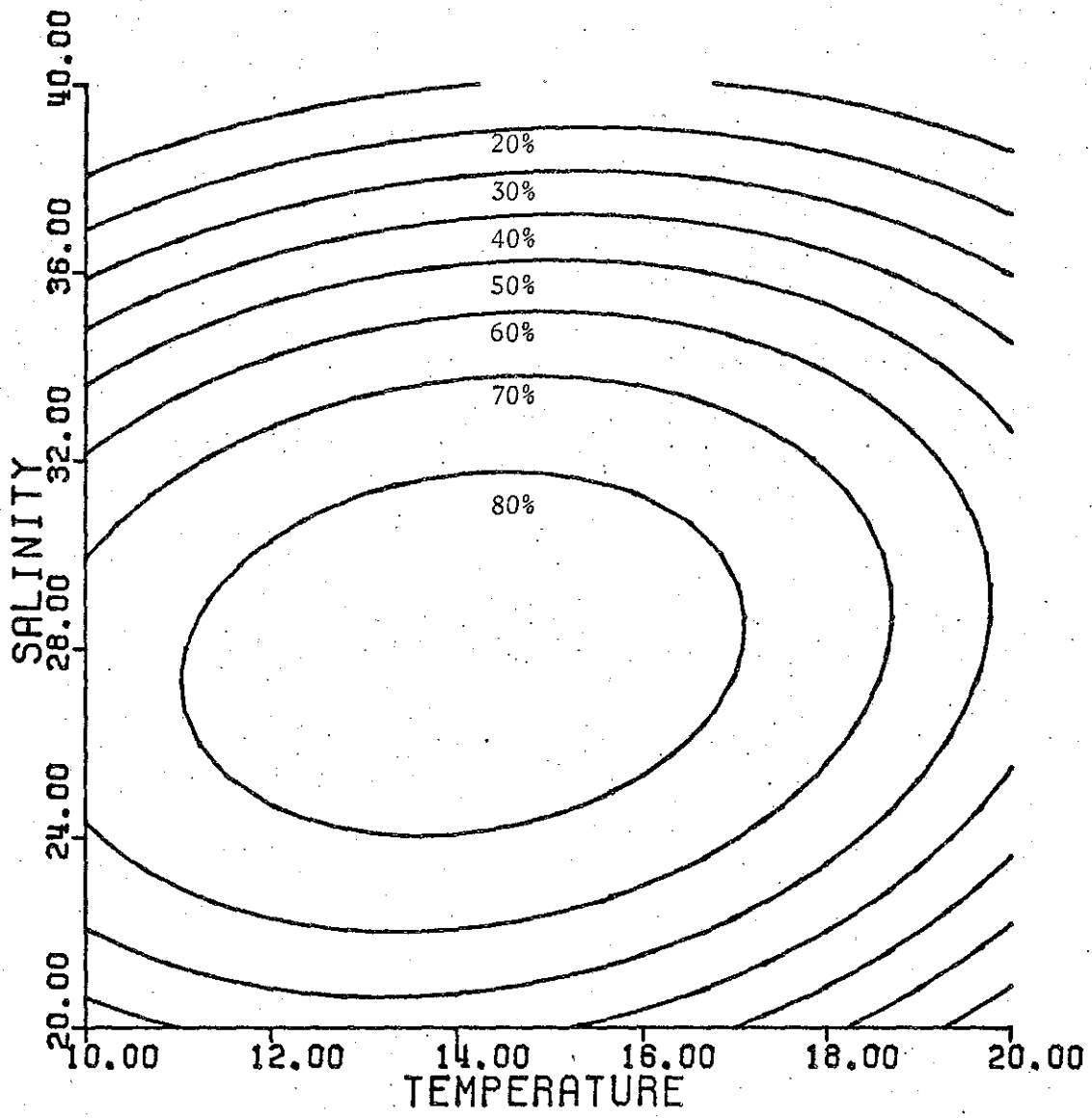

FIGURE 34

POLYDORA GIARDI

18 DAY SURVIVAL ISOPLETHS

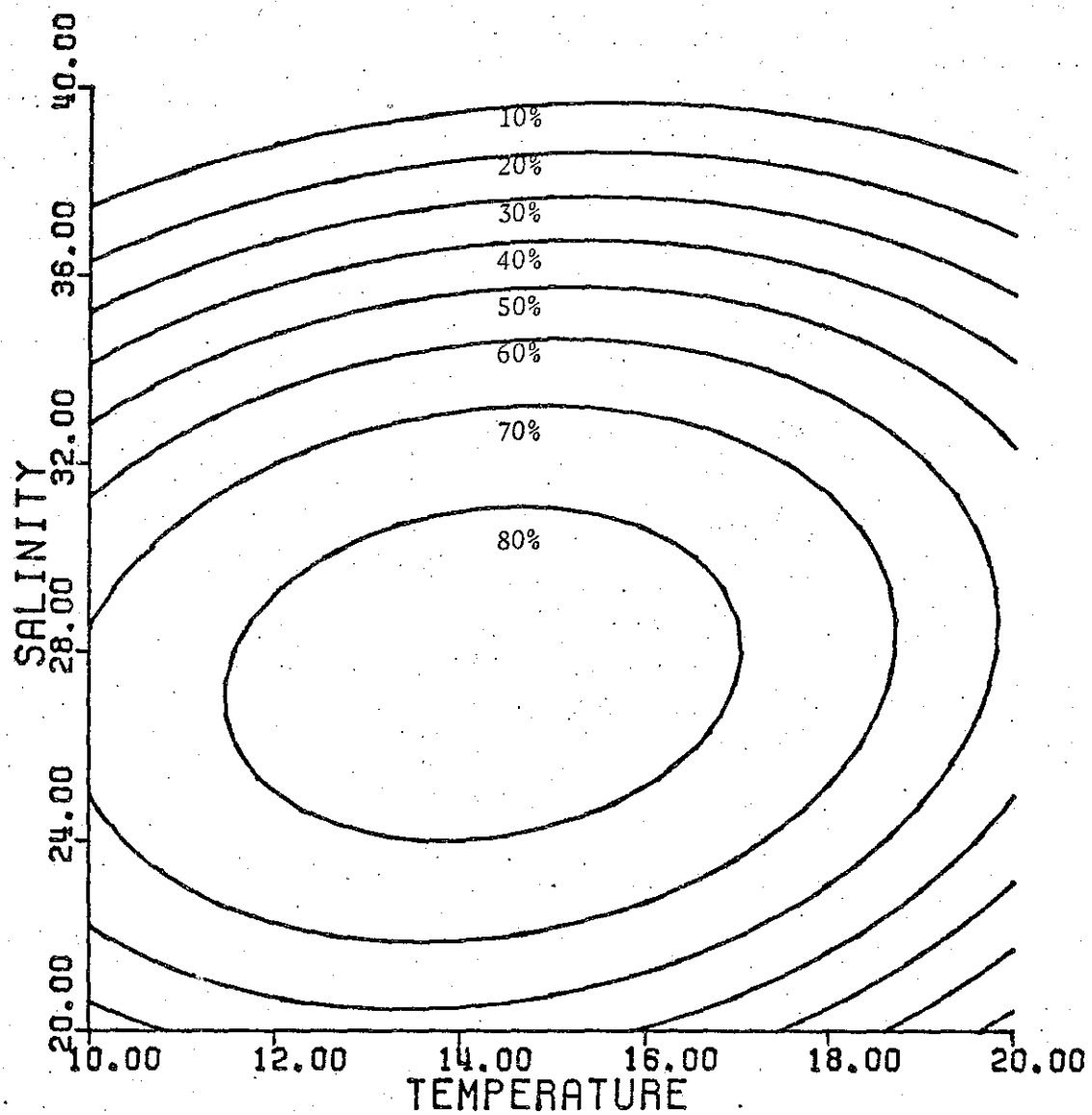

FIGURE 35

POLYDORA GIARDI

18 DAY SURVIVAL ISOPLETHS

FOOD CONC. = 1000 CELLS/ML

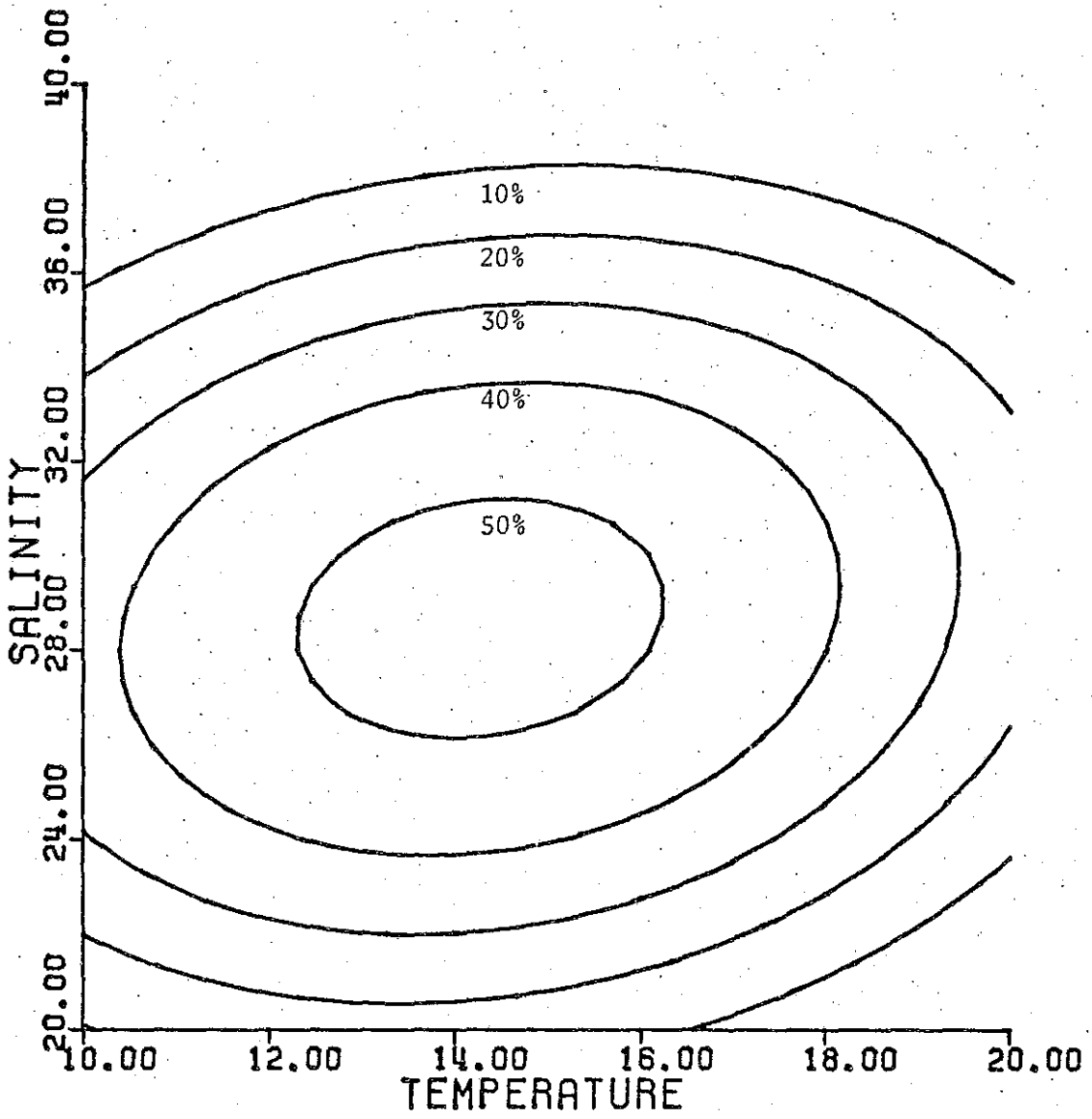

FIGURE 36

POLYDORA GIARDI

27 DAY SURVIVAL ISOPLETHS

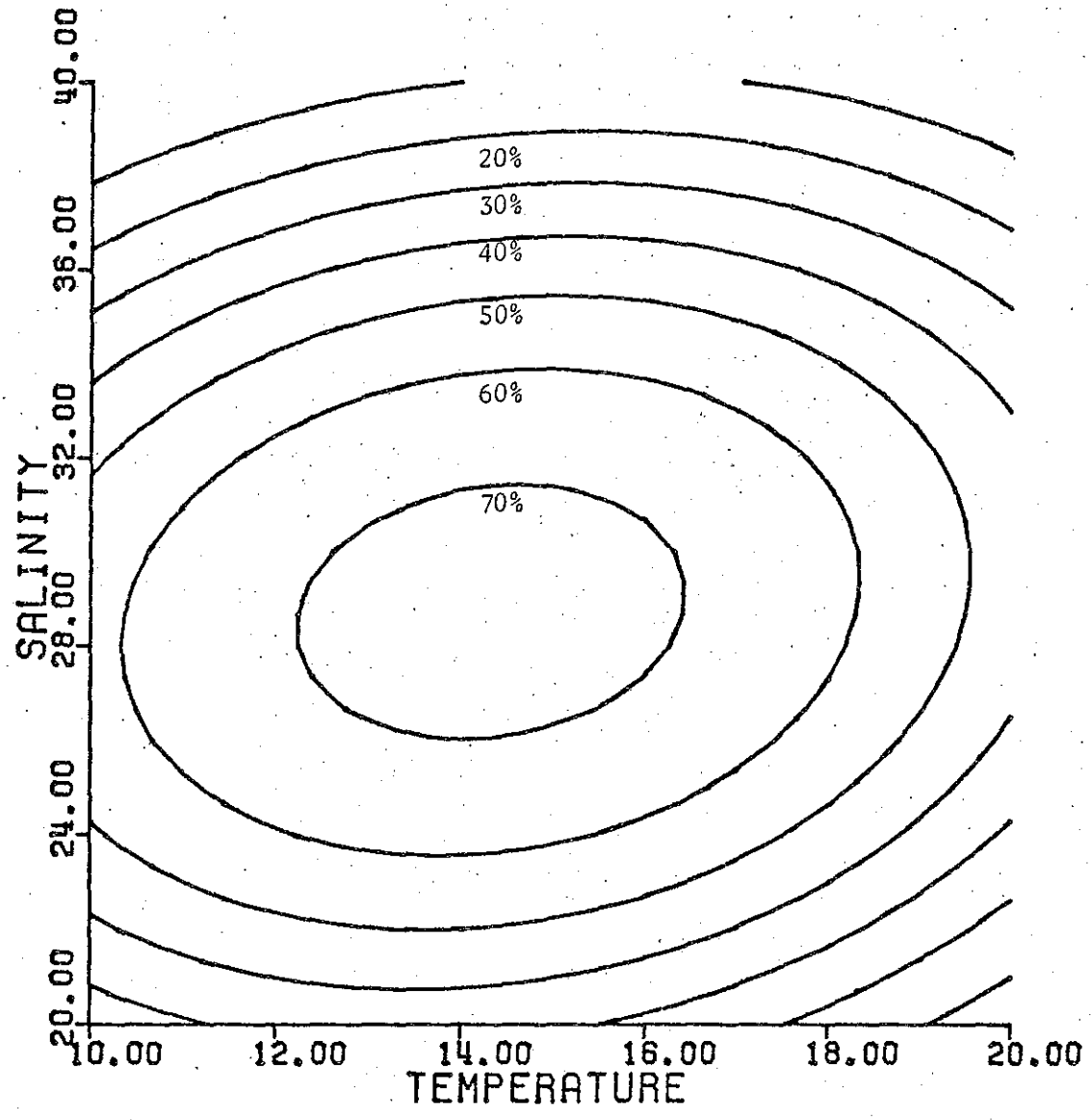

FIGURE 37

POLYDORA GIARDI

27 DAY SURVIVAL ISOPLETHS

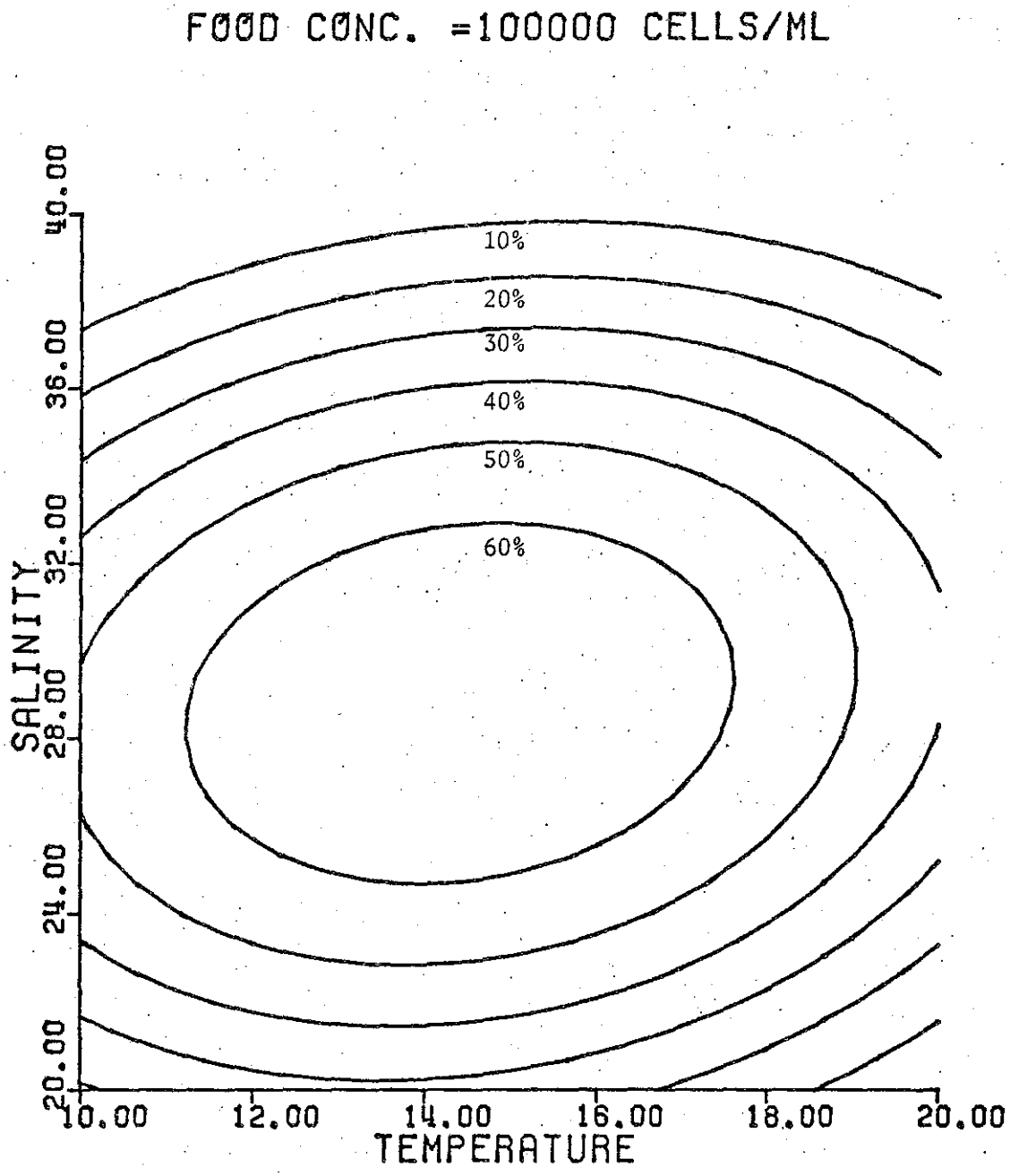

FIGURE 38

POLYDORA GIARDI

27 DAY SURVIVAL ISOPLETHS

FOOD CONC. = 1000 CELLS/ML

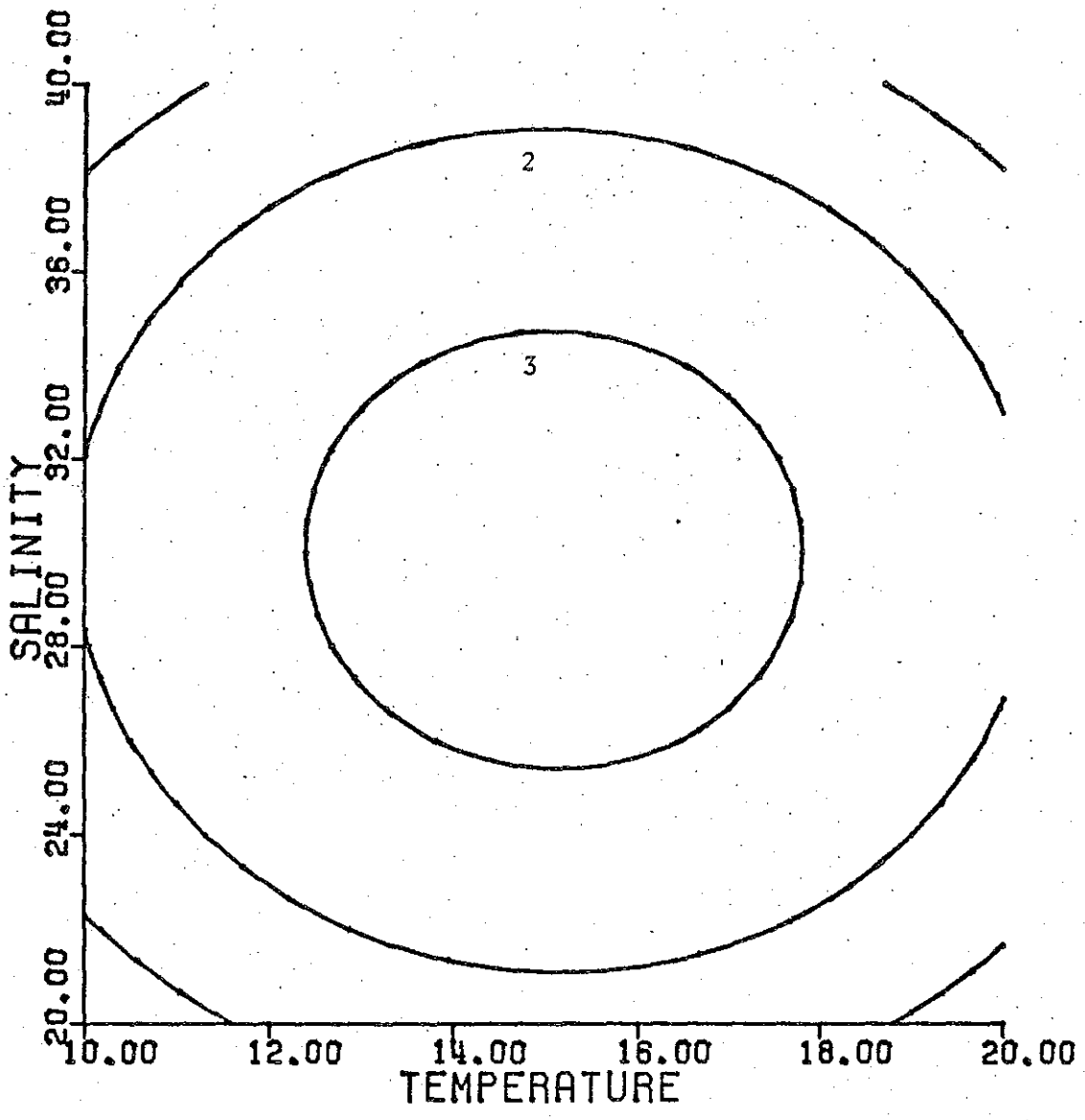

FIGURE 39

POLYDORA GIARDI

9 DAY GROWTH (SETIGERS)

FOOD CONC. = 50000 CELLS/ML

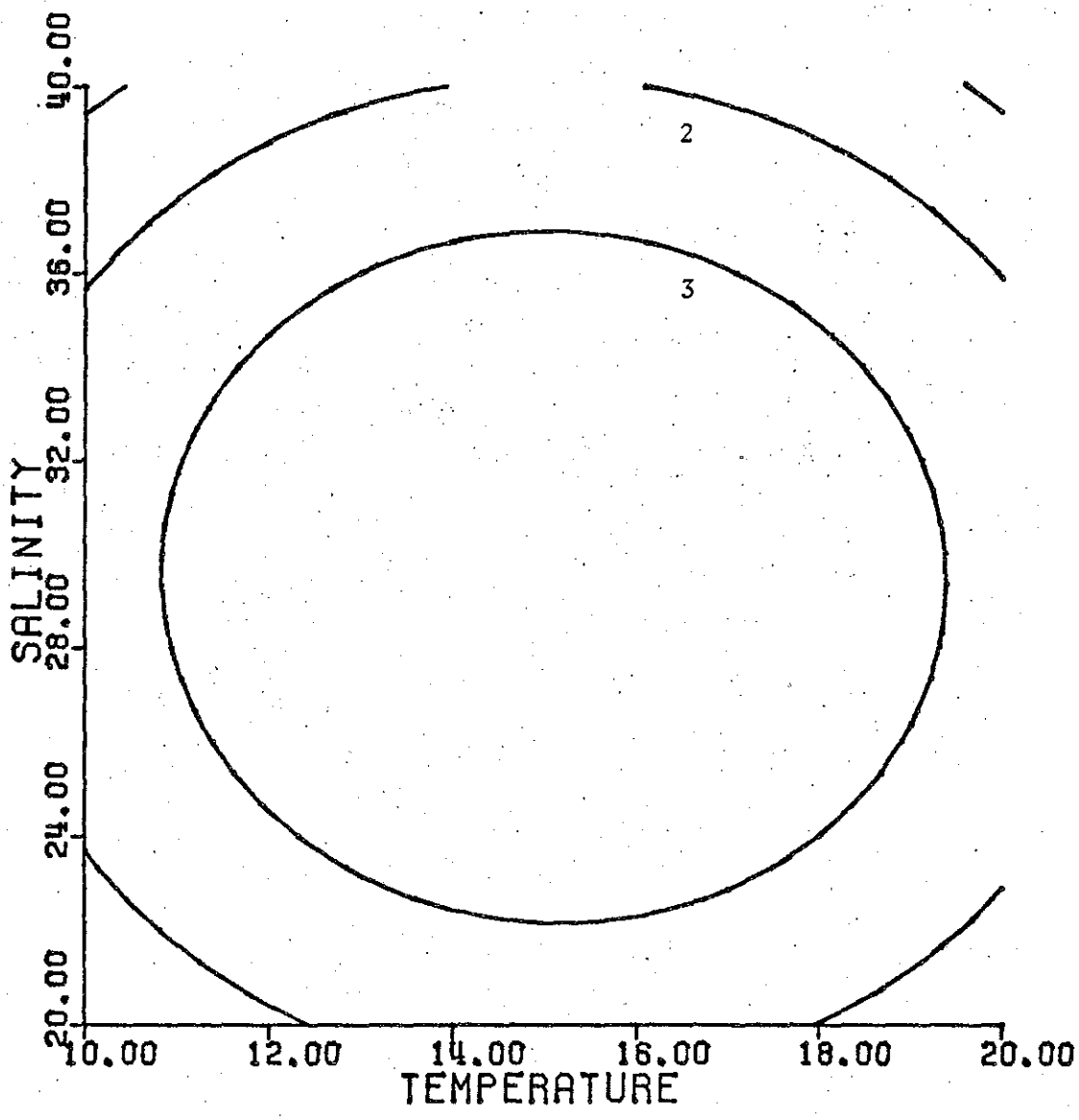

FIGURE 40

POLYDORA GIARDI

9 DAY GROWTH (SETIGERS)

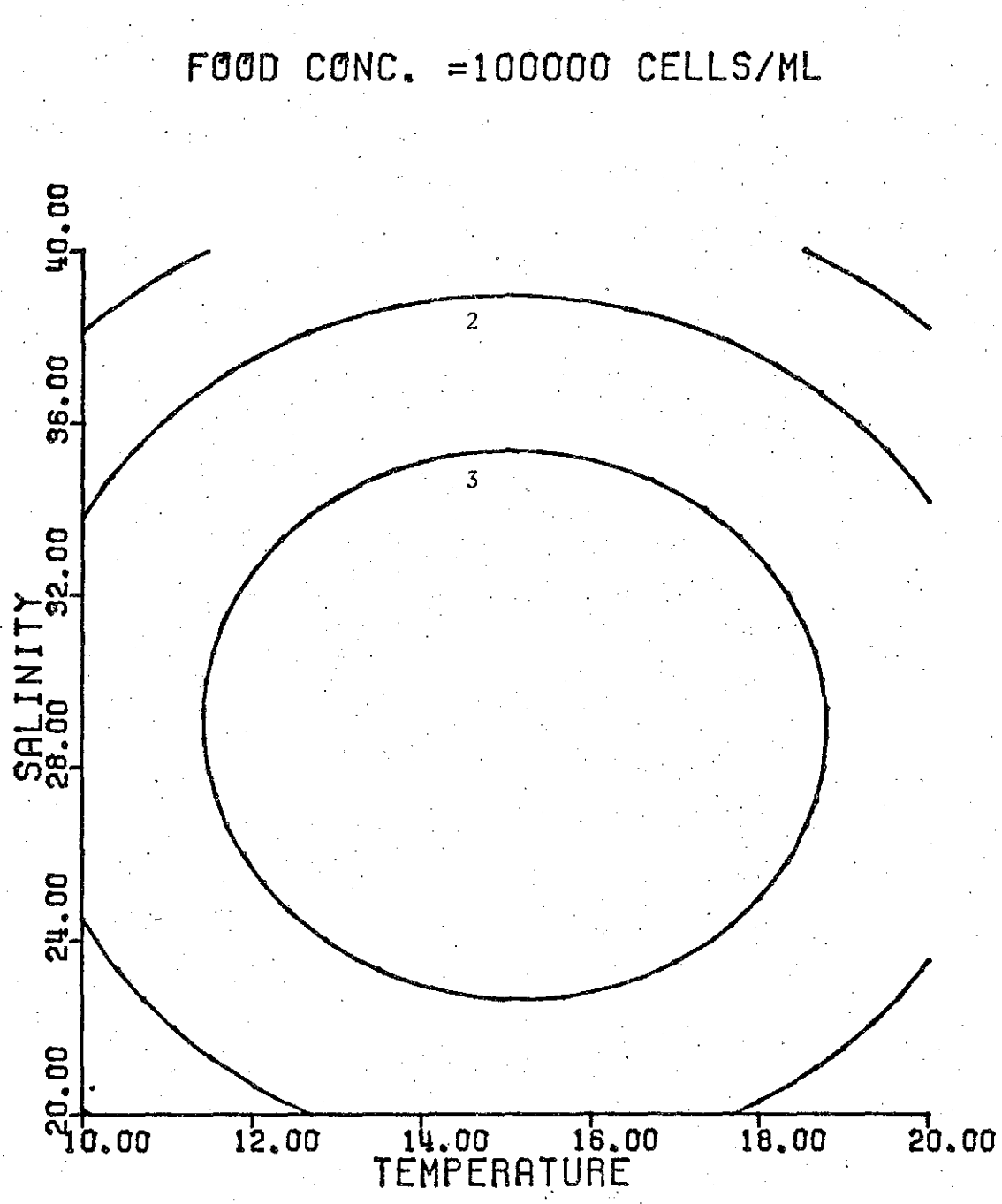

FIGURE 41

POLYDORA GIARDI

9 DAY GROWTH (SETIGERS)

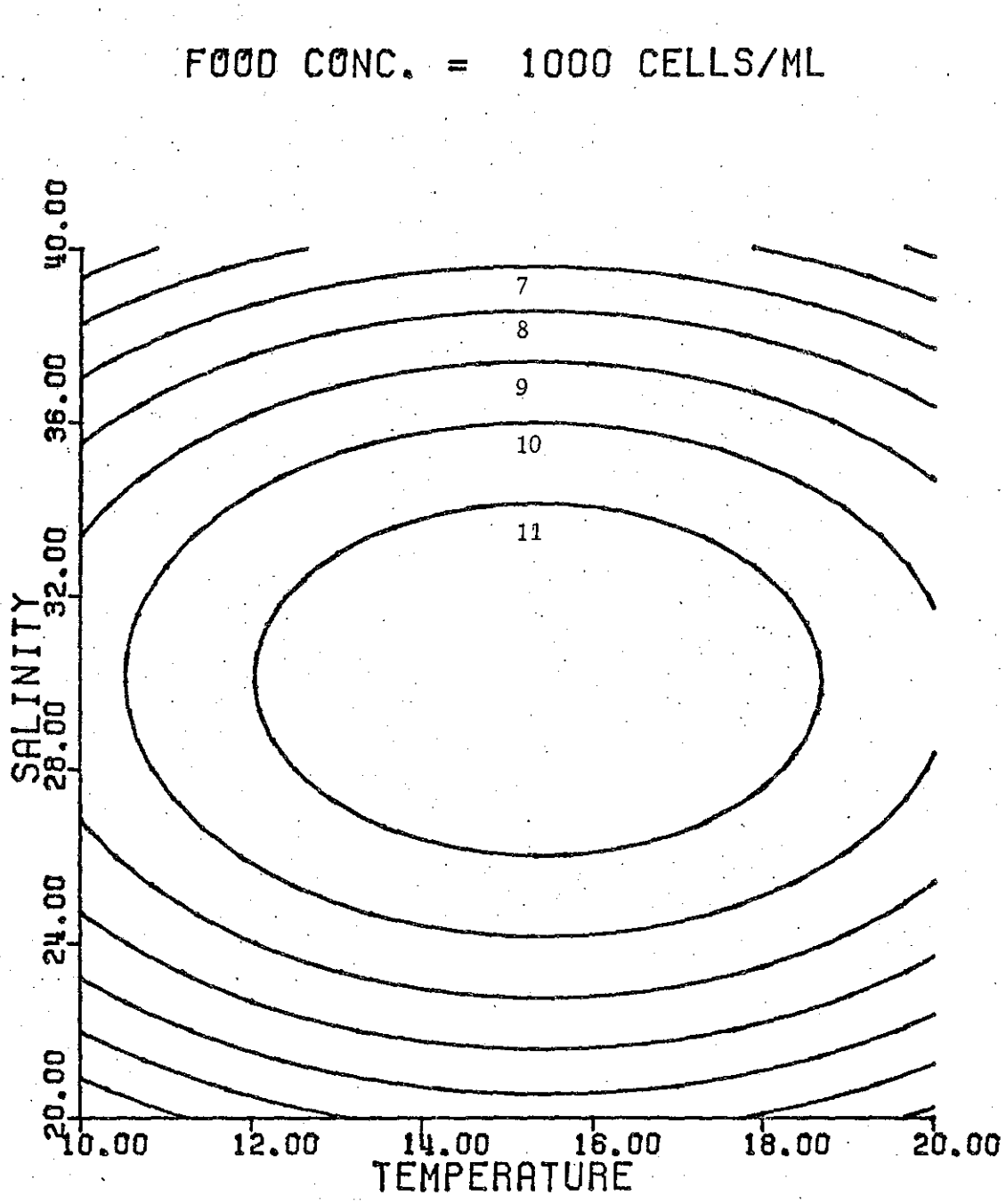

FIGURE 42

POLYDORA GIARDI

18 DAY GROWTH (SETIGERS)

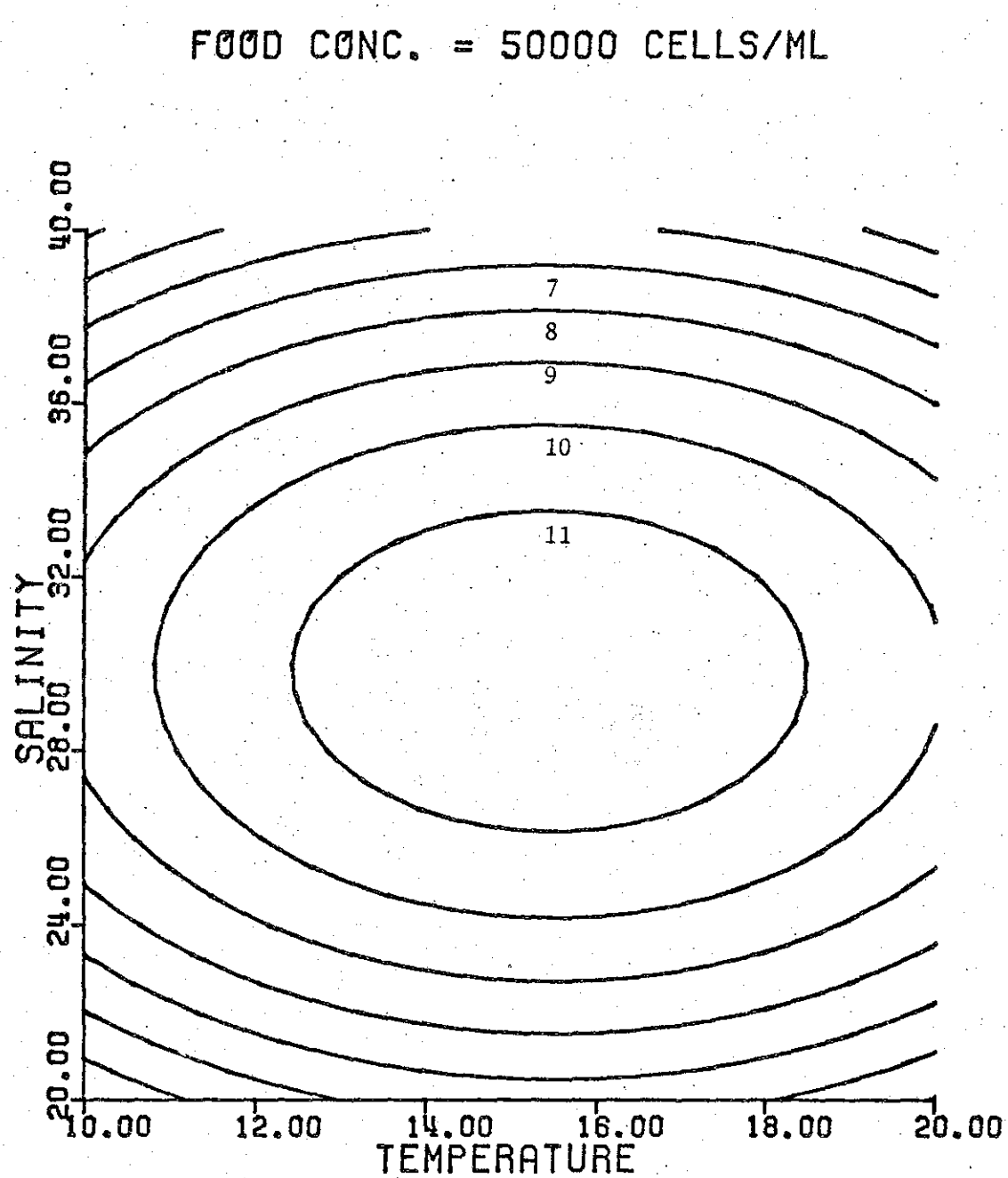

FIGURE 43

PØLYDØRA GIARDI

18 DAY GRØWTH (SETIGERS)

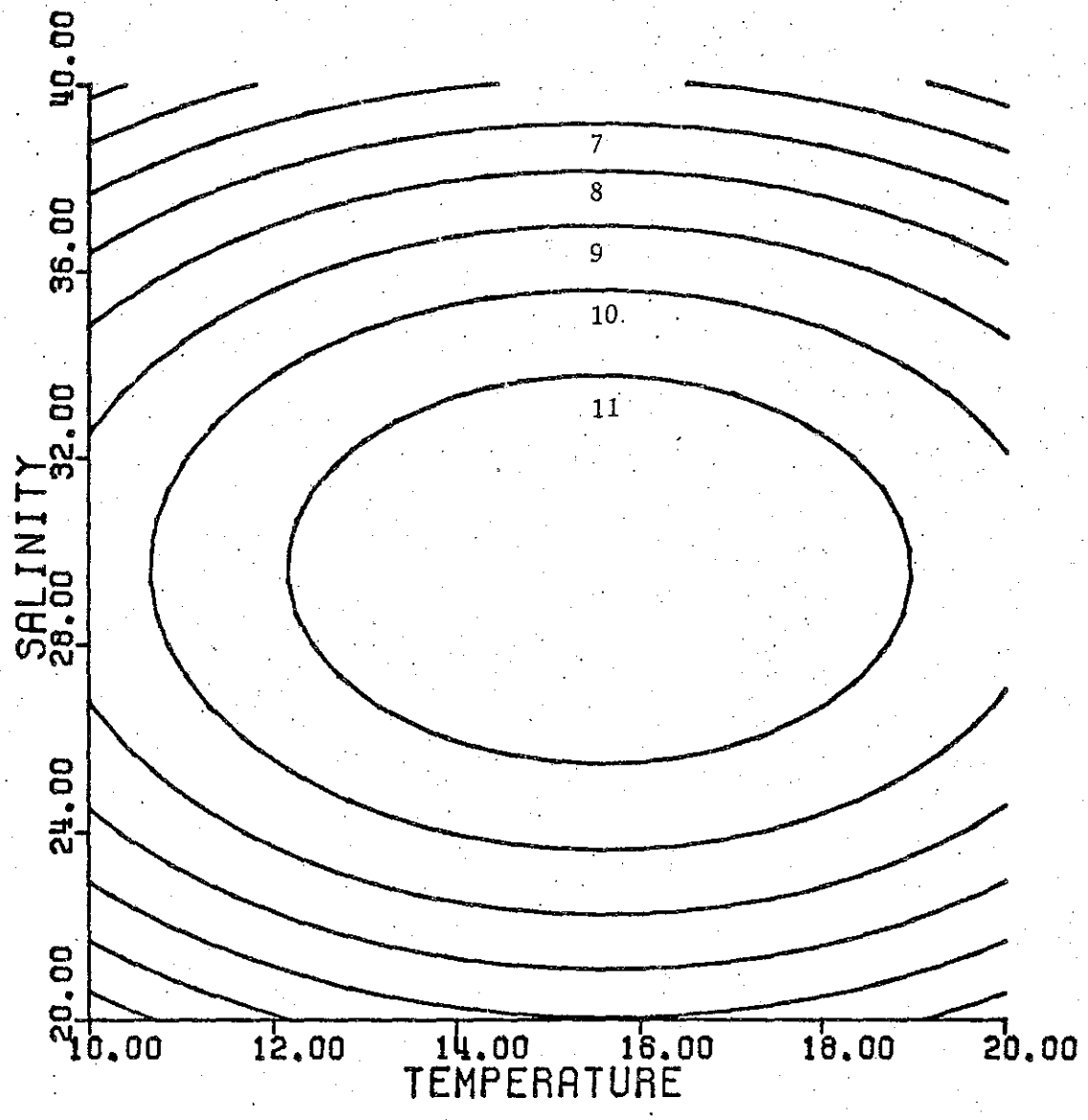

FIGURE 44

POLYDORA GIARDI

18 DAY GROWTH (SETIGERS)

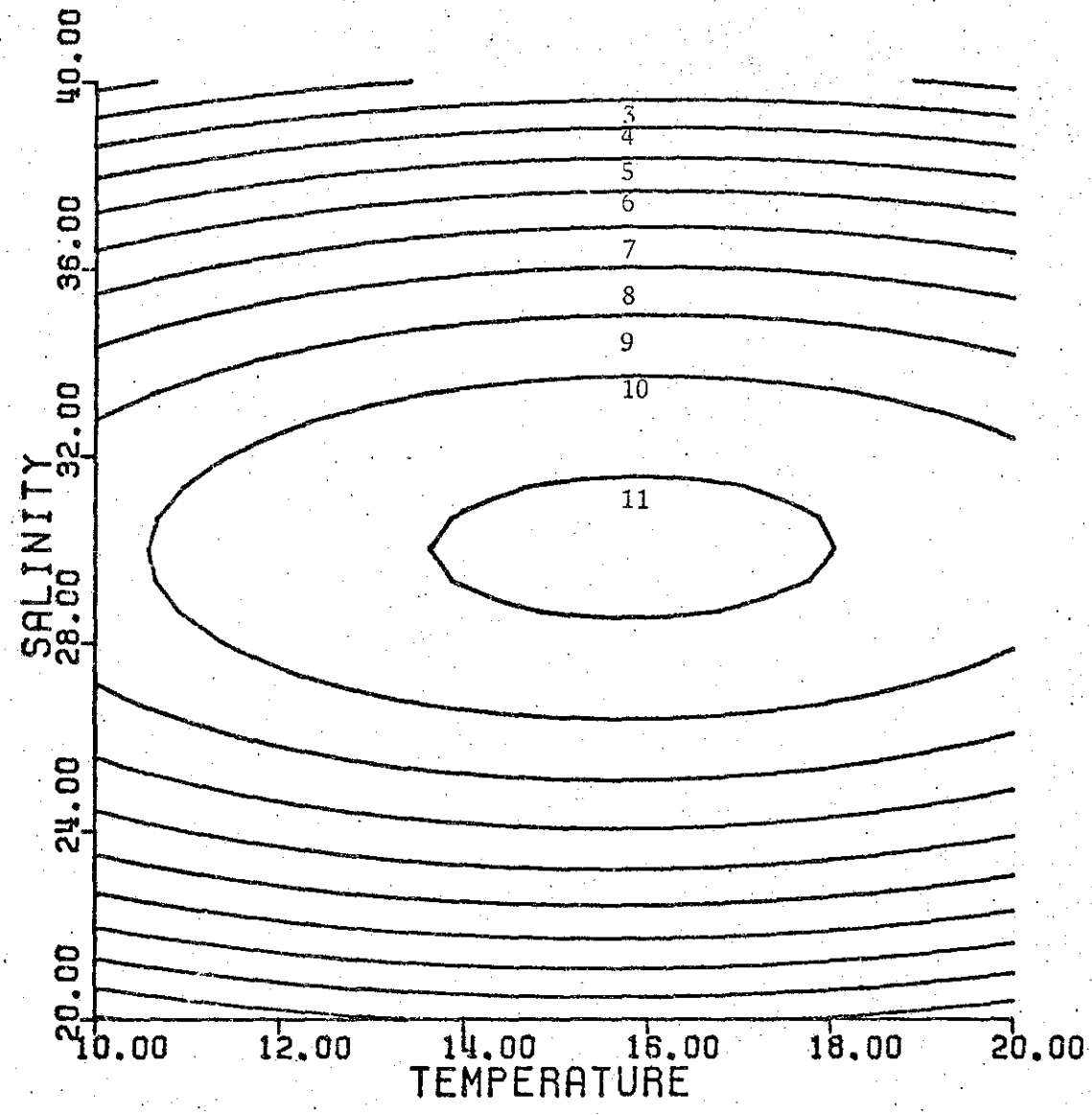

FIGURE 45

POLYDORA GIARDI

27 DAY GROWTH (SETIGERS)

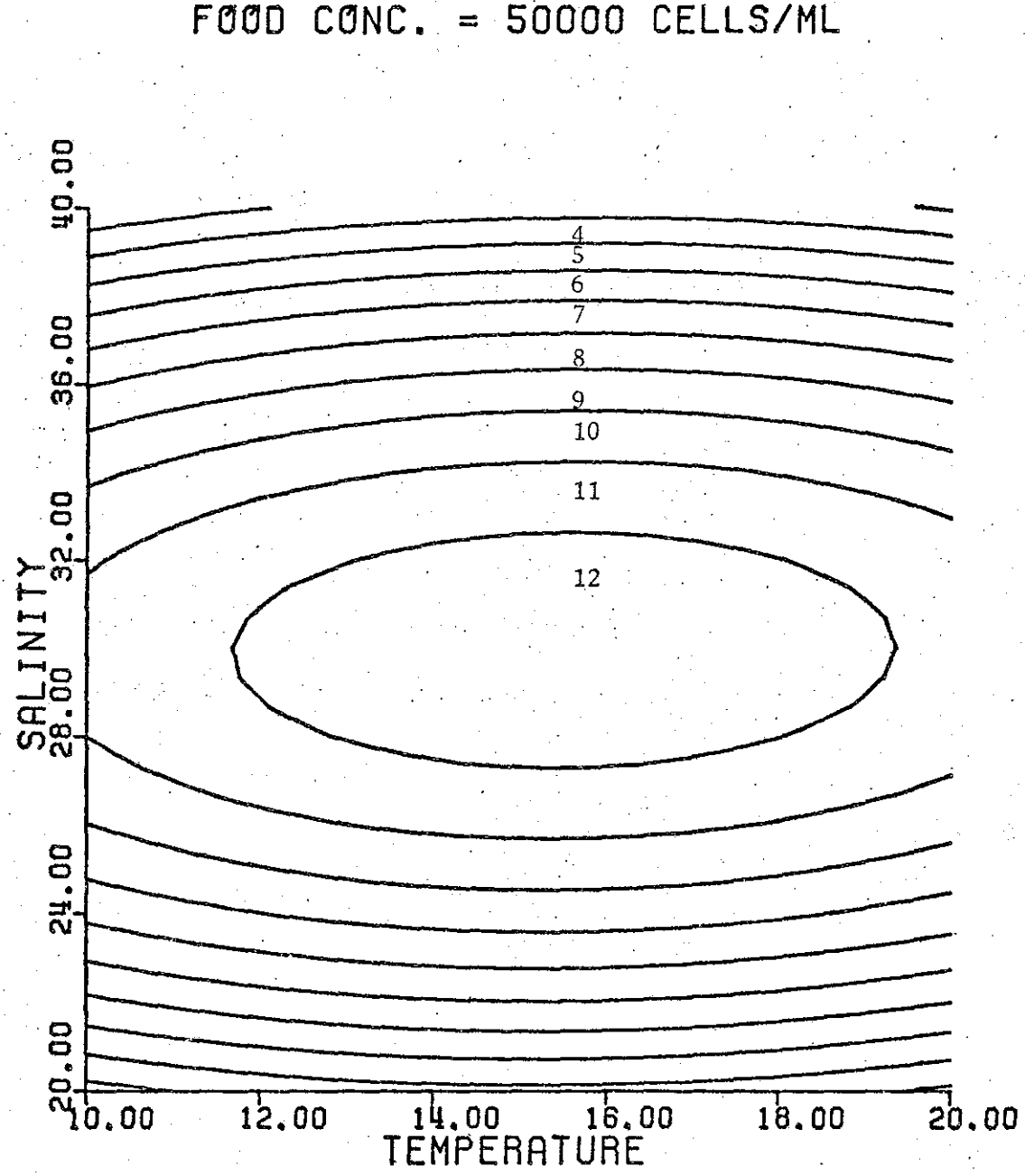

FIGURE 46

POLYDORA GIARDI

27 DAY GROWTH (SETIGERS)

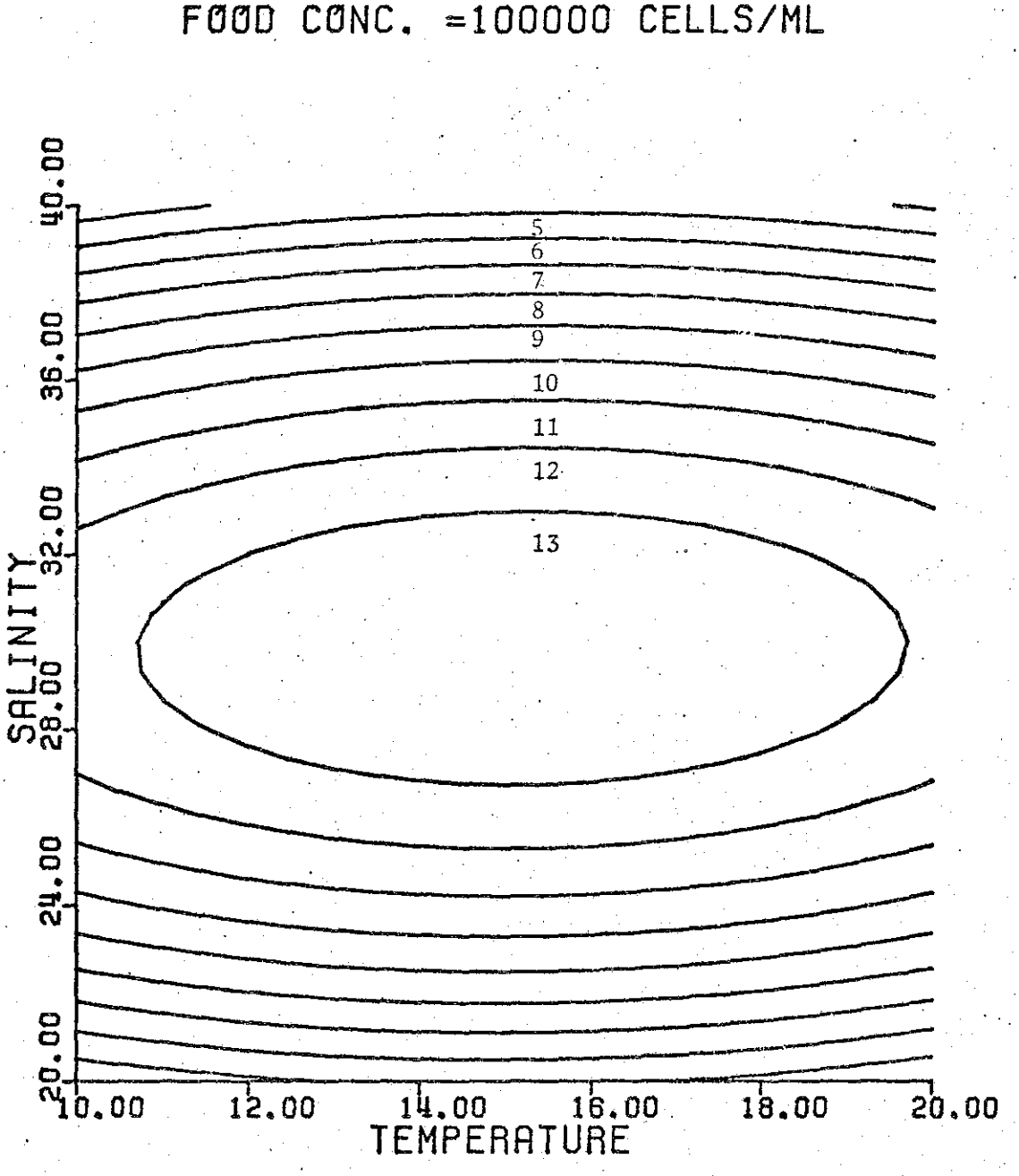

FIGURE 47

PØLYDØRA GIARDI

27 DAY GRØWTH (SETIGERS)

APPENDIX I

A response surface is a graphical representation of the expression:

$$y = f(x_1, x_2, \cdots x_k)$$

where the observed response (y) is a function of a suite of k independent variables. The actual form of the function f is unknown but for this study it was assumed that it could be approximated by a second order polynomial function. The choice of a polynomial function to approximate the unknown function was made because A) it possesses a simply defined optimum, B) the method of least squares provides a relatively simple and straightforward method of calculating estimates of the model coefficients and C) it is easy to expand to a multidimensional relationship between the dependent variable and several independent variables. Other possible choices were rectangular hyperbolae, inverse polynomial and exponential functions (Mead and Park, 1975).

The calculation or "fitting" of a response surface has two goals: 1) Finding a suitable approximation of the unknown function in order to predict future response and 2) Determining what levels of the independent variables are required in order to optimize the response. An example of the methods used to fit a response surface will now be illustrated.

Let us assume that the response we are interested in examining can be approximated by a second order polynomial containing two independent variables:

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

where y is the observed response; x_1 and x_2 are the independent variables, and E is the experimental error which is assumed to be independent from run to run with a mean = 0 and a variance = σ^2. The model can be written in matrix notation as follows:

$$Y = XB + E$$

where:

$$Y = \begin{vmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{vmatrix} \qquad X = \begin{vmatrix} 1 & x_{11} & \cdots & x_{1k} \\ 1 & x_{21} & & x_{2k} \\ \vdots & \vdots & & \vdots \\ 1 & x_{n1} & \cdots & x_{nk} \end{vmatrix} \qquad B = \begin{vmatrix} b_o \\ b_1 \\ \vdots \\ b_k \end{vmatrix}$$

Since the observed responses (y_1) are determined by the experimenter and the levels of the independent variables are fixed, the fitting of the response surface requires only the estimation of the model coefficients (b's) These coefficients can be determined by a least squares procedure, which results in a minimum value for the sum of squares associated with the deviations of the estimated value of the response from the actual observed response. We can express this sum of squares (L) in terms of our previously defined vectors and matrix and then minimizing it and solving for B.

$$L = (Y - XB)' (Y - XB)$$

$$L = Y'Y - (XB)' Y - Y' (XB) + (XB)' (XB)$$

$$L = Y'Y - B'X'Y - Y'XB + B'X'XB$$

$$L = Y'Y - 2B'X'Y + B'X'XB$$

$$\frac{\partial L}{\partial B} = -2X'Y + 2(X'X)\ B$$

$$0 = 2X'Y + 2(X'X)\ B$$

$$B = (X'X)^{-1}\ X'Y$$

The last espression is the least square estimator of the model coefficients that is required to fit the response surface.

In order to simplify many of the calculations involved in the estimation of the model coefficients, the independent variables are coded in the following manner. It is apparent that choosing coded values of -1, 0 and +1 for a 3^k design will greatly simplify the necessary calculations and will maintain the basic orthogonality of the design. The following formula allows this conversion:

$$\text{coded variable} = \frac{\text{original variable - central level}}{\text{spacing of the levels}}$$

It can be seen that for example if x_1 is temperature in degrees Centigrade, as it is in this study; the original levels are 10° C, 15° C, and 20° C they will indeed code to the desired -1, 0, and 1 with the central value equal to 15° C and the spacing equal to 5° C.

As previously mentioned the experimental error is assumed to be independent from run to run and the model coefficeints therefore uncorrelated. This assumption and the assumption that the residuals (deviations of expected responses from observed) are normally distributed allow significance testing of the type:

$$H_0 : B_i = 0$$

$$H_1 : B_i \neq 0 \quad (i = 1,2 \ldots k)$$

using an ANOVA. The ANOVA is performed very simply using information already available from the calculations involved in estimating the model coefficients.

$$SS_{REG} = B'X'Y$$

$$SS_{TOTAL} = Y'Y$$

$$SS_{ERROR} = SS_{TOTAL} - SS_{REG}$$

In addition if the design is orthogonal in nature; that is if each of the estimators is uncorrelated with one another, a simple partitioning of the regression sum of squares into independent components can be performed, each describing the contribution to the regression of an individual parameter in the model (Draper and Smith, 1966).

Response surface techniques were proposed by Box and Wilson (1951) as a statistical method for optimizing industrial processes in response to varying conditions. Several design refinements were proposed by Box (1954) and Box and Youle (1955). Costlow, Bookhout and Monroe (1960) applied these techniques to biology in a study investigating the survival of the larvae of the crab *Sesarma cineraum* under varying temperature and salinity combinations. Following that work and other studies on crab larvae by Costlow, Bookhout and their students, response surface techniques have been employed in studies on a variety of organisms. These include investigations on the bivalves, *Adula californiensis* (Lough and Gonor, 1973a,b), *Crassostrea virginica*, *Mercenaria mercenaria* and *Mulinia lateralis* (Lough, 1975), *Trichomya hirsuta* (Wallis, 1976a,b), *Mytilus galloprovincialis* (Hrs-Brenko et. al., 1977), *Mytilus edulis* (Widdows, 1978 a,b) and *Rangia cuneata*

(Cain, 1973), a prosobranch gastropod *Hydrobia ulvae* (Fish and Fish, 1977), the crabs *Hemigrapsus edwardsi* and *H. crenulatus* (Hicks, 1973) and *Pagurus longicarpus* (Briggs and McDermott, 1973). A variety of fish species, have also been investigated including the coho salmon (*Oncarhyncus kisutch*) (Alderdice, 1963), the Pacific cod (*Gadus macrocephalus*) (Forrester and Alderdice, 1965) and the petrale sole (*Eopsetta jordani*) (Alderdice and Forrester, 1971). In addition, an excellent review of response surface methodology as applied to the study of marine organisms has been provided by Alderdice (1972).

APPENDIX II

The experimental design employed in this study upon the addition of the third independent variable (i.e. food concentration), was a central composite design. This design was chosen for two reasons: 1) The central composite design exhibits an economy of design points with which to fit a quadratic polynomial in three independent variables as compared to a full 3^3 design (15 design points vs 27 design points for each set of replicates. 2) The central composite design can be made orthogonal with an appropriate choice of the axial points (α's) resulting in a diagonal $X'X$ matrix which, in turn provides uncorrelated estimates of the model coefficients (Myers, 1971). The group of designs known as composite designs are composed of first order factorial designs augmented by additional design points, known as the axial points of the design, which allows the coefficients of a second order surface to be determined. The addition of a design point at the center of the design space results in a central composite design of the type used in this study. A geometric representation of a central composite design with three independent variables is illustrated in figure 48. The axial points (α's) can be chosen, as in this study, to make the resulting central composite design orthogonal. This results in a design matrix for three independent variables ($k = 3$) of the form:

$$
D = \begin{array}{c}
\begin{array}{ccc} x_1 & x_2 & x_3 \end{array} \\
\left|\begin{array}{ccc}
-1 & -1 & -1 \\
-1 & -1 & 1 \\
-1 & 1 & -1 \\
-1 & 1 & 1 \\
1 & -1 & -1 \\
1 & -1 & 1 \\
1 & 1 & -1 \\
1 & 1 & 1 \\
0 & 0 & 0 \\
-\infty & 0 & 0 \\
+\infty & 0 & 0 \\
0 & -\infty & 0 \\
0 & +\infty & 0 \\
0 & 0 & -\infty \\
0 & 0 & +\infty
\end{array}\right|
\end{array}
$$

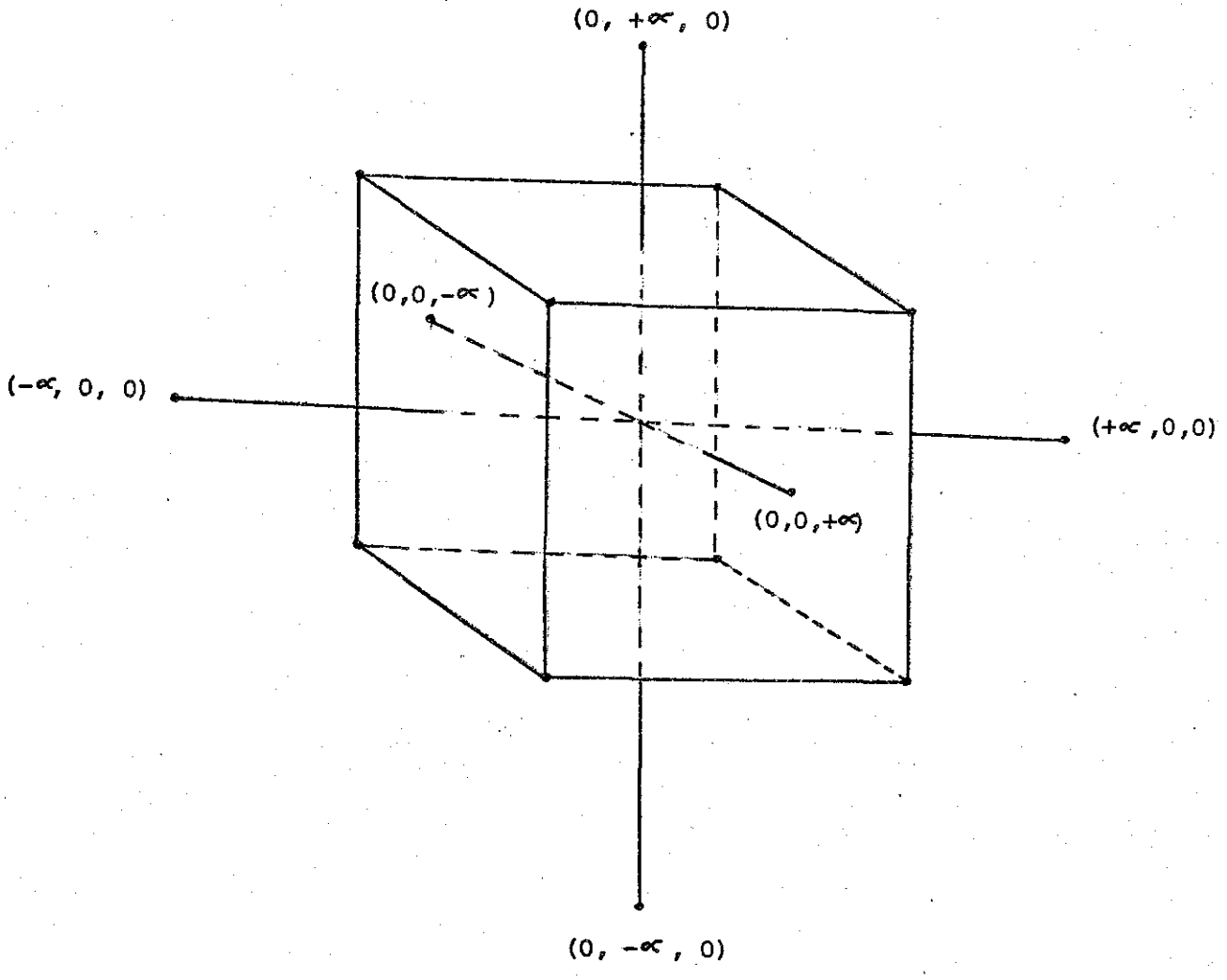

FIGURE 48

A geometric representation of the central composite design (K = 3)

(Redrawn after Myers)

APPENDIX III

```
$RESET FREE
FILE  6=OUTPUT,UNIT=REMOTE,RECORD=22
FILE  20=DESGNC,UNIT=DISK,RECORD=14,BLOCKING=30
FILE  21=DESGN2,UNIT=DISK,RECORD=14,BLOCKING=30
FILE  22=DESGN6,UNIT=DISK,RECORD=14,BLOCKING=30
FILE  23=RESPONSE,UNIT=DISK,RECORD=14,BLOCKING=30
FILE  24=DESGN1,UNIT=DISK,RECORD=14,BLOCKING=30
FILE  25=DESGNX,UNIT=DISK,RECORD=14,BLOCKING=30
FILE  26=DESGN3,UNIT=DISK,RECORD=14,BLOCKING=30
C
C
C
C
C  RESPONSE SURFACE PROGRAM
C
C
C
C
C
C
C
C
C
C  PROGRAM DEVELOPED BY ALAN L. HILLYARD
C
C
C  THIS PROGRAM WILL PLOT A TWO OR THREE VARIABLE
C  RESPONSE SURFACE.  THE POSSIBLE RESPONSES THAT
C  CAN BE PLOTTED ARE - PERCENT, ANGULAR TRANSFORM
C  OF PERCENT DATA, GROWTH IN MICRONS, GROWTH IN
C  IN SETIGERS, OR DAYS ELAPSED.  THE INDEPENDENT
C  VARIABLES ARE TEMPERATURE AND SALINITY OR
C  TEMPERATURE, SALINITY AND FOOD CONCENTRATION.
C  ADDITIONALLY INPUT FILES FOR THE VARIOUS DESIGN
C  MATRICES MUST BE SUPPLIED.  THESE ARE AS FOLLOWS:
C     DESGN1 = 2 VARIABLE UNCORRECTED FOR MEAN
C     DESGN2 = 3 VARIABLE UNCORRECTED FOR MEAN
C     DESGN3 = CCP UNCORRECTED FOR MEAN
C     DESGNC = 2 VARIABLE CORRECTED FOR MEAN
C     DESGNX = 3 VARIABLE CORRECTED FOR MEAN
C     DESGN6 = CCP CORRECTED FOR MEAN
C  THE MEASURED RESPONSE DATA IS ENTERED IN
C  RESPONSE.  THIS PROGRAM YIELDS AS OUTPUT EITHER
C  AN ANOVA TABLE OR AN ANOVA TABLE AND A PLOT.
C
C
C
C
C
C
C
```

```
C
      DIMENSION XX(100,100) ,X(100,100)
      DIMENSION XTRANS(100,100), DATA(100,1)
      DIMENSION XPROD(100,100), XXPROD(100,100)
      DIMENSION B(30),BB(100,100),BX(100,100)
      DIMENSION BC(100,100)
      DIMENSION ARAY(160,120),IPLOT(10),IBORD(15)
      DIMENSION ILBL(11),JLBL(8),KLBL(5),LLBL(5)
      DIMENSION BS(100,100),SSPAR(15),MSPAR(15)
      DIMENSION NBORD(15),FPAR(15),DFPAR(15)
      DIMENSION SSR(100,100)
      DIMENSION BF(100,100)
      DIMENSION IARC(10),IGROW(40),ISET(30)
      DIMENSION EIGENV(3,3)
      INTEGER DFREG,DFPAR,DFERR,DFTOT
      REAL MSPAR,MSREG,MSERR
      WRITE(6,101)
      READ(5,/)NVAR
      WRITE(6,102)
      READ(5,/)MM
      WRITE(6,3000)
      READ(5,/)NPLOT
      WRITE(6,444)
      READ(5,/)ICOR
      WRITE(6,996)
      READ(5,/)IDEP
      WRITE(6,302)
      READ(5,/)D1,D2
      WRITE(6,303)
      READ(5,/)D4,D3
      IF(NVAR .EQ. 2)GO TO 306
      WRITE(6,305)
      WRITE(6,304)
      READ(5,/)NC1,NC2,NC3
      READ(5,/)D5,D6
      GO TO 307
  306 NC1=10
      NC2=10
      NC3=10
      X3FIX=0
      M=-1
      IF(NVAR .EQ. 2)D6=1
  307 CONTINUE
      KLAST=0
      IF(NVAR .EQ. 2)GO TO 95
      IF(NVAR .EQ. 3) GO TO 96
      IF(NVAR .EQ.4)GO TO 97
   95 CONTINUE
      M=9
      N=6
      MX=9*MM
      DO 9 I=1,9
      DO 16 J=1,6
      IF(ICOR .EQ. 0)READ(20,/)XX(I,J)
      IF(ICOR .EQ. 1)READ(24,104)XX(I,J)
```

```
      DO 8 LL=1,MM
      K=LL+KLAST
      LLL=LL
      X(K,J)=XX(I,J)
      XTRANS(J,K)=X(K,J)
    8 CONTINUE
   16 CONTINUE
      KLAST=LLL+KLAST
    9 CONTINUE
      GO TO 111
   96 CONTINUE
      M=27
      N=11
      MX=27*MM
      DO 6 I=1,27
      DO 7 J=1,11
      IF(ICOR .EQ. 0)READ(25,/)XX(I,J)
      IF(ICOR .EQ. 1)READ(21,892)XX(I,J)
      DO 5 LL=1,MM
      K=LL+KLAST
      LLL=LL
      X(K,J)=XX(I,J)
      XTRANS(J,K)=X(K,J)
    5 CONTINUE
    7 CONTINUE
      KLAST=LLL+KLAST
    6 CONTINUE
      GO TO 111
   97 CONTINUE
      M=15
      N=10
      MX=15*MM
      DO 2 I=1,15
      DO 3 J=1,10
      IF(ICOR .EQ. 0)READ(26,/)XX(I,J)
      IF(ICOR .EQ. 1)READ(22,106)XX(I,J)
      DO 4 LL=1,MM
      K=LL+KLAST
      LLL=LL
      X(K,J)=XX(I,J)
      XTRANS(J,K)=X(K,J)
    4 CONTINUE
    3 CONTINUE
      KLAST=LLL+KLAST
    2 CONTINUE
  111 CONTINUE
      CALL MMULT(N,MX,N,X,XTRANS,XPROD)
      CALL INVERT(XPROD,N)
      READ(23,*)(DATA(NN,1),NN=1,MX)
      NB=1
      CALL MMULT(N,MX,NB,DATA,XTRANS,XXPROD)
   37 CALL MMULT(N,N,NB,XXPROD,XPROD,8)
      DATA IBLANK, ISTAR/' ', '*'/
      DATA(IPLOT(I), I=1,10)/'1','2','3','4','5','6',
     1'7','8','9','0'/
```

```
      DATA(IARC(J),J=1,10)/'0','1','2','3','4','5','6'
     1,'7','8','9'/
      DATA(IGROW(I), I=1,40)/'A','B','C','D','E','F',
     1'G','H','I','J','K','L','M','N','O','P',
     2'Q','R','S','T','U','V','W','X','Y','Z',
     3'1','2','3','4','5','6','7','8','9','0',
     4'*','#','@','$','&','+'/
      DATA(ISET(I), I=1,30)/'1','2','3','4','5','6',
     1'7','8','9','0','A','B','C','D','E','F',
     2'G','H','I','J','K','L','M','N','O','P',
     3'Q','R','S','T'/
      DATA(NBORD(I), I=1,15)/2*' ',10*'1',3*'2'/
      DATA(IBORD(I), I=1,15)/'8','9','0','1','2','3',
     1'4','5','6','7','8','9','0','1','2'/
      DATA(ILBL(I), I=1,11)/'E','R','U','T','A','R',
     1'E','P','M','E','T'/
      DATA(JLBL(I), I=1,8)/'S','A','L','I','N','I','T'
     1,'Y'/
      DATA(KLBL(I), I=1,5)/2*'2',2*'3','4'/
      DATA(LLBL(I), I=1,5)/'0','5','0','5','0'/
      DATA B0,B1,B2,B3,B11,B22,B33,B12,B23,B13,B123/
     111*0/
      WRITE(6,130)
      IF(NVAR .EQ. 2)GO TO 71
      IF(NVAR .EQ. 3)GO TO 72
      B0=B(1)
      B1=B(2)
      B2=B(3)
      B3=B(4)
      B11=B(5)
      B22=B(6)
      B33=B(7)
      B12=B(8)
      B13=B(9)
      B23=B(10)
      WRITE(6,*)B0,B1,B2,B3,B11,B22,B33,B12,B13,B23
      GO TO 73
   71 B0=B(1)
      B1=B(2)
      B2=B(3)
      B11=B(4)
      B22=B(5)
      B12=B(6)
      WRITE(6,*)B0,B1,B2,B11,B22,B12
      GO TO 73
   72 B0=B(1)
      B1=B(2)
      B2=B(3)
      B3=B(4)
      B11=B(5)
      B22=B(6)
      B33=B(7)
      B12=B(8)
      B13=B(9)
      B23=B(10)
```

```
      B123=B(11)
      WRITE(6,*)B0,B1,B2,B3,B11,B22,B33,B12,B13,B23,
     1B123
   73 CONTINUE
C CALCULATE THE STATIONARY POINT
      BB(1,1)=B11
      BB(1,2)=B12/2
      BB(2,1)=B22
      BB(2,2)=B22
      BX(1,1)=-(B1/2)
      BX(2,1)=-(B2/2)
      NBOMB=2
      IF(NVAR .EQ. 2)GO TO 501
      BX(3,1)=-(B3/2)
      BB(1,3)=B13/2
      BB(2,3)=B23/2
      BB(3,1)=B13/2
      BB(3,2)=B23/2
      BB(3,3)=B33
      NBOMB=3
      DET1=(BB(1,1)*BB(2,2)*BB(3,3))+(BB(1,2)*BB(2,3)*
     1BB(3,1))+(BB(1,3)*BB(2,1)*BB(3,2))
      DET2=(BB(1,3)*BB(2,2)*BB(3,1))+(BB(1,1)*BB(2,3)*
     1BB(3,2))+(BB(1,2)*BB(2,1)*BB(3,3))
      DETR=DET1-DET2
      BD(1,1)=((BB(2,2)*BB(3,3))-(BB(2,3)*BB(3,2)))/
     1DETR
      BD(1,2)=((BB(2,1)*BB(3,3))-(BB(2,3)*BB(3,1)))*
     1(-1)/DETR
      BD(1,3)=((BB(2,1)*BB(3,2))-(BB(2,2)*BB(3,1)))/
     1DETR
      BD(2,1)=BD(1,2)
      BD(2,2)=((BB(1,1)*BB(3,3))-(BB(1,3)*BB(3,1)))/
     1DETR
      BD(2,3)=((BB(1,1)*BB(3,2))-(BB(1,2)*BB(3,1)))*
     1(-1)/DETR
      BD(3,1)=BD(1,3)
      BD(3,2)=BD(2,3)
      BD(3,3)=((BB(1,1)*BB(2,2))-(BB(1,2)*BB(2,1)))/
     1DETR
      GO TO 709
  501 DETR=(BB(1,1)*BB(2,2))-(BB(1,2)*BB(2,1))
      BD(1,1)=BB(2,2)/DETR
      BD(1,2)=-BB(1,2)/DETR
      BD(2,1)=BD(1,2)
      BD(2,2)=BB(1,1)/DETR
  709 CALL MMULT(NBOMB,NBOMB,NB,BX,BD,BF)
      S1=(BF(1,1)*D3)+D4
      S2=(BF(2,1)*D2)+D1
      S3=(BF(3,1)*D6)+D5
      WRITE(6,107)
      IF(NVAR .EQ. 2) GO TO 502
      WRITE(6,108)S1,S2,S3
      GO TO 503
  502 WRITE(6,109)S1,S2
```

```
  503 CONTINUE
      DO 15 M=NC1,NC2,NC3
C BLANK THE ARRAY
      DO 52 I=1,160
      DO 53 J=1,120
   53 ARAY(I,J)=IBLANK
   52 CONTINUE
C READ IN THE BORDER
      ICOUNT=0
      DO 1 I=9,151
      DO 62 J=9,111
      ARAY(I,9)=ISTAR
      ARAY(I,111)=ISTAR
      ARAY(9,J)=ISTAR
      ARAY(151,J)=ISTAR
   62 CONTINUE
    1 CONTINUE
      DO 63 I=10,150,10
      ARAY(I,8)=ISTAR
      ARAY(I,112)=ISTAR
      II=I/10
      ARAY(I,7)=IEORD(II)
      ARAY(I,6)=NBORD(II)
   63 CONTINUE
      DO 64 I=10,110,25
      ARAY(8,I)=ISTAR
      ARAY(152,I)=ISTAR
      J=I+1
      ICOUNT=ICOUNT+1
      ARAY(7,I)=KLBL(ICOUNT)
      ARAY(7,J)=LLBL(ICOUNT)
   64 CONTINUE
      DO 65 I=65,75
      II=I-64
      ARAY(I,3)=ILBL(II)
   65 CONTINUE
      DO 66 I=47,54
      II=I-46
   66 ARAY(5,I)=JLBL(II)
      X3FIX=((M*1000)-D5)/D6
      X3FIX=((M*1000)-D5)/D6
C CALCULATE THE PERCENT RESPONSE
   38 CONTINUE
      GO TO (801,802,803,804,805),IDEP
  801 DO 80 IIC=20,110,10
      YFIX=(FLOAT(IIC)-10.)/100.
      MQ=(IIC-10)/10
      GO TO 999
  802 DO 865 IIA=10,100,10
      YFIX=IIA-10
      MQ=IIA/10
      GO TO 999
  803 DO 866 IIB=50,2000,50
      YFIX=IIB
      MQ=IIB/50
```

```
      GO TO 999
  804 DO 867 IID=1,25
      YFIX=IID
      MQ=IID
      GO TO 999
  805 DO 868 IIE=1,30
      YFIX=IIE
      MQ=IIE
  999 CONTINUE
C INCREMENT SALINITY AND CALCULATE TEMPERATURE
      DO 11 J=100,200
      X2FIX=((FLOAT(J)/5.)-D1)/D2
      KKK=J-90
      A=B11
      BBB=B1+B12*X2FIX+B13*X3FIX+B123*X2FIX*X3FIX
      C=B0+B2*X2FIX+B3*X3FIX+B22*X2FIX*X2FIX+B33*
     1X3FIX*X3FIX+B23*X2FIX*X3FIX-YFIX
      Q=(BBB*BBB)-(4*A*C)
      IF(Q)11,36,36
   36 S1PLOT=((-BBB+SQRT(Q))/(2*A))
      S2PLOT=((-BBB-SQRT(Q))/(2*A))
C UNCODE THE TEMPERATURES
      S1PLOT=((S1PLOT*D3)+D4)*10.
      S2PLOT=((S2PLOT*D3)+D4)*10.
      IY1=S1PLOT
      ROUND=S1PLOT-IY1
      IF(ROUND .GE. 0.5)IY1=IY1+1
      IY2=S2PLOT
      ROUND=S2PLOT-IY2
      IF(ROUND .GE. 0.5)IY2=IY2+1
      IF((IY1 .LT. 80) .OR. (IY1 .GT. 220)) GO TO 67
      LK=IY1-70
      GO TO (806,807,808,809),IDEP
  806 ARAY(LK,KKK)=IPLOT(MQ)
      GO TO 998
  807 ARAY(LK,KKK)=IARC(MQ)
      GO TO 998
  808 ARAY(LK,KKK)=IGROW(MQ)
      GO TO 998
  809 ARAY(LK,KKK)=ISET(MQ)
  998 CONTINUE
   67 IF((IY2 .LT. 80) .OR. (IY2 .GT. 220))GO TO 11
      LK=IY2-70
      GO TO(810,811,812,813),IDEP
  810 ARAY(LK,KKK)=IPLOT(MQ)
      GO TO 997
  811 ARAY(LK,KKK)=IARC(MQ)
      GO TO 997
  812 ARAY(LK,KKK)=IGROW(MQ)
      GO TO 997
  813 ARAY(LK,KKK)=ISET(MQ)
  997 CONTINUE
   11 CONTINUE
      IF(IDEP .EQ. 1)GO TO 80
      IF(IDEP .EQ. 2) GO TO 865
```

```
      IF(IDEP .EQ. 3) GO TO 866
      IF(IDEP  .EQ. 4) GO TO 867
  868 CONTINUE
      IF(IDEP .EQ. 5)GO TO 994
  867 CONTINUE
      IF(IDEP .EQ. 4) GO TO 994
  866 CONTINUE
      IF(IDEP .EQ. 3) GO TO 994
  865 CONTINUE
      IF(IDEP .EQ. 2) GO TO 994
   80 CONTINUE
  994 CONTINUE
      IF(NPLOT .EQ. 2) GO TO 3001
      DO 54 L=1,160
      KK=161-L
   54 WRITE(6,200)(ARAY(KK,J), J=1,120)
      IF(NVAR .EQ. 2) GO TO 69
      NN=M*1000
      WRITE(6,300)NN
   15 CONTINUE
   69 CONTINUE
 3001 CONTINUE
      TOTAL=0.
      DFREG=N
      DFERR=MX-N
      DFTOT=MX
      DO 88 I=1,N
   88 DFPAR(I)=1
      IF(ICOR .EQ. 1) GO TO 665
      DO 84 I=1,MX
   84 TOTAL=DATA(I,1)*DATA(I,1)+TOTAL
      DO 85 I=1,N
   85 BS(1,I)=B(I)
      CALL MMULT(N8,N,N8,XXPROD,BS,SSR)
      SSE=TOTAL-SSR(1,1)
      WRITE(6,*)(XXPROD(I,1), I=1,N)
      DO 86 I=1,N
      SSPAR(I)=(XXPROD(I,1)*BS(1,I))
   86 MSPAR(I)=SSPAR(I)/DFPAR(I)
      MSREG=SSR(1,1)/DFREG
      MSERR=SSE/DFERR
      FREG=MSREG/MSERR
      DO 87 I=1,N
   87 FPAR(I)=SSPAR(I)/MSERR
      WRITE(6,401)
      WRITE(6,402)
      WRITE(6,403)DFREG,SSR(1,1),MSREG,FREG
      NREG=N-1
      IF(NVAR .EQ. 2) GO TO 901
      WRITE(6,406)(DFPAR(I),SSPAR(I),MSPAR(I),FPAR(I),
     1I=1,N)
      IF(NVAR .NE. 3) GO TO 903
      WRITE(6,407)DFPAR(11),SSPAR(11),MSPAR(11),
     1FPAR(11)
      GO TO 903
```

```
901 WRITE(6,404)(DFPAR(I),SSPAR(I),MSPAR(I),FPAR(I),
   1I=1,N)
903 CONTINUE
    WRITE(6,632)DFERR,SSE,MSERR
    WRITE(6,408)DFTOT,TOTAL
665 CONTINUE
401 FORMAT('0',//,5X,'ANOVA TABLE',/,5X,'SOURCE',
   110X,'DF',15X,'SS',15X,'MS',15X,'F')
402 FORMAT(' ',80('-'))
403 FORMAT('0',5X,'REGRESSION',I8,2X,F15.6,2X,F15.6,
   11X,F15.6)
404 FORMAT('0',5X,'MEAN',I15,2X,F15.6,1X,F15.6,/,
   15X,'LINEAR TEMP.',I6,2X,F15.6,2X,F15.6,1X,
   2F15.6,/,5X,'LINEAR SAL.',I7,2X,F15.6,2X,F15.6,
   31X,F15.6,/,5X,'QUADRATIC TEMP.',I3,2X,F15.6,
   42X,F15.6,1X,F15.6,/,5X,'QUADRATIC SAL.',I4,
   5F15.6,2X,F15.6,1X,F15.6,/,5X,'TEMP * SAL',I8,
   62X,F15.6,2X,F15.6,1X,F15.6)
405 FORMAT('0',5X,'ERROR',I13,2X,F15.6,2X,F15.6,1X,
   1F15.6)
406 FORMAT('0',5X,'MEAN',I15,2X,F15.6,2X,F15.6,1X,
   1F15.6,/,5X,'LINEAR TEMP.',I6,2X,F15.6,2X,
   2F15.6,1X,F15.6,/,5X,'LINEAR SAL.',I7,2X,F15.6,
   32X,F15.6,1X,F15.6,/,5X,'LINEAR FOOD',I7,2X,
   4F15.6,2X,F15.6,1X,F15.6,/,5X,'QUADRATIC TEMP.'
   5,I3,2X,F15.6,2X,F15.6,1X,F15.6,/,5X,
   6'QUADRATIC SAL.',I4,2X,F15.6,2X,F15.6,1X,
   7F15.6,/,5X,'QUADRATIC FOOD',I4,2X,F15.6,2X,
   8F15.6,1X,F15.6,/,5X,'TEMP * SAL',I8,2X,F15.6,
   92X,F15.6,1X,F15.6,/,5X,'TEMP * FOOD',I7,2X,
   0F15.6,2XF15.6,1X,F15.6,/,5X,'SAL * FOOD',
   1I8,2X,F15.6,2X,F15.6,1X,F15.6)
407 FORMAT(' ',5X,'TEMP*SAL*FOOD',I5,2X,F15.6,
   12X,F15.6,1X,F15.6)
632 FORMAT('0',5X,'ERROR',I13,2X,F15.6,2X,F15.6)
408 FORMAT('0',5X,'TOTAL',I13,2X,F15.6)
130 FORMAT('0','THE MODEL COEFFICIENT VALUES = ')
200 FORMAT(' ',120A1)
300 FORMAT('0','FOOD CONCENTRATION = ',I7)
101 FORMAT(' ','CHOSE DESIGN 2=2VAR,3=3VAR.,4=CCP')
102 FORMAT(' ','ENTER NUMBER OF REPLICATES')
103 FORMAT(2I3)
104 FORMAT(I3)
892 FORMAT(F6.4)
105 FORMAT(6(F7.4,1X))
106 FORMAT(F18.11)
107 FORMAT('0','THE STATIONARY POINT IS:')
108 FORMAT('0','TEMPERATURE =',F10.7,' SALINITY =',
   1F13.7,'FOOD CONCENTRATION =',F18.8)
302 FORMAT('0','ENTER CENTRAL SALINITY VALUE, ENTER',
   1 'SALINITY SPACING')
303 FORMAT('0','ENTER CENTRAL TEMPERATURE VALUE ',
   1'ENTER TEMPERATURE SPACING')
304 FORMAT('0','ENTER INITIAL FOOD CONC./1000 ',
   1'ENTER FINAL FOOD CONC./1000, ENTER ',
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     2'INTERVALS/100'
  305 FORMAT('0','ENTER CENTRAL FOOD CONC., ENTER  ',
     1'FOOD CONC. SPACING')
  996 FORMAT(' ','ENTER DEPENDENT VARIABLE CODE --1',
     1'=RAW PERCENT;  2=ANGULAR TRANSFORMATION; 3= ',
     2'GROWTH IN MICRONS;  4=GROWTH IN SETIGERS; ',
     3'  5=DAYS')
  109 FORMAT('0','TEMPERATURE= ',F10.7,'SALINITY = ',
     1F10.7)
  444 FORMAT('0','ENTER 0 FOR MEAN CORRECTED DESIGN',
     1' ENTER 1 FOR AN UNCORRECTED DESIGN')
 3000 FORMAT('0','ENTER 1 FOR PLOTS OR 2 FOR ANOVA ',
     1'TABLE')
      CALL EIGEN(BB,EIGENV,NBCMB,NBCMB)
      END
      SUBROUTINE MMULT(NA,NC,ND,E,F,G)
      DIMENSION E(100,100),F(100,100),G(100,100)
      DO 10 I=1,NA
      DO 10 J=1,ND
      G(I,J)=0
      DO 10 II=1,NC
   10 G(I,J)=G(I,J)+(F(I,II)*E(II,J))
      RETURN
      END
      SUBROUTINE INVERT(H,NNN)
      DIMENSION H(100,100)
      NP=NNN+1
      DO 100 I=1,NNN
      DO 50 J=1,NNN
   50 H(J,NP)=0
      H(I,NP)=1
      DIV=H(I,I)
      DO 60 J=1,NP
   60 H(I,J)=H(I,J)/DIV
      DO 99 J=1,NNN
      IF(I .EQ. J) GO TO 99
      FAC =H(J,I)
      DO 98 K=1,NP
   98 H(J,K)=H(J,K)-H(I,K)*FAC
   99 CONTINUE
      DO 80 J=1,NNN
   80 H(J,I)=H(J,NP)
  100 CONTINUE
      RETURN
      END
      SUBROUTINE EIGEN(A,B,N,N1)
      DIMENSION A(100,100),B(3,3)
      ANORM=0
      DO 100 I =1,N
      DO 101 J=1,N
      IF(I-J)2,1,2
    1 B(I,J)=1
      GO TO 101
    2 B(I,J)=0
      ANORM=ANORM+A(I,J)*A(I,J)
```

```
  101 CONTINUE
  100 CONTINUE
      ANORM =SQRT(ANORM)
      FNORM=ANORM*1.0E-09/FLOAT(N)
      THR=ANORM
   23 THR = THR/FLOAT(N)
    3 IND=0
      DO 102 I=2,N
      I1=I-1
      DO 103 J=1,I1
      IF(ABS(A(J,I))-THR)103,4,4
    4 IND=1
      AL=-A(J,I)
      AM=(A(J,J)-A(I,I))/2
      AO=AL/SQRT(AL*AL+AM*AM)
      IF(AM)5,6,6
    5 AO=-AO
    6 SINX=AO/SQRT(2.*(1.+SQRT(1.-AO*AO)))
      SINX2=SINX*SINX
      COSX=SQRT(1.-SINX2)
      COSX2=COSX*COSX
      DO 104 K=1,N
      IF(K-J)7,10,7
    7 IF(K-I)8,10,8
    8 AT=A(K,J)
      A(K,J)=AT*COSX-A(K,I)*SINX
      A(K,I)=AT*SINX+A(K,I)*COSX
   10 BT=B(K,J)
      B(K,J)=BT*COSX-B(K,I)*SINX
      B(K,I)=BT*SINX+B(K,I)*COSX
  104 CONTINUE
      XT=2.*A(J,I)*SINX*COSX
      AT=A(J,J)
      BT=A(I,I)
      A(J,J)=AT*COSX2+BT*SINX2-XT
      A(I,I)=AT*SINX2+BT*COSX2+XT
      A(J,I)=(AT-BT)*SINX*COSX+A(J,I)*(COSX2-SINX2)
      A(I,J)=A(J,I)
      DO 105 K=1,N
      A(J,K)=A(K,J)
      A(I,K)=A(K,I)
  105 CONTINUE
  103 CONTINUE
  102 CONTINUE
      IF(IND)20,20,3
   20 IF(THR-FNORM)25,25,23
   25 DO 110 I=2,N
      J=I
   29 IF(A(J-1,J-1)-A(J,J))30,110,110
   30 AT=A(J-1,J-1)
      A(J-1,J-1)=A(J,J)
      A(J,J)=AT
      DO 111 K=1,N
      AT=B(K,J-1)
      B(K,J-1)=B(K,J)
```

```
      B(K,J)=AT
  111 CONTINUE
      J=J-1
      IF(J-1)110,110,29
  110 CONTINUE
      WRITE(6,*)A(1,1),A(2,2),A(3,3)
      WRITE(6,*)((B(I,J),J=1,N),I=1,N1)
      RETURN
      END
```