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Chlorinated Cooling Waters in the Marine Environment: Development of Effluent Guidelines

JUDITH M. CAPUZZO, JOEL C. GOLDMAN, JOHN A. DAVIDSON and SARAH A. LAWRENCE
Woods Hole Oceanographic Institution, Woods Hole, MA 02543, U.S.A.

The effects of free chlorine and chloramine on stage I lobster larvae and juvenile killifish were investigated in continuous flow bioassay units. In comparing mortality and changes in standard respiration rates during and after exposure to either chlorine form, significant respiratory stress was observed with exposure to sublethal levels. Sublethal responses to free and combined chlorine should be considered when establishing regulations for chlorine residuals in cooling waters.

The impact of environmental pollutants on aquatic life has traditionally been assessed in standard bioassays. In these assays the major criterion used to determine an organism's response to a toxicant stress is the measurement of an LC_{50} value (Sprague, 1969); viability after a designated time is thus the only data collected. Although these data are useful in detecting the sensitivity of an organism to a toxicant, important physiological and behavioural changes that may occur with sublethal exposure are not measured. The need for sublethal tests is obvious, particularly in assessing an organism's chance for survival after exposure (Sprague, 1971; Perkins, 1972). Translating these data to effluent guidelines, however, is a difficult problem.

The objective of the present study was to assess the toxic effects of free chlorine and chloramine on stage I larvae of the American lobster *Homarus americanus* and juveniles of the killifish *Fundulus heteroclitus* through a comparison of observed mortality data and changes in standard metabolic activity after exposure to the toxicants. Our interest in chlorine toxicity is a result of its extensive use in fouling control of once-through cooling water systems at coastal power stations. Chlorine in seawater may exist in a free form (HOCl or OCl^-) and in combination with ammonia and organic compounds, or result in the formation of its bromine counterparts (Lewis, 1966; Dove, 1970). Power generating stations vary in their chlorination procedures from continuous low-level (0.1 mg/l) chlorination, commonly practised in eat Britain (Beauchamp, 1969), to intermittent ulorination (2–3 h per day), commonly practised in the United States (Becker & Thatcher, 1973). In the latter instance, levels of chlorine residuals typically range from 0.05 to 5.00 mg/l to ensure removal of mussels and barnacles from condensers (Brungs, 1973; Marshall, 1971). Other marine organisms entrained in these cooling waters or residing in adjacent receiving waters are exposed to this chlorine stress, accompanied by the thermal and mechanical stresses of condenser passage.

Materials and Methods

Our bioassays were conducted in a system of 24 500 ml units with seawater and toxicant supplied separately to each unit by peristaltic pumps. The seawater (salinity = 30–31‰; pH = 7.9–8.1) used in the assays was filtered through activated charcoal and 1.2 μm membrane filters and aerated for 48 h with ammonia free air for removal of organics and ammonia. Chlorine, added as sodium hypochlorite (NaOCl) or chloramine ($ClNH_2$, prepared from equimolar amounts of NH_4OH and NaOCl), was measured as total residual chlorine by amperometric titration (APHA, 1971); concentrations ranged from 0.01 to 10.00 mg/l total residual chlorine applied as free chlorine and 0.01 to 5.00 mg/l applied as chloramine and there were three replicate samples at each exposure concentration.

Test organisms were acclimated to laboratory conditions before use in the assays. Ten lobster larvae or 8 killifish were added to each assay unit and exposed to either toxicant for 30 or 60 min at 25°C; after the designated exposure period, chlorine was chemically removed by the addition of sodium thiosulfate. Organisms were maintained for 48 h and observations on mortality made during that time period. Respiration rates of individual organisms were measured using all glass differential microrespirometers (Grunbaum *et al.*, 1955). Animals were not fed for at least 4 h before being placed in microrespirometer units and were allowed to acclimate to test conditions for 15 min before O_2 uptake was measured. Respiration rates of quiescent animals were measured at 25°C during exposure to the toxicants and following exposure in the bioassay units; significant differences in respiration rates between control and exposed organisms were determined by analysis of variance. A more detailed account of the bioassay procedure is reported elsewhere (Capuzzo *et al.*, in press).

We observed a differential effect of free chlorine and chloramine that was species specific. Applied chloramine was more toxic to lobster larvae than corresponding concentrations of free chlorine: LC_{50} values at 25°C, estimated by log-probit analysis (Finney, 1971) were 0.30 ± 0.05 mg/l and 2.90 ± 0.10 mg/l total residual chlorine, respectively (Fig. 1A). A gradual increase in lobster mortality was observed with increases in concentrations of both toxicants; ~20% mortality was observed at the lowest detectable level of residual chlorine (0.01 mg/l), whereas, <10% mortality was observed among control organisms. In contrast, juvenile killifish were more susceptible to free chlorine than to chloramine and a significant threshold effect was observed (Fig. 1C). Complete survival at 25°C occurred at concentrations <0.4 mg/l total residual chlorine, applied as free chlorine, and <0.8 mg/l total residual

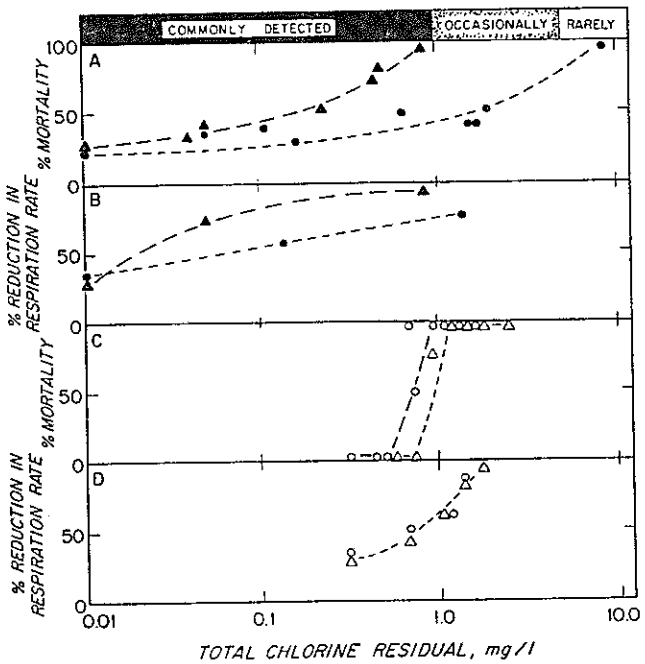

Fig. 1 The effect of chlorine residuals on stage I lobster larvae *Homarus americanus* and juvenile killifish *Fundulus heteroclitus*. Concentrations tested were within the range detected in marine cooling waters (Brungs, 1973; Marshall, 1971). Commonly detected residuals range from 0.05 to 1.0 mg/l, but may occasionally reach higher levels— ~5 mg/l to ensure removal of mussels and barnacles and ~12 mg/l to deter eels and jellyfish. In this study total residual chlorine equalled 18% of the applied chlorine or chloramine level due to the chlorine demand of seawater (A) *Homarus americanus*—% mortality 48 h after 30 or 60 min exposure to chlorine residuals at 25°C; closed circles—chlorine applied as free chlorine, closed triangles—chlorine applied as chloramine; no significant difference in mortality was observed between the 30 and 60 min exposure periods. (B) *Homarus americanus*—% reduction in respiration rate from that of control organisms (1.1 μl O_2/h/mg) 48 h after 60 min exposure to chlorine residuals at 25°C; control and exposed animals averaged 2.6 mg dry wt; closed circles—chlorine applied as free chlorine; closed triangles—chlorine applied as chloramine. (C) *Fundulus heteroclitus*—% mortality 48 h after 30 min exposure to chlorine residuals at 25°C; open circles—chlorine applied as free chlorine; open triangles—chlorine applied as chloramine. (D) *Fundulus heteroclitus*—% reduction in respiration rate from that of control organisms (1.2 μl O_2/h/mg) during 30 min exposure to chlorine residuals at 25°C; control and exposed animals averaged 18.4 mg dry wt; the respiration rate of test organisms exposed to 0.3 mg/l was not restored to the control level even 48 h after exposure, whereas control animals maintained a stable respiration rate during the 48 h period; open circles—chlorine applied as free chlorine; open triangles—chlorine applied as chloramine.

observed at higher concentrations.

The results of our respiration studies are indicative of significant respiratory stress with exposure to both toxicants. Respiration rates of lobster larvae measured 48 h after exposure to each toxicant were significantly lower ($p < 0.05$) than control organisms (Fig. 1B); the per cent reduction in respiration rates observed was proportional to the concentration of each toxicant. Respiration rates of juvenile killifish monitored during and 48 h after exposure to each toxicant were significantly reduced only with exposure to concentrations approaching lethal levels (Fig. 1D). Initial respiratory stress was detected with exposure to 0.3 mg/l total residual chlorine, applied as free chlorine or chloramine; the respiration rates of exposed organisms were not restored to the control level even 48 h after exposure. A more drastic decrease in respiration rate was observed with exposure to higher concentrations, correlated with the increases in mortality observed.

Discussion

The action of each toxicant appears to be an alteration of standard metabolic activity as revealed by changes in respiration rates with exposure to sublethal levels; the actual toxicants, however, may not be chlorine compounds alone. Free chlorine will release bromine from the bromide ions of seawater forming hypobromous acid (HOBr) and hypobromite ion (OBr^-), or in the presence of high ammonia concentrations form chloramine (Lewis, 1966; Dove, 1970). Because of the low ammonia levels detected in the seawater used in this study (<0.4 μg atom/l), applied free chlorine probably resulted in a significant production of free bromine species, whereas applied chloramine persisted as a toxicant. The amperometric titration does not distinguish between chlorine and bromine compounds; levels reported as mg/l total residual chlorine include all chlorine and bromine species.

Decreased metabolic activity of sensitive species, such as larval lobsters and juvenile fish, important marine food resources, could result in serious changes in growth and maturation and increased susceptibility to other environmental stresses including disease and predation. The differences in response of lobster larvae and juvenile killifish to the toxicants probably reflect differences in uptake and metabolic regulation. Juvenile killifish are apparently unaffected by acute exposure to toxicant concentrations <0.3 mg/l, whereas lobster larvae experience significant metabolic stress even at the lowest toxicant concentrations tested (0.01 mg/l).

We believe these findings substantiate our concern that standard bioassay data have limited applicability in establishing 'safe' guidelines for marine organisms. Levels of chlorine residuals currently being detected in chlorinated cooling waters are high enough to cause significant stress to some organisms without immediate mortality. Thus, measurements of viability at the discharge point of entrainments or in receiving waters adjacent to chlorine discharges do not provide us with an accurate picture of chlorine toxicity. Sublethal effects of free and combined chlorine on marine organisms—

including fish and invertebrate species—should be considered when establishing regulations for chlorine residuals in cooling waters from power plant operations. Low level chlorination combined with dechlorination and rapid dilution of cooling waters would provide the greatest protection to entrained organisms and organisms residing in receiving waters.

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Relationships Between Heavy Metals and Major Cations Along Pollution Gradients

C. R. C. SHEPPARD

Department of Botany, University of Durham, Durham, U.K.

Along some pollution gradients, animal tissue concentrations of several major, physiologically important cations vary greatly in a way that corresponds with levels of toxic metals. Changes in concentration from normal of these major cations can be as much as fourfold which may be an underlying cause of much of the stress experienced by these species in polluted environments.

It is known that the effects of heavy metals on organisms are often at least as harmful as many of the more visible forms of pollution (Ruivo, 1972). Because of the importance of their effects numerous base-line studies of heavy metal concentrations have been undertaken in most areas of the world.

In most laboratories where analysis of heavy metals by atomic absorption methods is undertaken, it is now routine to obtain estimates of the concentrations of the major physiological cations—calcium, sodium, potassium and magnesium—as well, so that the interferences superimposed on the heavy metal readings by these four elements can be adjusted for.

In a base-line study carried out along the Italian coast using the urchins *Arbacia lixula* and *Paracentrotus lividus*, pollution gradients for several heavy metals have been recorded (Sheppard & Bellamy, 1974). In a similar study using *Patella vulgata* along the British North Sea coast (unpublished data) similar gradients have been recorded. However it was noticed in both cases that the concentrations of the major cations in the species varied in conjunction with the heavy metals, either with a positive or with a reciprocal relationship. This variation was examined.

Methods

In the Italian transect about 40 of each species of urchin were collected from each of 14 sites spread between Naples and a marine park about 100 km to its south. In the British transect about 8 limpets were collected from 25 sites along the East coast. In all cases analysis, of the soft parts only, was carried out by atomic absorption spectrophotometry, for the heavy metals lead, copper, nickel and zinc, and for the major cations calcium, sodium, potassium and magnesium. Mean values for each site were obtained. Correlation coefficients were then computed for each heavy metal with each of the major cations, for each species. In all cases the Spearman ranked coefficient was used since the data was not always normally distributed and was often more bimodal.

Results

Table 1 presents all correlation coefficients that were significant to $p = 0.05$. Almost three quarters of the total number of coefficients showed at least this significance. Where the coefficient was not significant to $p = 0.05$ only