SYNERGISTIC EFFECTS OF ENVIRONMENTAL VARIABLES ON THE METABOLISM OF THE COPEPOD *EUTERPINA ACUTIFRONS* FROM TWO DIFFERENT AREAS OFF THE COAST OF THE STATE OF SÃO PAULO, BRAZIL[1]

G. SOARES MOREIRA[2] AND W. B. VERNBERG[3]

ABSTRACT

The combined effects of temperature and salinity on the respiratory rate of two populations of the copepod *Euterpina acutifrons* have been determined. One population was taken from a nonpolluted area, São Sebastião Channel, and the other from a polluted area, Santos Bay, both off the coast of the State of São Paulo, Brazil. Four groups of copepods were used in the experiments: 1) São Sebastião animals kept in São Sebastião water (35‰ salinity); 2) Santos animals kept in Santos water (28‰ salinity); 3) São Sebastião animals kept in Santos water; and 4) São Sebastião animals kept in diluted São Sebastião water (28‰ salinity). Results showed that São Sebastião copepods in either full strength seawater (35‰) or lower salinity seawater (28‰) could metabolically regulate over a wider range of salinities than could Santos copepods in Santos water or São Sebastião copepods maintained in Santos water. It was concluded that the water quality of the Santos Bay was responsible for changes in the metabolic regulatory capacity of the copepods exposed to Santos water.

The planktonic harpacticoid *Euterpina acutifrons* (Dana) is distributed in the warm waters of the world between lat. 66°N and 40°S (Haq 1972). It is a euryhaline species and has been reported in salinities ranging from 8‰ (Cananeia Estuary, southern Brazil, Tundisi 1972) to 39‰ (Mediterranean Sea, El-Maghraby 1965). Laboratory studies have shown that reproduction can occur over a salinity range of 15 to 45‰ (Moreira and Yamashita 1975). *Euterpina acutifrons* is an important link in the marine trophic web serving as food source for both adult and larval fishes (Pouchet and de Guerne 1887; Lebour 1918; Blin 1923; Carvalho 1945; Marques 1951; Thayer et al. 1974).

In an earlier paper, Moreira (1975) reported that salinity tolerances for Brazilian populations of *E. acutifrons* from Santos were very different from those of populations of this species from São Sebastião. This in itself is not surprising since the salinity regimes of the two areas are different. The salinity in the Santos Estuary varies widely from 17 to 30‰ depending on the tide and season of the year, while in São Sebastião Channel the salinity is approximately 35‰ throughout the year. Water temperatures in both areas are essentially the same, ranging from 19° to 30°C depending upon season. It was not determined, however, if the observed differences in salinity tolerances of the two populations were genetically or environmentally induced. Subsequently, a study was initiated to resolve this question by measuring metabolic response patterns of specimens from both populations to different thermal-salinity regimes. It soon became apparent that environmental parameters other than temperature and salinity were factors in determining the metabolic response patterns of these copepods.

A detailed chemical analysis of the water in Santos Bay is not available, but great numbers of tankers and other vessels continuously operate near shore, discharging ballast water and contaminating seawater and adjacent regions with petroleum. In addition, there are a large number of industries that discharge wastes directly into the water. One sample analysis of Santos Bay seawater was found to contain 270 ppb lead and 200 ppb nickel (unpublished data). Furthermore, to minimize the effects of human waste or degradation products, approximately 400 tons of chlorine are added monthly near shore. The data presented in this paper demonstrate that

[1]Contribution no. 214 of the Belle W. Baruch Institute for Marine Biology and Coastal Research.

[2]Physiology Department, Institute of Biosciences, and Institute of Marine Biology, University of São Paulo, Brazil.

[3]School of Public Health, Belle W. Baruch Institute for Marine Biology and Coastal Research and Department of Biology, University of South Carolina, Columbia, SC 29208.

Manuscript accepted September 1977.
FISHERY BULLETIN: VOL. 76, NO. 2, 1978.

metabolic response patterns of *E. acutifrons* to the normal fluctuations found in estuarine systems were significantly altered by the water quality of Santos Bay water.

MATERIALS AND METHODS

The copepods were collected in two fixed locations off the coast of the State of São Paulo, one in Santos Bay (lat. 23°59'S; long. 46°19'W), the other in São Sebastião Channel (lat. 23°50'S; long. 45°25'S). Collections were made with a nylon plankton net (20 mμ) during the winter when water temperatures averaged approximately 21°C. All samples were brought immediately into the laboratory whereupon *E. acutifrons* were sorted from the plankton using a mouth pipette under a binocular microscope. The copepods were placed in 2-l crystallizing dishes, 20 cm in diameter and 15 cm high. In the first two series of experiments, the copepods were placed in the water obtained from the collection points, i.e., copepods from São Sebastião Channel were placed in São Sebastião water (35‰) and copepods from Santos were placed in Santos water (28‰). In the last two series of experiments, copepods from São Sebastião were placed either in Santos water or in São Sebastião water diluted to 28‰. The copepods were maintained under temperature and photoperiod regimes approximating field conditions: 19°-24°C and 11 L:13 D. The copepods were fed with *Phaedactylum* and *Platymonas* daily and kept in the laboratory at least 1 wk before being used in the respiration experiments.

Oxygen uptake was determined using Cartesian diver respirometers (Holter 1941), which have a total volume of 8-13 μl. Only nongravid females were used. Two or three copepods were placed in each diver, depending upon the salinity/temperature regime of the experiment. The oxygen uptake was determined during a 2-h interval. The first 30-min reading was discarded; after this initial reading, uptake rates remained constant.

Oxygen uptake rates were measured under the following environmental conditions: São Sebastião animals maintained in São Sebastião water, Santos animals in Santos water, and São Sebastião animals in Santos water, 15°, 20°, 25°, 30°, and 32°C at 15, 25, 35, 45, and 55‰ salinities. The oxygen uptake of São Sebastião animals maintained in São Sebastião water diluted to 28‰ was determined at 15°, 25°, and 30°C over the same salinity ranges used in the other experiments. Ten determinations were made under each set of environmental conditions. Distilled water or freeze-concentrated brine was added to filtered seawater to attain the desired salinities. Salinities were determined by titrating against silver nitrate (Harvey 1955).

Dry weights for the copepods were obtained using a Torbal[3] torsion balance, 0.01 mg sensitivity. The copepods were rinsed with distilled water and dried at 70°C for 24 h before they were weighed. Three replicates of 200 nongravid females from each area of collection were used. Results were expressed as microliters of oxygen per milligram per hour. Significant difference of means was calculated by the method of Simpson et al. (1960) for small samples.

The metabolic data obtained in the first three series of experiments were analyzed statistically using multiple regression techniques. The basic experimental design used in this study is usually referred as a factorial design. Specifically, the plan was a 5 × 5 factorial using five levels of temperature and five levels of salinity, making in all 25 combinations of experimental conditions. Since 10 determinations of oxygen uptake were made in each combination, a total of 250 observations were made in each series. Thus, the 250 observations may reasonably be considered as continuous responses of a function of the two factors and interactions.

Oxygen uptake data were analyzed as percentage of oxygen consumption relative to that at 25°C and 35‰ salinity for São Sebastião animals in São Sebastião water and at 25°C and 25‰ salinity for Santos animals in Santos water and São Sebastião animals in Santos water, i.e., the rate under these "standard" conditions was assumed to be 100%, and rates obtained under other regimes were calculated as the percent deviation from that rate. Since the observations are treated as percentage measurements generated by data from binomial populations, the transformation Y = arc sin $\sqrt{x}$, where x is observed percent respiration, is appropriate to stabilize variances (Mendenhall 1968). Analysis of variance for this data indicated which of the factors (temperature, salinity, or temperature-salinity interactions) had significant effects on the metabolism of the copepods. The program was run on an IBM 360 computer.

[4]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

RESULTS

Animals From São Sebastião in São Sebastião Water (35‰)

The metabolic rate of *Euterpina acutifrons* from São Sebastião which were maintained in water from São Sebastião Channel was less influenced by changes in salinity than were the other groups of *E. acutifrons* (Figure 1). Rate did, however, increase with increasing temperature up to 30°C; at 32°C, rates either leveled off or decreased. Greatest increases in respiration rates were observed between 15° and 20°C. These increases are reflected in the relatively high metabolic rates within this thermal range obtained from this group of animals.

At 20°C metabolic rates were not significantly different over the entire salinity range of 15-55‰, and at 15°C the copepods were able to regulate their metabolism over a range of 25-55‰. At higher temperatures (25°, 30°, and 32°C) rates generally were lower at the salinity extremes (15, 45,55‰) and highest at 35‰. Rates varied from a minimum of 4.87 to a maximum of 22.36 μl/mg h^{-1} dry weight (Figure 1).

Statistical analysis indicated that 66% of the observed variability in the rates could be explained by the temperature-salinity combinations (Table 1), although the linear effect of temperature was the single significant factor (1% level). The linear effect of salinity, the quadratic effects of the temperature and salinity, and the temperature-salinity interaction did not contribute significantly to the observed changes in respiration rates. Figure 2A shows the response surface contours fitted over the experimental design.

TABLE 1.—Analysis of variance of data for *Euterpina acutifrons* (São Sebastião animals in São Sebastião water, 35‰). T = temperature, S = salinity.

Variable	r^2	Significance level
T	0.54953	1%
T^2	0.65769	Not significant
S	0.65796	Not significant
S^2	0.65885	Not significant
T × S	0.65933	Not significant

Animals From Santos in Santos Water (28‰)

In the Santos animals maintained in Santos water, the rate of oxygen uptake also increased over the temperature range to 30°C for the entire salinity range. In most of the salinities, the largest metabolic increase occurred between 25° and 30°C. This contrasts with São Sebastião copepods which exhibited the largest increase between 15° and 20°C. The Santos animals did not show the metabolic regulation observed in the São Sebastião animals maintained in São Sebastião water, and tended to have low metabolic rates at the salinity extremes, i.e., 15, 45, and 55‰. Highest rates occurred at salinities of 25-35‰. The rates varied from a minimum of 7.97 to a maximum of 28.20 μl/mg h^{-1} dry weight (Figure 1).

Statistical analysis indicated that only 46% of the observed variability in the respiration rates of these animals could be explained by the temperature-salinity combinations (Table 2). The significant factors were the quadratic effects of temperature and salinity (0.05% level) and the linear effect of salinity (0.05% level). The temperature-salinity interaction was not a significant factor. Figure 2B shows the response surface contours fitted over the experimental design.

TABLE 2.—Analysis of variance of data for *Euterpina acutifrons* (Santos animals in Santos water 28‰). T = temperature, S = salinity.

Variable	r^2	Significance level
T^2	0.24350	0.05%
S^2	0.28955	0.05%
S	0.46193	0.05%
T × S	0.46198	Not significant

Animals From São Sebastião in Santos Water

Transfer of São Sebastião copepods into water from Santos markedly altered their metabolic responses, especially their response to salinity. Respiration rates increased with temperature up to 25°C at the extreme salinities (15, 45, 55‰) and up to 30°C at salinities of 25 and 35‰, before leveling off or decreasing. The copepods which were transferred to Santos water did not regulate metabolically at any temperature at the salinity extremes. Lowest rates were obtained at salinities of 15 and 55‰, and the highest rates were observed at 25‰ at 15° and 25°C. At 30° and 32°C, peak metabolic rates occurred at 35‰ (Figure 1). Rates varied from a minimum of 6.80 to a maximum of 37.23 μl/mg h^{-1} dry weight (Figure 1).

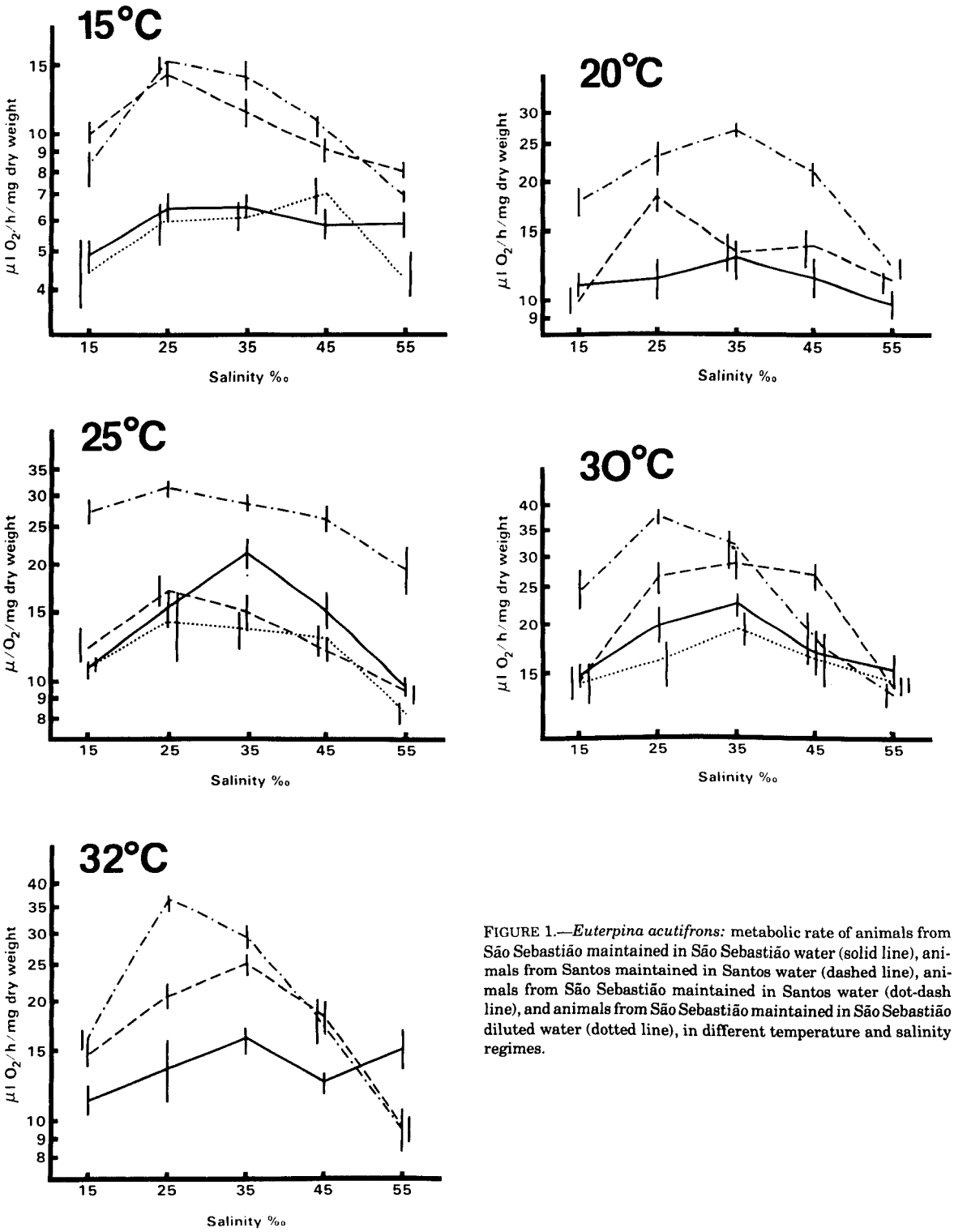

FIGURE 1.—*Euterpina acutifrons:* metabolic rate of animals from São Sebastião maintained in São Sebastião water (solid line), animals from Santos maintained in Santos water (dashed line), animals from São Sebastião maintained in Santos water (dot-dash line), and animals from São Sebastião maintained in São Sebastião diluted water (dotted line), in different temperature and salinity regimes.

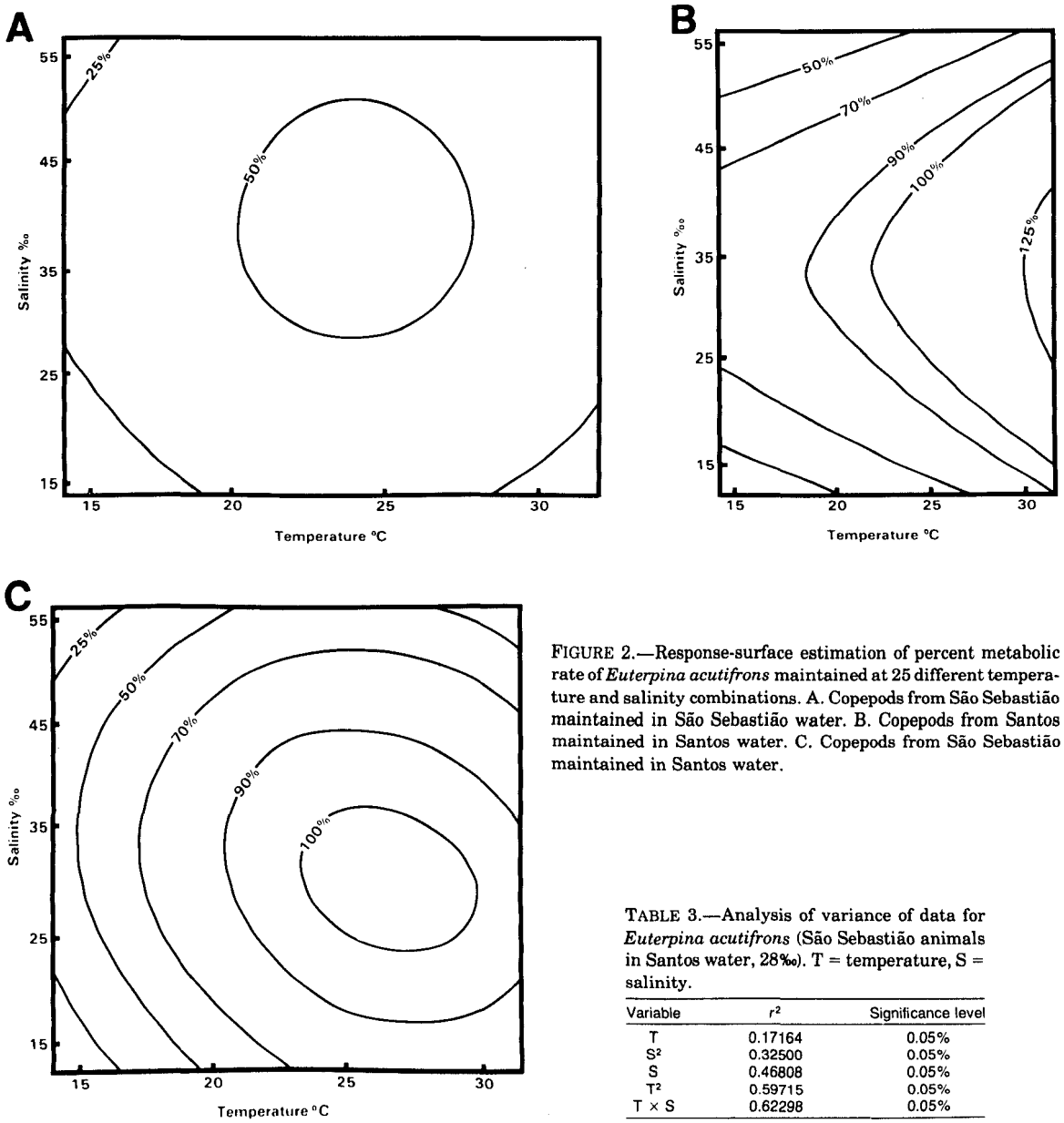

FIGURE 2.—Response-surface estimation of percent metabolic rate of *Euterpina acutifrons* maintained at 25 different temperature and salinity combinations. A. Copepods from São Sebastião maintained in São Sebastião water. B. Copepods from Santos maintained in Santos water. C. Copepods from São Sebastião maintained in Santos water.

TABLE 3.—Analysis of variance of data for *Euterpina acutifrons* (São Sebastião animals in Santos water, 28‰). T = temperature, S = salinity.

Variable	r^2	Significance level
T	0.17164	0.05%
S^2	0.32500	0.05%
S	0.46808	0.05%
T^2	0.59715	0.05%
T × S	0.62298	0.05%

All of the analyzed factors, i.e., linear temperature and salinity, as well as the quadratic effect of these factors, and the temperature-salinity interaction, contributed significantly (0.05% level) to the observed variability in respiration rates (Table 3). A total of 62% of the variability could be explained by these various factors. Figure 2C shows the response surface contours fitted over the experimental design.

Animals From São Sebastião in São Sebastião Diluted Water

In this series the respiration experiments were run at three temperatures to test whether or not the results obtained for Santos animals were the result of acclimation to a lower salinity. The respiration rates at 28‰ were essentially the same as those for animals maintained in undiluted São

Sebastião water. At 15°, 25°, and 30°C, metabolic rates were not significantly different over the range of 15-45‰. The rates varied from a minimum of 4.32 to a maximum of 19.17 μl/mg h^{-1} dry weight (Figure 1).

DISCUSSION

In Brazilian waters, populations of *E. acutifrons* thrive over a wide range of salinities and variable salinity alone does not seem to be a limiting factor in their distributional patterns (Tundisi 1972; Moreira and Yamashita 1975). Indeed, of the various environmental variables tested, temperature alone significantly affected the metabolic rates of these copepods. The present data demonstrate that specimens of copepods from the unpolluted São Sebastião Channel have the capability of metabolic regulation over a wide range of salinities when tested using São Sebastião water. On the other hand, marked diminution in the capability to regulate metabolically at salinity extremes was noted in *E. acutifrons* from the Santos population and specimens from São Sebastião maintained in Santos water. For both groups of animals salinity, as well as temperature, proved to exert a statistically significant effect at the 5% level (or less) on their oxygen uptake rates. These marked changes in metabolic control in the copepods taken from or exposed to Santos water compared with that of copepods from São Sebastião are depicted in Figure 2.

While we did not measure population densities of the *E. acutifrons* in our two study areas (Santos Bay and São Sebastião Channel), there is some indication in the literature that population size is sensitive to polluted waters. Gabriel et al. (1975) reported a decrease in abundance of this species in the Milford Haven Estuary following its development into the largest oil port in the United Kingdom in the 1960's, and there are several examples that indicate that pollutants can affect the survival of copepods and planktonic larvae. Barnes and Stanbury (1948) have studied the toxic action of copper and mercury salts on the copepod *Nitocra spinipes* and verified that mercuric chloride is a very effective poison; in contrast, these animals are very resistant to copper. D'Agostino and Finney (1974) have found that copper and cadmium inhibit growth and development of the copepod *Tigriopus japonicus* at 0.064 mg/l and 0.044 mg/l, respectively. Heinle (1969) suggested that the high mortality rate of *Acartia tonsa* in a power plant effluent was due to the chlorination of the cooling water, correlating the apparent periodicity in the mortality rate with the chlorination schedule. Latimer et al. (1975) studied the toxicity of 30-min exposures of residual chlorine to two species of copepods, *Limnocalanus macrurus* and *Cyclops bicuspidatus thomasi*. The predicted "safe" concentrations were 0.9 mg/l for *L. macrurus* and 0.5 mg/l for *C. b. thomasi*. Roberts et al. (1975) studied the acute toxicity of chlorine to some estuarine species, including molluscan larvae, copepods, shrimps, and fishes. They found that molluscan larvae and *Acartia tonsa* were the most sensitive species tested, with 48-h TL_{50} values at chlorine levels <0.005 ppm. Gray (1974) demonstrated that lead ($Pb(NO_3)_2$) at 0.3 ppm reduced the growth rate of the marine ciliate protozoan *Cristigera* by 11.7% and at 0.15 ppm by 8.46%. Mercury was found to have an effect on survival, metabolism, and behavior of the planktonic larvae of *Uca pugilator* (DeCoursey and Vernberg 1972; Vernberg et al. 1973). Generally, larvae are much more sensitive to toxicants than are adults and very low concentrations of a toxicant can interact with environmental factors to cause increased mortalities among larvae (Vernberg 1975).

Detailed chemical analyses of Santos water obviously are needed, but the very high concentration of lead and nickel which were found in one sample, plus the oil and other industrial effluents that are being discharged, leave little doubt that the Santos Estuary is highly polluted. Data presented in this paper strongly suggest that specimens living in the Santos Estuary do so at a high cost energetically. This high metabolic cost for survival following exposure to salinity extremes would almost certainly be a factor limiting the distribution of *E. acutifrons* in polluted estuaries, since fluctuating salinity regimes are characteristic of this environment. Results obtained in this study highlight the fact that the physiological responses of marine organisms may be markedly modified if test animals are taken from or exposed to polluted waters.

LITERATURE CITED

BARNES, H., AND F. A. STANBURY.
1948. The toxic action of copper and mercury salts both separately and when mixed on the harpacticid copepod, *Nitocra spinipes* (Boeck). J. Exp. Biol. 25:270-275.

BLIN, [F.]
1923. Note sur l'alimentation de la Sardine. Euterpes et Sardines. Bull. Soc. Zool. Fr. 48:99-105.

CARVALHO, J. DE PAIVA.
1945. Copepodos de Caioba e Taia de Guaratuba. Arq. Mus. Parana. 4:83-116.

D'AGOSTINO, A., AND C. FINNEY.
1974. The effect of copper and cadmium on the development of *Tigriopus japonicus*. *In* F. J. Vernberg and W. B. Vernberg (editors), Pollution and physiology of marine organisms, p. 445-463. Academic Press, N.Y.

DECOURSEY, P. J., AND W. B. VERNBERG.
1972. Effect of mercury on survival, metabolism and behaviour of larval *Uca pugilator* (Brachyura). Oikos 23:241-247.

EL-MAGHRABY, A. M.
1965. The seasonal variations in length of some marine planktonic copepods from the eastern Mediterranean at Alexandria. Crustaceana 8:37-47.

GABRIEL, P. L., N. S. DIAS, AND A. NELSON-SMITH.
1975. Temporal changes in the plankton of an industrialized estuary. Estuarine Coastal Mar. Sci. 3:145-151.

GRAY, J. S.
1974. Synergistic effects of three heavy metals on growth rates of a marine ciliate protozoan. *In* F. J. Vernberg and W. B. Vernberg (editors), Pollution and physiology of marine organisms, p. 465-485. Academic Press, N.Y.

HAQ, S. M.
1972. Breeding of *Euterpina acutifrons*, a harpacticid copepod, with special reference to dimorphic males. Mar. Biol. (Berl.) 15:221-235.

HARVEY, H. W.
1955. The chemistry and fertility of sea waters. Camb. Univ. Press, N.Y., 224 p.

HEINLE, D. R.
1969. Temperature and zooplankton. Chesapeake Sci. 10:186-209.

HOLTER, H.
1941. Technique of the Cartesian diver. C. R. Trav. Lab. Carlsberg (Chim.) 24:399-478.

LATIMER, D. L., A. S. BROOKS, AND A. M. BEETON.
1975. Toxicity of 30-minute exposures of residual chlorine to the copepods *Limnocalanus macrurus* and *Cyclops bicuspidatus thomasi*. J. Fish. Res. Board Can. 32:2495-2501.

LEBOUR, M. V.
1918. The food of post-larval fish. J. Mar. Biol. Assoc. U.K. 11:433-469.

MARQUES, E.
1951. Copepodes encontrados no conteudo gastrico de algun clupeideos da Guine Portuguesa. Anais Junta Invest. Colon. 6:11-18.

MENDENHALL, W.
1968. Introduction to linear models and the design and analysis of experiments. Wadsworth, Belmont, Calif., 465 p.

MOREIRA, G. S.
1975. Studies on the salinity resistance of the copepod *Euterpina acutifrons* (Dana). *In* F. J. Vernberg (editor), Physiological ecology of estuarine organisms, p. 73-79. Belle W. Baruch Libr. Mar. Sci. 3, Univ. S.C. Press, Columbia.

MOREIRA, G. S., AND C. YAMASHITA.
1975. Influencia de la salinidad en la reproduction y desarrollo de *Euterpina acutifrons* (Dana). *In* Memorias I Simposion Latino-americano sobre Oceanografia Biologica (Mexico), p. 236-245.

POUCHET, G., AND J. DEGUERNE.
1877. Sur la nourriture de la Sardine. C. R. Acad. Sci., Paris 104:712-715.

ROBERTS, M. H., JR., R. J. DIAZ, M. E. BENDER, AND R. J. HUGGETT.
1975. Acute toxicity of chlorine to selected estuarine species. J. Fish. Res. Board Can. 32:2525-2528.

SIMPSON, G. G., A. ROE, AND R. C. LEWONTIN.
1960. Quantitative zoology. Revised ed. Harcourt, Brace and Co., N.Y., 450 p.

THAYER, G. W., D. E. HOSS, M. A. KJELSON, W. F. HETTLER, JR., AND M. W. LACROIX.
1974. Biomass of zooplankton in the Newport River estuary and the influence of postlarval fishes. Chesapeake Sci. 15:9-16.

TUNDISI, T. M.
1972. Aspectos ecologicos do zooplankton da jegiao lagunas de Cannaneia com especial referencia aos Copepoda (Crustacea). Ph.D. Thesis, São Paulo Univ., São Paulo.

VERNBERG, W. B.
1975. Multiple factor effects on animals. *In* F. J. Vernberg (editor), Physiological adaptation to the environment, p. 521-537. Intext Educational Publishers, N.Y.

VERNBERG, W. B., P. J. DECOURSEY, AND W. J. PADGETT.
1973. Synergistic effects of environmental variables on larvae of *Uca pugilator*. Mar. Biol. (Berl.) 22:307-312.