Journal of Applied Bacteriology 1992, **73**, 465-471

Susceptibility of the brine shrimp *Artemia* and its pathogen *Vibrio parahaemolyticus* to chlorine dioxide in contaminated sea-water

M. Esther Puente, F. Vega-Villasante, Gina Holguin and Y. Bashan
Department of Microbiology, Division of Experimental Biology, The Center for Biological Research (CIB), La Paz, B.C.S. 23000, Mexico

4142/02/92: accepted 27 May 1992

M.E. PUENTE, F. VEGA-VILLASANTE, G. HOLGUIN AND Y. BASHAN. 1992. Adults and nauplii of the brine shrimp, *Artemia*, together with *Vibrio parahaemolyticus*, were placed in sewage-contaminated sea-water which had been treated with chlorine dioxide (Hallox E-100™) to test its potential as a disinfectant for salt water aquaculture. The nauplii were very susceptible to low concentrations of chlorine dioxide (47 mg/l Cl^-), but the adults were slightly more resistant. Sterile sea-water treated with lower concentrations of chlorine dioxide (less than 47 mg/1 Cl^-) had no effect on the shrimp, but inhibited the growth of *V. parahaemolyticus*. In sewage-contaminated sea-water, chlorine dioxide levels of 285-2850 mg/l, necessary for the inactivation of *V. parahaemolyticus* and any native bacteria, destroyed the *Artemia* culture. Hallox E-100™ persisted in sea-water for 18 h, but later decayed. We conclude that: (i) *Artemia* nauplii are a sensitive and convenient test-organism to determine low concentrations of chlorine dioxide in sea-water; (ii) chlorine dioxide is efficient for controlling *V. parahaemolyticus* in sea-water; and (iii) chlorine dioxide should be further evaluated as a potential disinfectant for aquaculture, but, for higher organisms than *Artemia*.

INTRODUCTION

*Vibrio*sis is a common disease of economically important marine invertebrates (Egidius 1987; Chan et al. 1989). The causative organisms are *Vibrio parahaemolyticus* in shrimp, oysters, mussels and other molluscs (Gunther & Catena 1980; Vanderzant et al. 1970; Nolan et al. 1984), and *Listonella anguillarum* (*V. anguillarum*) in shrimp (Lightner 1983), as well as many marine fishes (Fraser & Mays 1982). *Vibrio alginolyticus* also infects shrimp under cultivation (Lightner 1983). Vibriosis in marine animals also offers a serious threat to public health as several strains of *Vibrio* spp. cause human gastroenteritis (Nolan *et al*. 1984).

Sea-water can be disinfected by ultraviolet irradiation, filtration, heat treatment, exposure to ozone and/or by the application of oxidative agents or surface-active chemicals (Ledo *et al*. 1983; Lightner 1983; Jacobson & Liltved 1988). As many of these treatments are also harmful to higher forms of life, new strategies and chemical treatments are constantly being sought. Although chlorine dioxide can be toxic to some early stages of marine life (Wilde *et al.* 1983; Hose *et al.* 1989) it is used in the treatment of potable water with no reported hazardous effects (Abdel-Rahman et al. 1985; Ames & Stratton 1987; Guttman-Bass *et al*. 1987). Several commercial preparations of chlorine dioxide are considered to be safe for human consumption, but lethal to harmful bacteria (de Guevara 1955; Berg *et al.* 1986; Foegeding et al. 1986; Kenyon *et al*. 1986; Harakeh 1987; Harakeh *et al*. 1988).

The brine shrimp *Artemia* is a small aquatic invertebrate which is used worldwide as a live food in aquaculture. Unlike most other marine organisms, it is an ideal species for toxicity tests because: (i) it does not require the maintenance of stock cultures; (ii) it can be cultivated in massive numbers in a limited amount of water; (iii) it has a very short life-span, enabling many experiments to be conducted in a short time; (iv) as it is transparent, microscopic examinations of live specimen are possible; and (v) its abundance facilitates replication for statistical analyses (Vanhaecke *et al.* 1980). For these reasons the brine shrimp is widely used

Correspondence to: Dr Y. Bashan, Department of Microbiology, CIB, La Paz, PO Box 128, B.C.S., Mexico 23000.

by pharmaceutical, agricultural and petro-chemical industries for toxicity tests (Grosch 1980; Leonhard & Lawrence 1980).

In this study the susceptibility of *V. parahaemolyticus* and the brine shrimp *Artemia* to commercial preparation of chlorine dioxide was evaluated.

MATERIALS AND METHODS

Organisms and culture conditions

Vibrio parahaemolyticus strain MMF6 was isolated from sea-water from the coast of Guerrero Negro, northern Baja California Sur, Mexico, on Marine Agar (Difco, 2216E) and identified according to Baumann & Baumann (1981) and Lightner (1983) by the following characteristics: rod shape; Gram-negative, -positive tests; growth on McConkey agar; growth on TCBS agar; congo red incorporation; oxidase, catalase, glucose fermentation; orange fluorescence in McConkey agar with u.v. light; negative results for haemolysis; sucrose fermentation, lactose fermentation and methyl red reaction.

Artemia sp. nauplii were cultivated from dry commercial cyst preparation (Ocean Star International, Snowville, UT) as follows: Cysts were suspended in 1 l of u.v.-treated sea-water in an Erlenmeyer flask, fluorescent-illuminated with 70 $\mu E/m^2/s$ at ambient temperature and ventilated with a small, standard, commercial aquarium air-pump. Two days after hatching the nauplii were transferred to a 20 l glass container under the same conditions. They were fed daily with 1 ml of the microalgae *Isochrysis galbana* (inoculum of 2×10^8 cells/ml, yielding 10^4 cells/ml culture). The sea-water was replaced every 2 d. A new culture was initiated routinely every 40 d, and organisms from 7 to 35-d-old were used for our experiments.

Sea-water

Sea-water samples were obtained 20 m offshore in the Ensenada de La Paz, southern Baja California, Mexico. This large, lagoon-type bay continuously receives diluted sewage water from an oxidation plant located 10 km from the sampling site, and raw sewage from numerous vacation yachts anchored in the bay as well as from the over-flooded sewage system of the city during rain storms. The level of dissolved organic nitrogen in the bay water can reach a high of 50 mg/1, declining rapidly within a few days because of the strong tides of the bay. The natural *V. parahaemolyticus* population in La Paz bay is unknown, but the number of coliforms is stable and ranged from 47 300 cfu/ml (high tide) to 52 000 cfu/ml (low tide) on 9/7/1988. Sea-water for this study was taken in August, 1991. Course particles were removed by filtration through Whatmann No. 1 filter paper. In one experiment, the sea-water samples were sterilized with u.v. irradiation before inoculation. Naturally-occurring species of marine bacteria in the samples were not identified.

Disinfectant solution

A commercial preparation of Halox E-100™. (Halox American, Burlingame, CA) containing approximately 26 mg/ml of active chlorine was used. The chemical composition of fresh Halox E-100™ is: total Cl, 41-04 mg/ml; free Cl^-, 26·42 mg/ml (Hager unpublished). This solution was diluted in sea-water to give final concentrations (mg/l) of 2850, 285, 95 and 47 active chlorine (to treat *Vibrio*) and additional concentrations of 24, 12 and 6 (to treat *Artemia*). At each time-interval, total chlorine residue in all the samples was determined by a chlorine 'test kit' (Hach, Loveland, USA).

Infection of *Artemia* with *Vibrio parahaemolyticus*

Vibrio parahaemolyticus MMF6 was grown in marine broth (Difco, 2216) at 30°C for 18 h on a rotary shaker (Lab-line, Melrose Park, IL) at 100 rev/min. The inoculum was washed three times in sterile sea-water by centrifugation (7000 g for 10 min each time) and diluted to 10^9 cfu/ml. It was further diluted to the required concentration in 50 ml samples. *Artemia* nauplii were suspended in these suspensions under the same environmental and feeding conditions described above. The containers were monitored for three consecutive days and the mortality rate of *Artemia* was scored. Both dead and live organisms were sampled and prepared for microscopic examination.

To verify that the microalgal feed and the *Artemia* batch culture were free from vibrios before inoculation, samples (0-1 ml) were collected from the microalgae culture and spread on Thiosulphate Citrate Bile Salt Sucrose agar (TCBS, Difco). Batch *Artemia* nauplii (approx. 100 individuals per sample) were washed with sterile saline solution (0-85% NaCl), macerated in a mortar and pestle, re-suspended in 5 ml of the same solution and plated on TCBS agar. The same method was used to detect Vibrios in the inoculation experiments. The plates were incubated at 37°C for 24 h, and the *Vibrio* colonies counted.

Treatment of *V. parahaemolyticus* and the natural marine bacteria in sea-water with chlorine dioxide

Glass containers with 10 ml sea-water treated with chlorine dioxide were inoculated separately with 10^4-10^5 cfu/ml of *V. parahaemolyticus*. The numbers of bacteria in each inoculum were estimated immediately afterward by the plate count method on marine agar 2216E by sampling 50 ml volumes from each container. The containers were incubated at 30°C and sampled 6, 18, 24 and 48 h after inocu-

lation. Before counting the bacteria the residual chlorine was neutralized with several drops of 10% sodium-thiosulphate. At each time-interval, bacteria were counted by the plate count method on Marine Agar 2216E.

Treatment of *Artemia* nauplii and adults with chlorine dioxide

Artemia growing in batch culture were transferred to new glass containers containing fresh sea-water for the duration of the experiments. The size of the containers and the number of organisms per container varied. *Artemia* nauplii were placed in 15 ml containers containing 30 organisms. Adult *Artemia* were placed in 250 ml Erlenmeyer flasks, 10 per flask, or a single organism in a 15 ml container. All experiments were carried out at ambient temperature. After an acclimation time of 2-4 h, the respective concentrations of chlorine dioxide were added and immediately mixed. The containers were illuminated and ventilated as described above. Mortality of *Artemia* nauplii and adults was monitored daily.

Determination of organic matter In sea-water

This was done by the open reflux (potassium dichromate) method of Clesceri *et al.* (1989).

Experimental design and statistical analysis

All experiments were randomly designed in three to six replicates. A replicate consisted of one Erlenmeyer flask or 30 individual *Artemia*. Experiments with bacteria were repeated twice and those with *Artemia*, six times each. Results from all experiments for each organism were combined and were analysed statistically with significance values given at $P \leq 0\text{-}05$ with one-way analysis of variance (ANOVA), linear regression analysis and by calculation of standard error.

RESULTS

Infection of *Artemia* nauplii with V. *parahaemolyticus*

Artemia nauplii were found to be highly susceptible to infection by *V. parahaemolyticus*. The threshold inoculum which killed *Artemia* nauplii was 10^4 cfu/ml; 10^8 cfu/ml resulted in a complete destruction of the entire population (Fig. la). Half of the *Artemia* nauplii population died 48 h after inoculation and mortality usually climbed above 70% 24 h after that (Fig. lb). Light-microscopy of the transparent organism revealed several abnormal features in the inoculated animals: empty digestive tract despite the abundance of food, atypical swimming, thinner and smaller bodies, and at high inoculum levels, even the excretion of *Vibrio*-type bacteria (results not shown).

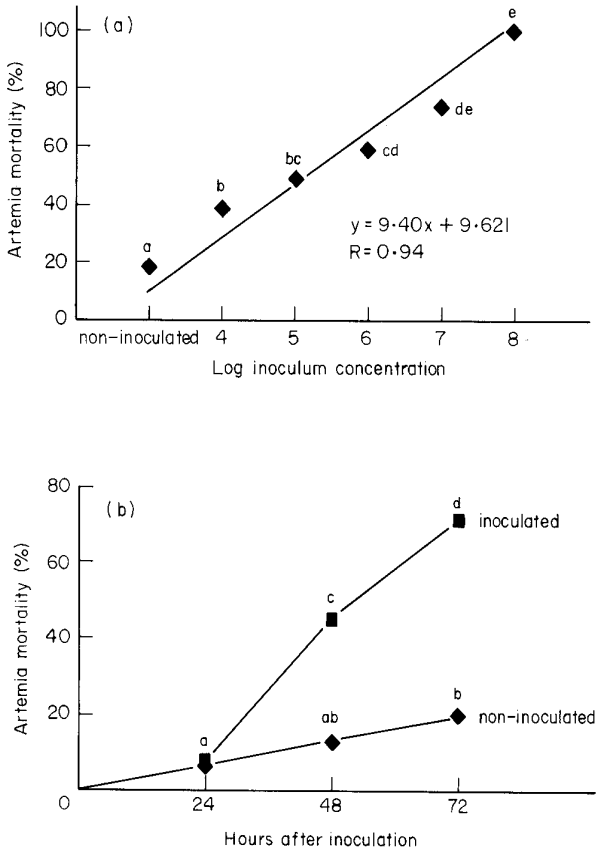

Fig. 1 Infection of 1-week-old *Artemia* nauplii with *Vibrio parahaemolyticus*. (a) Dose-response to bacteria. (b) Effect of incubation time. Points denoted by different letters within each sub-figure differ significantly at $P \leq$, 0-05 using linear regression analysis and ANOVA , respectively

Effect of chlorine dioxide on *Artemia* nauplii and adults

Artemia nauplii were found to be highly susceptible to chlorine dioxide. A low concentration of 47 *mg*/1 resulted in over 70% mortality of the nauplii. However, this level seemed to be a threshold concentration; lower levels had no effect on the viability of *Artemia* nauplii (Fig. 2a). Higher levels of chlorine dioxide eliminated the entire population (results not shown). *Artemia* adults tolerated up to 95 *mg*/1 of chlorine dioxide (Fig. 2b), but were as sensitive as nauplii to concentrations above this level (results not shown).

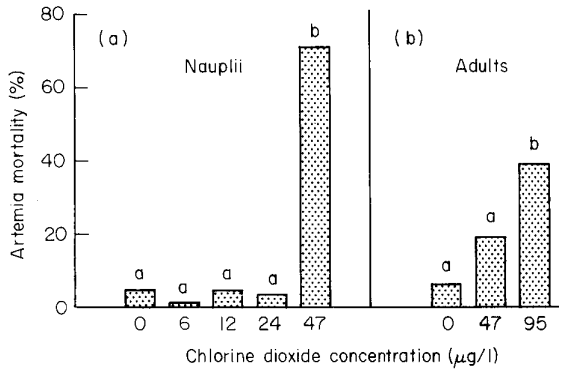

Fig. 2 Inactivation of *Artemia* nauplii (a) and adults (b) by chlorine dioxide. Columns denoted by different letters within each sub-figure differ significantly at $P \leq 0{\cdot}05$ using ANOVA

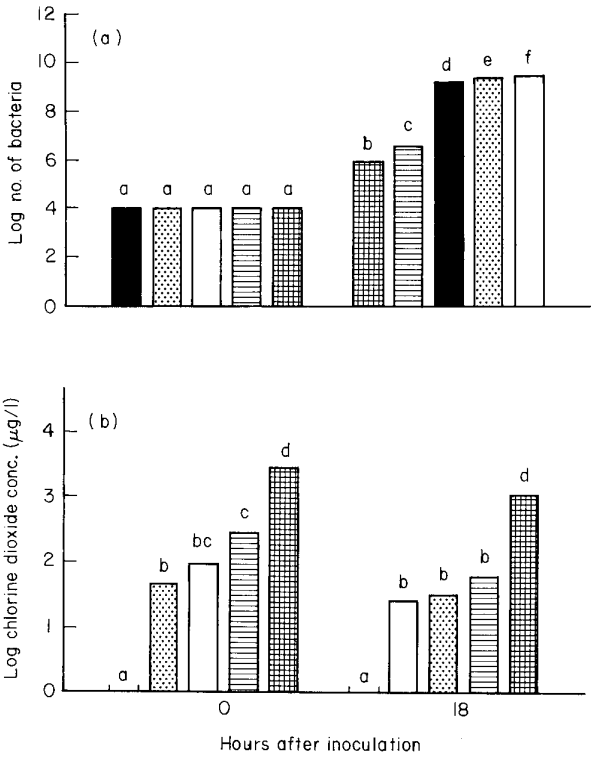

Fig. 3 (a) Inhibition of the growth of natural marine bacteria in natural sea-water after incubation in the presence of various concentrations of chlorine dioxide. (b) Chlorine dioxide concentration in the treated sea-water before incubation and 18 h after. (*mg*/1 chlorine dioxide): ■, 0; ⊡, 47; □, 95; ▤, 285; ▦, 2850. Columns denoted by different letters within each sub-figure differ significantly at $P \leq 0{\cdot}05$ using ANOVA

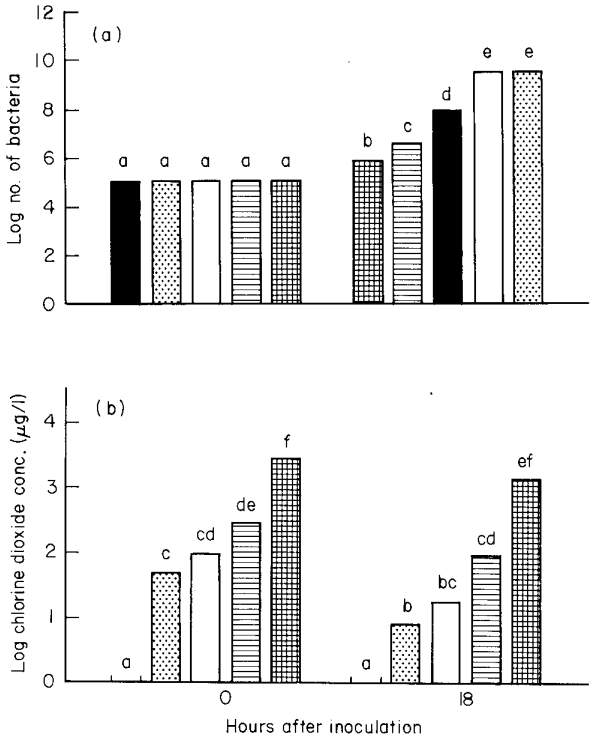

Fig. 4 (a) Inhibition of the growth of natural marine bacteria supplemented with *Vibrio parahaemolyticus* in natural sea-water after incubation in the presence of various concentrations of chlorine dioxide. (b) Chlorine dioxide concentration in the treated sea-water before incubation and 18 h after. (*mg*/l chlorine dioxide): ■, 0; ⊡, 47; □, 95; ▤, 285; ▦, 2850. Columns denoted by different letters within each sub-figure differ significantly at $P \leq 0{\cdot}50$ using ANOVA

Effect of chlorine dioxide on the native marine population in natural sea-water

Incubation of natural sea-water for 18 h without further treatment significantly increased total bacterial counts. Only two concentrations of chlorine dioxide (285 and 2850 *mg*/l) significantly inhibited the development of the bacterial population. However, they were unable to completely inhibit the bacteria, and bacterial counts remained significantly higher than at inoculation time (Fig. 3a). No significant effect on sea-water pH was observed during the incubation period and the pH after incubation for 18 h (controls and the different treatments), was 7·4 ± 0·4. The amount of organic matter in all samples was similar: 1·33 ± 0·125 mg/1. Furthermore, this incubation period had no effect on the residual chlorine dioxide in the water

and its content was almost identical after incubation for 18 h (Fig. 3b).

Almost identical results were obtained when,natural sea-water was inoculated with *V. parahaemolyticus*. Although inoculation increased the number of bacteria in the inoculated sea-water, the total bacterial population 18 h later showed a similar trend as the non-inoculated water (compare Fig. 4a with Fig. 3a). Also, the level of chlorine dioxide in sea-water containing *V. parahaemolyticus* was similar to that of the non-inoculated water (compare Fig. 4b with Fig. 3b).

Effect of chlorine dioxide on V. parahaemolytlcus in sterilized sea-water

Incubation of *V. parahaemolyticus* in sterile sea-water significantly increased the population with time, up to 48 h after inoculation (Fig. 5a). Native marine bacterial development in natural sea-water was similarly affected by inoculation. The addition of any concentration of chlorine dioxide (47-285 *m*g/l) significantly reduced the population of *V. parahaemolyticus* after 48 h, and the addition of the highest concentration (2850 *m*g/l) completely arrested further development of the initial inoculum (Fig. 5a, empty triangle). The level of chlorine dioxide in sterile sea-water inoculated with *V. parahaemolyticus* was stable only at its highest level, but decreased after 24 h of incubation with lower concentrations (Fig. 5b). The pH values of all sea-water treated with various concentrations of chlorine dioxide were stable throughout the experiments and were 7·93 ± 0·048 pH units.

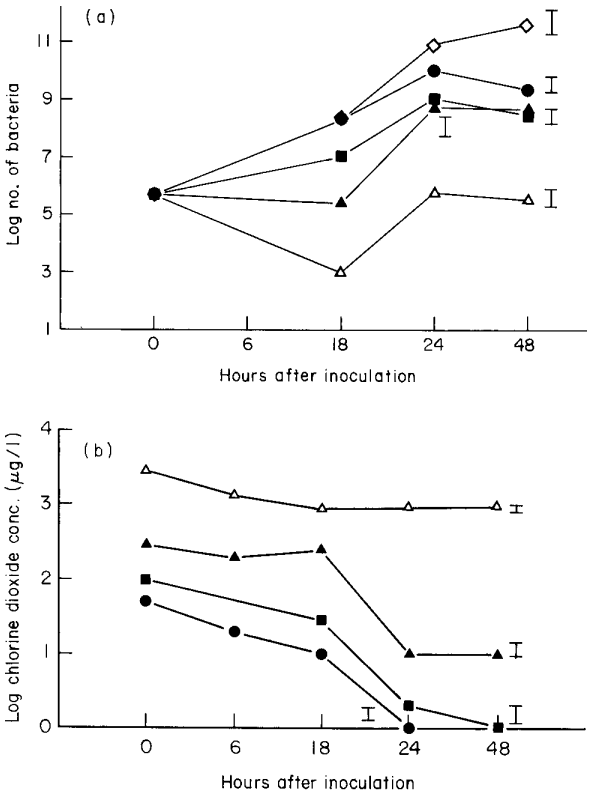

Fig. 5 (a) Inhibition of growth of *Vibrio parahaemolyticus* with time in sterile sea-water after incubation in the presence of various concentrations of chlorine dioxide. (b) Chlorine dioxide concentration during the period of the experiment. (*m*g/l chlorine dioxide): ◇, 0; ●, 47; ■, 95; ▲, 285; △, 2850. Bars represent the standard error of each line

DISCUSSION

Aquaculture is a large consumer of chlorine products (Lightner 1983). The evaluation of new products is therefore important to both sea-food producers and consumers. As stable chlorine dioxide compounds kill micro-organisms but pose a negligible health hazard to humans, we evaluated one of them (Hallox E-100™) as a potential disinfectant for aquaculture.

Hallox E-100™ efficiently inactivated naturally-occurring marine bacteria in sewage-contaminated sea-water, as well as *V. parahaemolyticus*, a short generation time bacterium (Ulitzur 1974) and one of the most destructive pathogens in aquaculture. The concentration required for decontamination was relatively low (285 *m*g/l seawater). Nevertheless, this low concentration destroyed *Artemia* nauplii and adults which are extremely sensitive to chlorine compounds, as well as other chemical compounds (Grosch 1980; Leonhard & Lawrence 1980). While chlorine dioxide may be a suitable disinfectant for the aquaculture of other shrimp, it seems that this specific preparation is not practical for use in *Artemia* cultivation. However, the extreme sensitivity of *Artemia* makes it an excellent test-organism for minute amounts of this compound in sea-water.

The soluble organic nitrogen in sea-water rarely exceeds 5-20 *m*g/l (Strickland & Parsons 1977). The nitrogen concentration in the study site varied greatly with the diurnal tides, but it was always much higher than this figure. This may explain the rapid multiplication of the native marine bacteria and *V. parahaemolyticus* when samples of sea-water were incubated. Presumably, these levels of bacteria cannot be reached under natural conditions because of: (i) the lower temperatures of the water in the bay (24-28°C in the summer); and (ii) the continuous predation by marine organisms which were filtered out of our samples. Sewage-free sea-water samples were not evaluated in this study because the entire bay is contaminated, and the surrounding area lacks efficient sewage-treatment facilities. However, it will be useful to compare these results with sea-water samples obtained from remote, uninhabited areas

of the peninsula which have been designated as future aquaculture sites.

Hallox E-100™ is known to be stable in fresh water (Hallox Corp., unpublished). This study gives further experimental evidence that the compound is also stable in sea-water. Further study is needed to evaluate the significance of organic matter in sea-water on the survivability of the compound in sea-water.

In conclusion, we suggest that: (i) chlorine dioxide has potential as an agent against *V. parahaemolyticus* and should be further tested for its toxicity against seafood organisms; and *(ii) Artemia* nauplii can serve as a highly sensitive bioassay for monitoring minute amounts of chlorine dioxide in disinfected sea-water.

ACKNOWLEDGEMENTS

Y. Bashan participated in this study in memory of the late Mr Avner Bashan from Israel. We thank Dr Jose-Luis Ochoa from our division for launching research on Hallox in marine pathology, Mr Roy Bowers for careful English corrections and Mr Robert King from Hallox California Co., USA, for donation of Hallox and a kit for detection of chlorine dioxide residues in water.

REFERENCES

ABDEL-RAHMAN, M.S., COURI, D. & BULL, R.J. (1985) Toxicity of chlorine dioxide in drinking water. *Journal of Environmental Pathology, Toxicology and Oncology* **6**, 105-114.

AMES, R.G. & STRATTON, J.W. (1987) Effect of chlorine dioxide water disinfection on hematologic and serum parameters of renal dialysis patients. *Archives in Environmental Health* **42**, 280-285.

BAUMANN, P. & BAUMANN, L. (1981) The marine Gram negative eubacteria. In *The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria* ed. Starr, M.P. pp. 1302-1331. Berlin: Springer-Verlag.

BERG, J.D., ROBERTS, P.V. & MATIN, A. (1986) Effect of chlorine dioxide in selected membrane functions of *Escherichia coli*. *Journal of Applied Bacteriology* **60**, 213-220.

CHAN, K.-Y., WOO, M.L., LAM, L.Y. & FRENCH, G.L. (1989) *Vibrio parahaemolyticus* and other halophilic *Vibrios* associated with seafood in Hong Kong. *Journal of Applied Bacteriology* **66**, 57-64.

CLESCERI, L.S., GREENBERG, A.E. & TRUSSELL, R.R. (ed.) (1989) *Standard Methods for the Examination of Water and Waste Water, pp.* 5-13, 17th edn. Washington DC: American Public Health Association.

DE GUEVARA, M. L. (1955) Aqueous chlorine dioxide antiseptic compositions and production thereof. US Patent 2 701 781.

EGIDIUS, E . (1987) *Vibrio*sis : pathogenicity and pathology. A review. *Aquaculture* **67**, 15-28.

FOEGEDING, P.M., HEMSTAPAT, V. & GIESBRECHT, F. G. (1986) Chlorine dioxide inactivation of *Bacillus* and *Clostridium* spores. *Journal of Food Science* **51**, 197-201.

FRASER, M.C. & MAYS, A. (ed.) (1982) *Merck Veterinary Manual*, 3rd edn, pp. 1072. (Spanish). New Jersey: Merck.

GROSCH, D . S . (1980) Alterations to the reproductive performance of *Artemia* caused by antifouling paints, algaecides, and an aquatic herbicide. In *The Brine Shrimp Artemia* ed. Persoone, G., Sorgeloos, P., Roels, O. & Jaspers, E. pp. 201-211, Vol. 1. Wetteren, Belgium: Universa Press.

GUNTHER, D.C. & CATENA, A. (1980) The interaction of *Vibrio* with *Artemia* nauplii. In *The Brine Shrimp Artemia* ed. Persoone, G., Sorgeloos, P., Roels, O. & Jaspers, E. pp. 213-221, Vol. 1. Wetteren, Belgium: Universa Press.

GUTTMAN-BASS, N., BAIREY-ALBUQUERQUE, M., ULIT-ZUR, S., CHARTRAND, A. & RAV-ACHA, C. (1987) Effects of chlorine and chlorine dioxide on mutagenic activity of lake Kinnereth water. *Environmental Science and Technology* **21**, 252-260.

HARAKEH, S. (1987) The behavior of viruses on disinfection by chlorine dioxide and other disinfectants in effluent. *FEMS Microbiology Letters* **44**, 335-341.

HARAKEH, S., ILLESCAS, A. & MATIN, A. (1988) Inactivation of bacteria by Purogene. *Journal of Applied Bacteriology* **64**, 459-463.

HOSE, J.E., DI FIORE, D., PARKER, H.S. & SCIARROTTA, T. (1989) Toxicity of chlorine dioxide to early life stages of marine organisms. *Bulletin of Environmental Contamination and Toxicology* **42**, 315-319.

JACOBSON, P. & LILTVED, H. (1988) Thermal disinfection of sea water for aquaculture purpose. *Aquaculture Engineering* **7**, 443-447.

KENYON, A.J., HAMILTON, S.G. & DOUGLAS D.M. (1986) Comparison of antipseudomonal activity of chlorine dioxide/chlorous acid-containing gel with commercially available antiseptics. *American Journal of Veterinarian Research* **47**, 1101-1104.

LEDO, A., GONZALEZ, E., BARJA, J.L. & TORANZO, A. E. (1983) Effect of depuration systems on the reduction of bacteriological indicators in cultured mussels (*Mytilus edulis* Linnaeus). *Journal of Shellfish Research* **3**, 59-64.

LEONHARD, S.L. & LAWRENCE, S.G. (1980) The brine shrimp *Artemia* as a laboratory bioassay organism. I. The effects of the heavy metal cadmium on reproduction. In *The Brine Shrimp Artemia* ed. Persoone, G., Sorgeloos, P., Roels, O. & Jaspers, E. p. 224, Vol. 1. Wetteren, Belgium: Universa Press.

LIGHTNER, D . V . (1983) Pathology and disease treatments for crustaceans. In *Crustacean Aquaculture. CRC Handbook of Marine Culture* ed. McVey, J.P. pp. 289-321, Vol. 1. Boca Raton: CRC.

NOLAN, C.M., BALLARD, J., KAYSNER, C.A., LILJA, J.L., WILLIAMS, L.P. & TENOVER, F.C. (1984) *Vibrio parahaemolyticus* gastroenteritis. An outbreak associated with raw oysters in the Pacific Northwest. *Diagnostic Microbiology and Infectious Diseases* **2**, 119-128.

STRICKLAND, J. & PARSONS, T.R. (1977) Determination of soluble organic nitrogen. In *Practical Handbook of Seawater Analysis* ed. Stevenson, J.C. & Watson, J. pp. 143-147. Canada: Alger Press.

ULITZUR, S. (1974) Vibrio parahaemolyticus and Vibrio alginolyticus: short-generation time marine bacteria. *Microbial Ecology* **1**, 127-135.

VANDERZANT, C., NICKELSON, R. & PARKER, J.C. (1970) Isolation of *Vibrio parahaemolyticus* from gulf coast shrimp. *Journal of Milk and Food Technology* **33**, 161-162.

VANHAECKE, P., PERSOONE, G., CLAUS, C. & SORGELOOS, P. (1980) Research on the development of a short term standard toxicity test with *Artemia* nauplii. In *The Brine Shrimp Artemia* ed. Persoone, G., Sorgeloos, P., Roels, O. & Jaspers, E. pp. *263-285, Vol.* 1. Wetteren, Belgium: Universa Press.

WILDE, E.W., SORACCO, R.J., MAYACK, L.A., SHEALY, R.L., BROADWELL, T.L. & STEFFEN, R.F. (1983) Comparison of chlorine and chlorine dioxide toxicity to fathead minnows and bluegill. *Water Research* **17**, 1327-1331.