MULTIPLE FACTOR EFFECTS OF ENVIRONMENTAL PARAMETERS ON THE PHYSIOLOGY, ECOLOGY AND DISTRIBUTION OF SOME MARINE MEIOFAUNA [1].

by

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Résumé

Dans la nature, la méiofaune n'est généralement pas exposée à un seul facteur à la fois et il est bien établi qu'un organisme qui est soumis à l'interaction d'un ou de plusieurs facteurs atteignant les limites de sa tolérance, sera affecté d'une manière plus dévastatrice que s'il était soumis à l'action d'un seul facteur.

Le but de ces recherches est d'étudier certains aspects de l'interaction de facteurs multiples sur la méiofaune d'estuaire.

La tolérance à diverses conditions de température et de salinité a été définie à la fois en aérobiose et en anaérobiose, chez plusieurs espèces de Copépodes et chez le Cilié *Trachelo*raphis. Les résultats ont été traités statistiquement au moyen de techniques de régression multiple.

Des tentatives pour corréler les modèles de répartition saisonnière avec les résultats obtenus par les mesures de tolérance ont été effectuées.

Introduction

Within recent years, there has been increasing interest in correlating laboratory and field studies in an attempt to better understand the distribution of meiofauna. Jansson (1967) utilized tolerance and preference experiments to aid in explaining field distributions of the copepod *Parastenocaris vicesima* and other workers have attempted to correlate the physiological requirements of various species of meiofauna to habitat preference (Wieser and Kanwisher, 1961 ; Lasserre, 1969, 1970, 1971 ; Coull and Vernberg, 1970 ; Lasserre et Renaud-Mornant, 1971a, 1971b, 1973 ; Vernberg and Coull, 1974).

One of the chief characteristics of the marine environment is fluctuation in salinity; in temperate zones, temperature also cycles over a relatively wide range. As was recently pointed out by Giere (1973), oxygen is another important environmental factor in delineating distributional patterns and oxygen levels also can change daily and seasonally. In nature, meiofauna generally are not exposed to one environmental factor at a time

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and it is well-recognized that the interaction of two or more factors nearing tolerance limits generally produces a more drastic effect on organisms than would any single factor (see recent review of Vernberg, 1975). The purpose of this study was to investigate some aspects of multiple factor interaction in estuarine meiofauna.

The meiofauna used in this study were obtained from subtidal areas in the North Inlet Estuary, Georgetown, South Carolina. One of the areas, Debidue Creek, is a well-sorted (S_o=1.2) fine sand (Md 175-200/0 lying subtidally to an intertidal sandflat approximately 1 km N-W from the mouth of the North Inlet Estuary at 33°20.1' N, 79°10.1 W. The other area, Bread and Butter Creek, is 1 km West of Debidue in a tidal creek where the sediment is a fine, silty mud (Md 40-52u). Temperatures are essentially the same in both areas, ranging from 30°C in the summer months to 9.5°C during the winter. Salinity fluctuations are generally greater in Bread and Butter Creek ranging from 22-36 per mil in Debidue Creek, they range from 28-37 per mil. In both areas, the lower salinities characteristically occur during the colder months of the year. Quantitative collections have been made over the past three years in both of these regions and are continuing.

Materials and methods

Plastic buckets were used as scoops to collect the upper 2-5 cm of sediment, filled to one half their volume with sea water, capped and transported to the laboratory in air-conditioned vehicles. In the laboratory, the buckets were placed in environmental chambers set at the field temperature. Copepods were obtained by sieving through 2 sieves (500 and 120 u) and the 120u fraction was then hand-sorted. The animals were placed in sea water at temperatures approximating that of the field and used immediately for either tolerance or metabolic studies. The ciliates, *Trachelorapbis* sp., were extracted from the sediment by the sea water ice technique (Uhlig, 1968). Each *Trachelorapbis* was sorted by mouth pipette and used experimentally within 24 hours after collection. (See Vernberg and Coull, 1974 for more on *Trachelorapbis.)*

For the temperature-salinity tolerance experiments, the copepods were maintained in 11.4 cm bowls and placed in the desired temperature-salinity regime. The bowls were kept in environmental chambers and checked at 15-20 min intervals until the animals were no longer living. Cessation of activity was used as the criteria of death. It was not possible to obtain LD_{50} values since this requires a relatively large number of animals collected at the same time. 10 to 20 animals were used in each experimental group, but in some cases only 4 or 5 animals would be available for use at one time. Animals were never used more than once.

Tolerance of the organisms to anaerobic conditions was determined by placing them in 80 ml of water in 125 ml Erlenmeyer flasks and bubbling 100 per cent nitrogen into the water for 1/2 hour. A total of 10 to 20 animals were used in these experiments, and again they were monitored until all activity ceased. To insure that this procedure did result in anaerobic conditions, a control flask containing methylene blue and yeast was subjected to the same procedure. Leucomethylene blue results under these conditions and the solution turns white. It was found that the experimental procedure produced anaerobic conditions which prevailed for several days. Parallel experiments were also made in which compressed air was bubbled into flasks containing control animals; no damage to the organisms was noted.

Survival data were analyzed statistically by means of multiple regression techniques. Response surfaces were fitted based on the percent mortality of the longest survival. That is, if 100 per cent of a particular species survived 10 days under a temperature-salinity regime of 20 °C, 25 per mil, but only 1 day at 15°C, 15 per mil, then mortality under this condition was entered as 10 per cent. The program was run on an IBM 360 computer.

RESULTS

Mortality of the three most commonly found copepods, *Thompsonula hyaenae, Pseudobradya pulchera* and *Scottolana canadensis* was measured at 15, 22 and 37.5° C in combination with salinities of 25, 30 and 35 per mil. Statistical analysis of this data is shown in Tables 1, 2 and 3. For *T. hyaenae,* 57.8 per cent of the mortality is accounted

TABLE 1

Survival of *Thompsonula hyaenae.* for **24 hours** under aerobic conditions and 8 hours anaerobically in 9 temperature-salinity combinations. T, T^2 and **S,** S^2 represent linear and quadratic effects **of** temperature and salinity respectively ; T **X** S indicates temperature-salinity interaction.

Regression Step No.	Variable	R-Square	Significance Level
AEROBIC			
1	T^2	.41473	10 per cent
2	T	.52718	3 per cent
3	T × S	.52746	N.S.
4	S	.52756	N.S.
5	S	.52758	N.S.
ANAEROBIC			
1	T	.51475	3 per cent
2	T^2	.79166	5 per cent
3	S	.82865	N.S.
4	T × S	.91583	N.S.
5	S^2	.92179	N.S.

TABLE 2

Survival **of *Pseu.dobradya pulchera*** for **24** hours aerobically and **4** hours anaerobically in **9** temperature-salinity combinations. T, T^2 and S, S^2 represent linear and quadratic effects **of** temperature and salinity respectively **;** T **X S** indicates temperature-salinity interaction.

Regression Step No.	Variable	R-Square	Significance Level
AEROBIC			
1	T^2	.95503	1 per cent
2	T	.99803	1 per cent
3	S	.99894	10 per cent
4	S^2	.99925	N.S.
5	T × S	.99954	N.S.
ANAEROBIC			
1	T × S	.85243	1 per cent
2	T^2	.86873	N.S.
3	S	.97566	5 per cent
4	T	.97931	N.S.
5	S^2	.98198	N.S.

TABLE 3

Survival of *Scottolana canadensis* for 24 hours under aerobic and anaerobic conditions in 9 temperature-salinity combinations. T, T^2 and S, S^3 represent linear and quadratic effects of temperature and salinity respectively; T X S indicates temperature-salinity interaction.

Regression Step No.	Variable	R-Square	Significance Level
AEROBIC			
1	T^2	.66079	1 per cent
2	T	.70912	N.S.
3	T × S	.71069	N.S.
4	S	.72010	N.S.
5	S	.80118	N.S.
ANAEROBIC			
1	T	.57688	3 per cent
2	T^2	.76149	N.S.
3	S^2	.80611	N.S.
4	T × S	.85604	N.S.
5	S	.88136	N.S.

for by the 5 variables which were tested. Only the linear and quadratic effects of temperature, however, were significant. The 5 variables accounted for 99 per cent of the mortality in *P. pulchera;* the

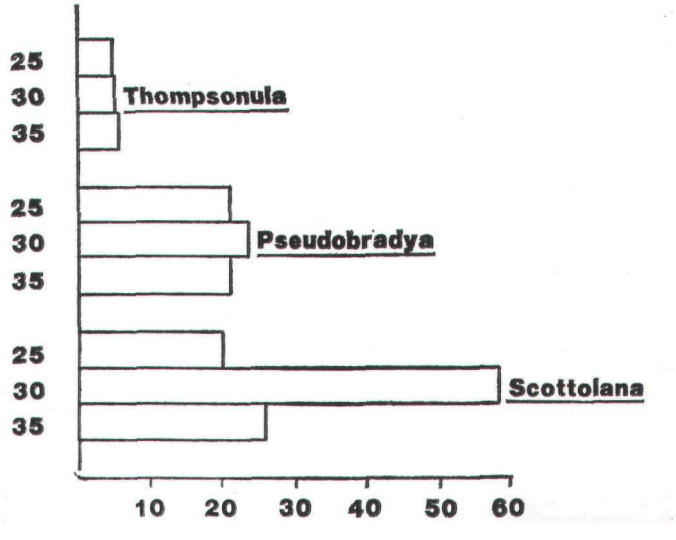

FIG. 1.

Tolerance of three species of copepods to 37.5° C under different salinity regimes (salinity per mil).

linear and quadratic effects of temperature were the most significant factors, but the linear effect of salinity also played a role. For S. *canadensis,* 80 per cent of the variability was explained by the

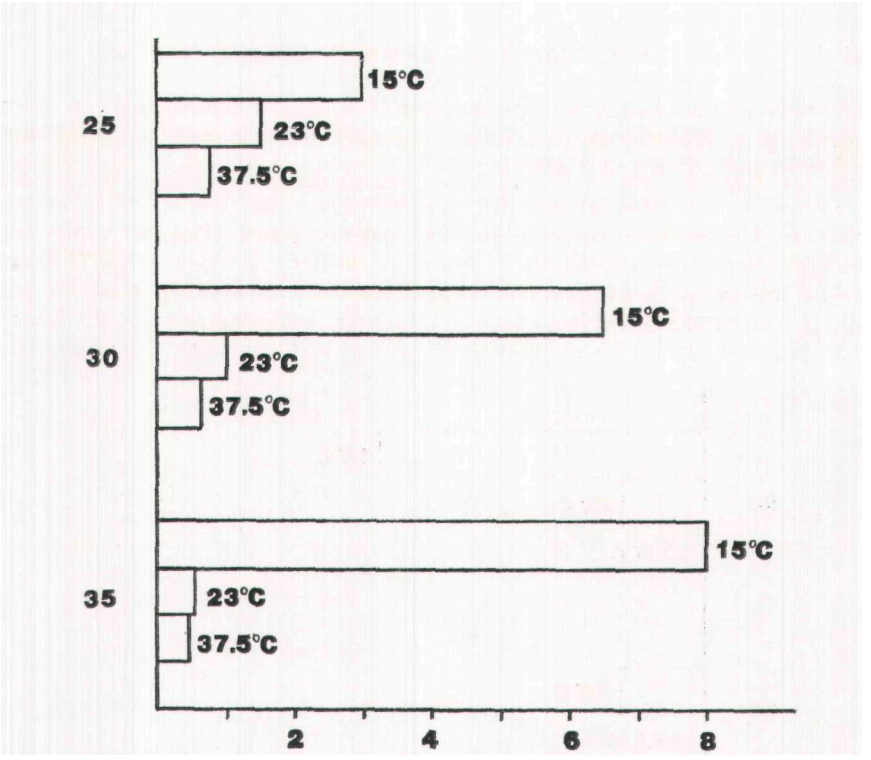

FIG. 2.

Tolerance of *Thompsonula hyaenae* to anaerobiosis under different temperature-salinity combinations (salinity per mil).

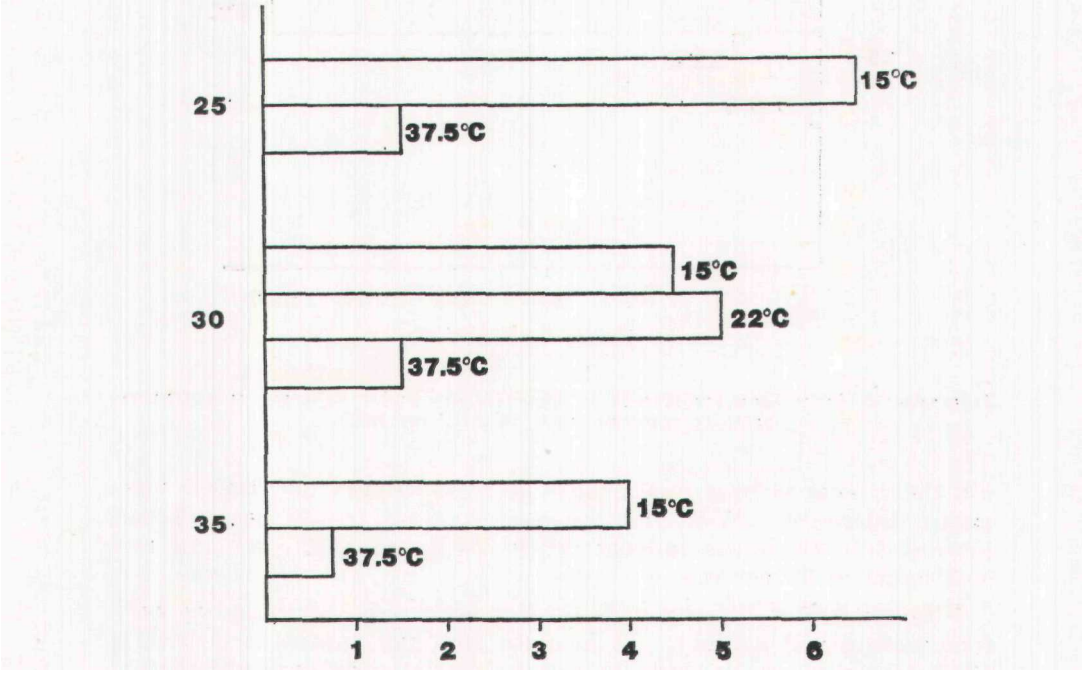

FIG. 3.

Tolerance of *Pseudobradya pulchera* to anaerobiosis under different temperature-salinity combinations (salinity per mil).

variables tested, but only the quadratic effects of temperature were significant. Temperature-salinity interaction was not a significant factor in any of these 3 species.

One of the most interesting findings was the difference in sensitivity of these three species to high temperature (Fig. 1). By far the most sensitive species is *T. hyaenae,* which survived 37.5° C from 4.5-5.5 hours, with longest survival occurring at a salinity of 35 per mil. P. *pulchera* survived exposure to high temperature for 23 hours at 30 per mil, 22 hours at 25 and 35 per mil. Only with S. *canadensis*

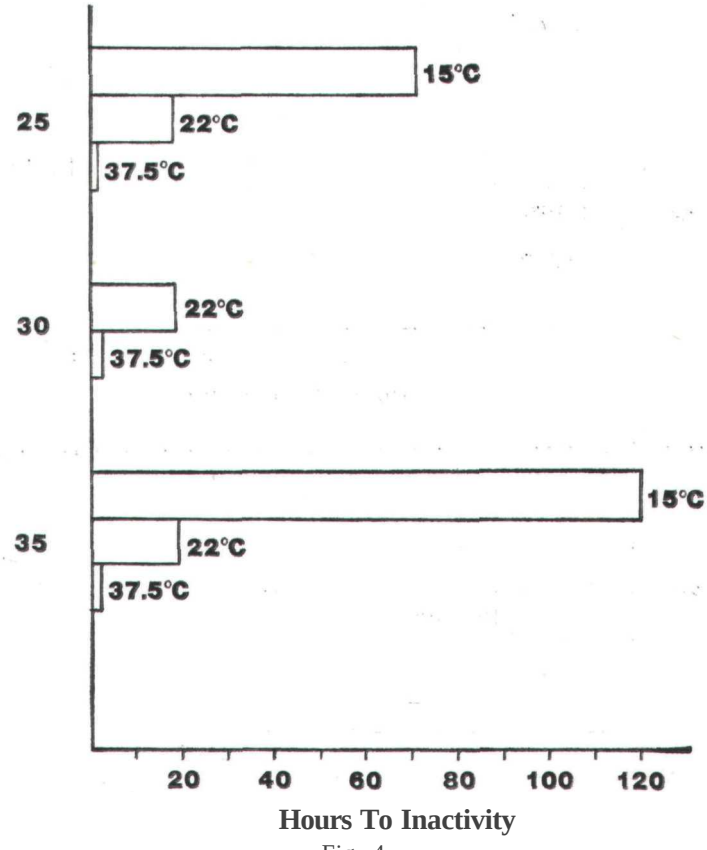

Fig. 4.
Tolerance of *Scottolana canadensis* to anaerobiosis under different temperature-salinity combinations (salinity per mil).

did there seem to be a relationship between salinity and high temperature tolerance. With this copepod, survival at 30 per mil was increased to 58 hours in contrast to 20 hours at 25 per mil and 26.5 hours at 35 per mil.

Resistance of the copepods to anaerobiosis is influenced by both temperature and salinity. *T. hyaenae* survived anaerobiosis best at 15° C regardless of the salinity, but greatest survival was obtained at 15° C, 35 per mil (Fig. 2). *P. pulchera,* on the other hand, survived best at 22° C, 30 per mil (Fig. 3), while highest survival of *S. canadensis* was observed at 15° C, 30 per mil (Fig. 4).

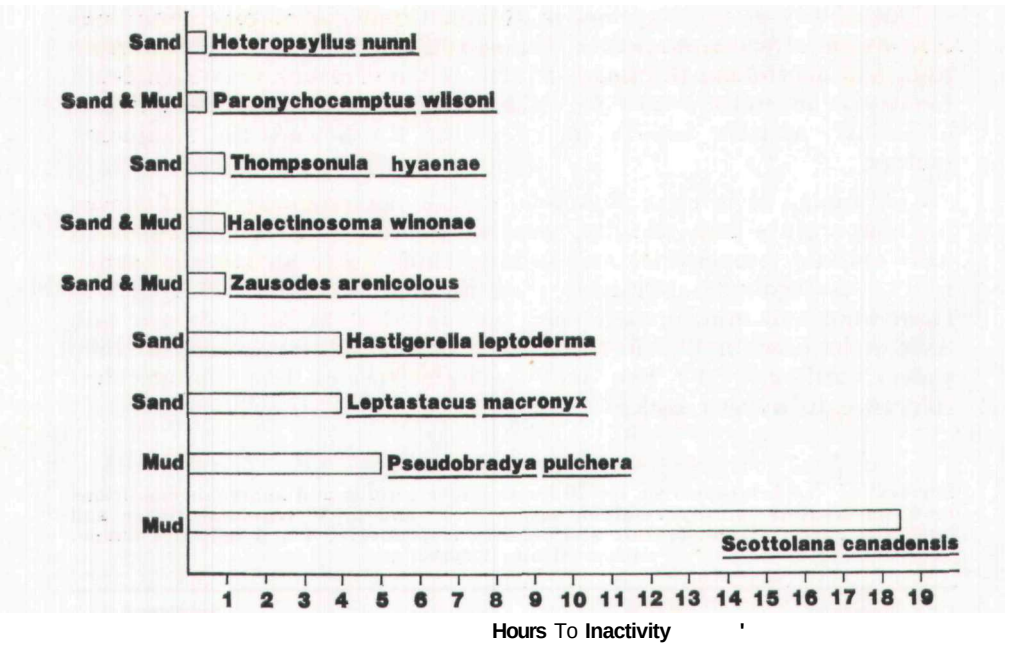

FIG. 5.

Tolerance of species of sand and mud-dwelling copepods to anaerobiosis at 21-23° C, 30 per mil.

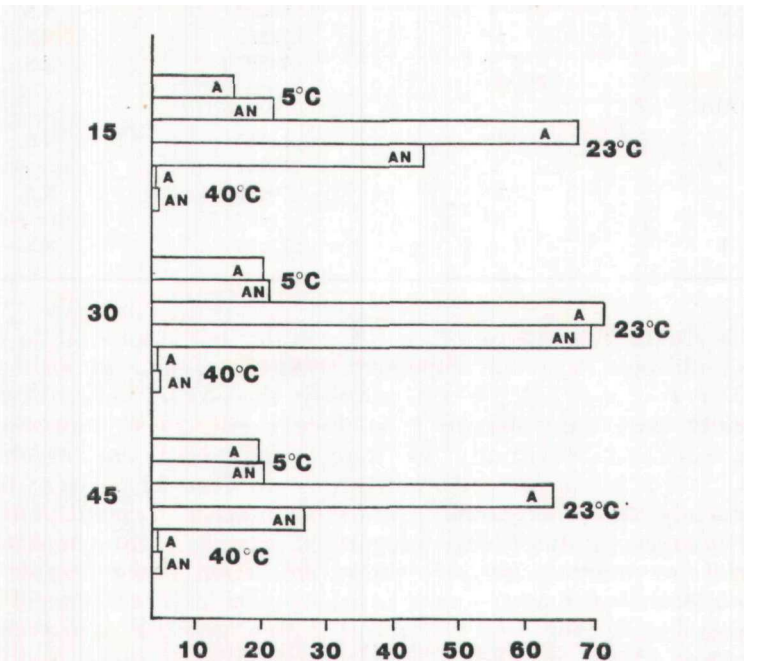

FIG. 6.

Survival of *Tracheloraphis* sp. maintained aerobically and anaerobically under different temperature-salinity combinations (salinity per mil).

Mortality of these species maintained under anaerobic conditions also was analyzed statistically (Tables 1, 2 and 3). For both *Thompsonula* and *Scottolana* the linear effects of temperature were significant factors in mortality. For *Pseudobradya,* however, the linear effects of salinity and the interaction effects of T X S were the significant factors.

Although it is clear that tolerance to anaerobiosis is influenced by temperature and salinity, relative tolerances to anaerobiosis at near optimal temperature and salinity should give an index of sensitivity. Accordingly, tolerance of a number of species of copepods from sand and mud or both were measured at 21-23° C, 30 per mil. As can be seen in Fig. 5, there was considerable variations in tolerances, although the two mud-dwelling species had the greatest tolerance to oxygen lack.

TABLE 4

Survival of *Tracheloraphis* sp. for 70 hours under aerobic and anaerobic conditions in 9 temperature-salinity combinations. T, T^2 and S, S^3 represent linear and quadratic effects of temperature and salinity respectively; T X S indicates temperature-salinity interaction.

Regression Step No.	Variable	R-Square	Significane* Level
AEROBIC			
1	T^2	.11567	N.S.
2	T	.58507	3 per cent
3	S	.66029	N.S.
4	S^2	.74269	N.S.
5	T X S	.74807	N.S.
ANAEROBIC			
1	T2	.14717	N.S.
2	T	.43954	5 per cent
3	S^2	.50147	N.S.
4	S^2	.71437	5 per cent
5	T X S	.73243	N.S.

The ciliate *Tracheloraphis* was generally more tolerant to anaerobic conditions than the copepods. At 23° C and at either low (15 per mil) or high (45 per mil) salinity, the ciliates survived longer aerobically than anaerobically. At 30 per mil, 23° C, however, the ciliates survived essentially as long anaerobically as aerobically (Fig. 6). At 5° C, 15 per mil, the ciliates survived 27 per cent longer anaerobically than aerobically; under the other stressful environmental regimes, no differences were noted between ciliates maintained aerobically or anaerobically. Mortality data from *Tracheloraphis* was analyzed statistically and is summarized in Table 4. Both aerobically and anaerobically, the 5 variables tested accounted for approximately 74 per cent of the mortality. Under aerobic conditions, the linear effects of temperature were the only single significant factor in explaining the mortality data. Anaerobically, however, both the linear effects of temperature and the quadratic effect of salinity were significant.

A response surface was estimated for survival of *Tracheloraphis* maintained under aerobic and anaerobic conditions. The 80 per cent survival contours for these curves are shown in Fig. 7. Maximum survival under aerobic conditions is predicted at 13-31° C, 20-50 per mil; under anaerobic conditions 18-23° C, 28-38 per mil.

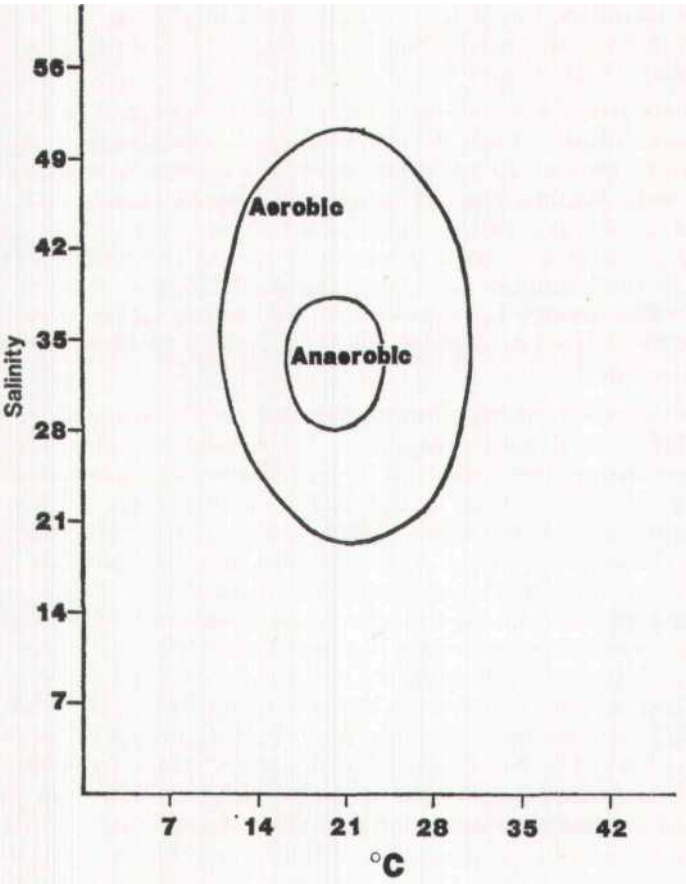

FIG. 7.
Comparison of 80 per cent survival contours of response surface estimation of survival of *Tracheloraphis* sp. maintained aerobically and anaerobically under different temperature-salinity combinations (salinity per mil).

DISCUSSION

Of the three most commonly found species of copepods, *T. hyaenae, P. pulchera* and *S. canadensis, P. pulchera* has the widest known latitudinal range. This mud-dwelling species has been reported from New Jersey to South Carolina, from California, and in the Caribbean. In South Carolina, population peaks occur in March and April when water temperatures range from approximately 14-22° C and salinities from 23-30 per mil. It occurs only rarely at other seasons of the

year. S. *canadensis* (see Coull, 1972, for taxonomic revision) has been reported from Nova Scotia to South Carolina and occurs as a burrower and epibenthic species. It is most abundant in late spring, but can be found from March to August. The temperature during this period ranges from 14-28° C, and salinities from 23-33 per mil. *T. hyaenae* lives in sand and has been reported from Massachusetts to South Carolina, but it is most abundant in spring, fall and winter. The thermal-salinity conditions when numbers of animals are highest range from 14-27 °C and 30-35 per mil.

Some correlation was observed between tolerance to high temperature and salinity. Both *P. pulchera* and *S. canadensis* survived high temperature best at 30 per mil; increased tolerance was particularly striking with *S. canadensis* (Fig. 1). Greatest tolerance in *T. hyaenae* occurred at 35 per mil. These differences in survival times can probably be correlated with the salinity ranges encountered in nature, for in the sand habitat salinities tend to be higher than in the mud habitat. The greater tolerance of *S. canadensis* to temperature compared to the other two species could be related to the greater habitat area it occupies.

The seasonal distributional pattern of 7'. *hyaenae* is an interesting one. It all but disappears during the summer months, but reappears during the early fall before the waters have cooled down to any extent. Statistical analysis of mortality rates of this copepod maintained in the laboratory under different temperature-salinity regimes showed that only 57.8 per cent of the mortality could be explained by these factors. One clue as to cause of their distributional pattern may lie in the decreased resistance of *T. hyaenae* to anaerobic conditions at higher temperatures (Fig. 2). During most of the year, the prevailing winds are from the northeast open ocean side of our Debidue Station with the result that the sand is well-mixed and oxygenated. During the summer months the prevailing winds are from the southwest, pushing in silt-clays from the Winyah Bay area with the result that oxygen levels are lowered, thus producing an undesirable environment for this species. Furthermore, from our monthly field measurements of Eh over the past two years, the redox layer migrates in the sediment. During the winter, spring and fall, reducing conditions (—50 to —250 mv) occur at 10-12 cm, whereas during the summer the redox discontinuity layer varies between 4-8 cm. It seems probable that the disappearance of *T. hyaenae* during the summer months is due to its greatly increased sensitivity to oxygen lack in the sediment (partially evidenced by the upward migration of the redox layer and the increased silt clay content) in combination with the higher temperatures.

The ciliate *Tracheloraphis* sp. is found in the North Inlet Estuary only from May to September when water temperatures range from 22-30° C and salinities from 22-35 per mil. In contrast to the copepods, this ciliate survives approximately the same length of time anaerobically as aerobically under a thermal-salinity regime of 23° C, 30 per mil (Fig. 6). Since this is the thermal-salinity range in which the greatest number of *Tracheloraphis* occurs, it may indicate that these organisms spend a portion of their lives in nature in anaerobic situations. Salinity in addition to temperature is a significant cause

of mortality in these organisms when they are subjected to anaerobic conditions (Table 4), thus their seasonal occurrence in Debidue Creek may be correlated with the seasonal temperature-salinity fluctuations occurring here.

Except for the work of Gray and Ventilla (1973), we are unaware of multiple factor analyses on marine micro-meiofauna. We have attempted to demonstrate that to more fully understand the organismal-environmental interactions, it is imperative to observe multiple factor effects. We have, by no means, answered all the questions regarding these multiple factor effects on our experimental organisms. We have, however, provided important data to help explain their distribution and hopefully such studies will stimulate others into this most fascinating and rewarding avenue of research. We are continuing our studies on other meiofauna with the goal of better answering some of the important questions regarding distribution of these omnipresent organisms.

Acknowledgments

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Summary

In nature, meiofauna generally are not exposed to one environmental factor at a time, and it is well-recognized that exposure to the interaction of one or more factors nearing tolerance limits usually produces a more drastic effect on organisms than would exposure to a single factor. The purpose of this study was to investigate some aspects of multiple factor interaction in estuarine meiofauna.

Tolerances to various conditions of temperature and salinity were determined both aerobically and anaerobically in several species of copepods and a species of the ciliate *Tracheloraphis*. Results were analyzed statistically by means of multiple regression techniques.

An attempt has been made to correlate seasonal distributional patterns with the results of the tolerance studies.

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