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Brian P. Bradley

Morrris H. Roberts Jr.

CONTAMINANT PROBLEMS AND MANAGEMENT OF LIVING CHESAPEAKE BAY RESOURCES

EDITED BY
SHYAMAL K. MAJUMDAR, Ph.D.
Professor of Biology
Lafayette College
Easton, Pennsylvania 18042

LENWOOD W. HALL, Jr., M.S.
Senior Staff Biologist
The Johns Hopkins University
Applied Physics Laboratory
Aquatic Ecology Section
Shady Side, Maryland 20764

HERBERT M. AUSTIN, Ph.D.
Professor of Marine Science
The College of William and Mary
Virginia Institute of Marine Science
Gloucester Point, Virginia 23062

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Chapter Eighteen

EFFECTS OF CONTAMINANTS ON ESTUARINE ZOOPLANKTON[1]

BRIAN P. BRADLEY[1] and MORRIS H. ROBERTS, JR.[2]

[1]Department of Biological Sciences
University of Maryland Baltimore County
Baltimore, Maryland 21228
and
[2]Virginia Institute of Marine Science
College of William and Mary
Gloucester Point, Virginia 23062

ABSTRACT

The objectives of the chapter are (1) to evaluate laboratory studies concerning effects of heavy metals, pesticides and oxidants on copepods, mysids, bivalve and decapod larvae (2) access field studies (mainly with copepods) on these and other contaminants which when coupled with laboratory data provide information on known and potential hazards of contaminants to zooplankton and (3) briefly review some bioassay methods used in these studies.

Mercury is the most toxic heavy metal by weight, followed by copper, silver and cadmium. Pesticides have been tested much less extensively than heavy metals. In general, bivalve larvae seem less sensitive than the crustacean taxa. Mysids, decapods and copepods seem comparable in sensitivity. Of the pesticides, tributyltin, an antifoulant, presents the greatest present or potential hazard. Chlorine, the most widely used oxidant in Chesapeake Bay, is highly toxic to all taxa reviewed, making zooplankton highly vulnerable. Lethal effects can be reduced or eliminated by dechlorination, but sublethal effects may persist.

Most field studies with copepods have dealt with uptake of heavy metals and pesticides, effects of oil residues and impacts of power plants. Acute lethal effects are rarely observed in the field. For this reason death is an inadequate basis

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from which to infer an ecotoxicological response. We believe that reliable sublethal endpoints for laboratory studies need to be developed to evaluate field observations on contaminant effects on zooplankton.

INTRODUCTION

The overall objective of this review is to evaluate the degree of potential or actual impact of contaminants on zooplankton within the Chesapeake Bay. To accomplish this, we made a comprehensive but not exhaustive review of both laboratory and field studies relating to contaminants and bay species. The review includes work done elsewhere on marine and estuarine species similar to those found in Chesapeake Bay.

To narrow the scope of the task, we focused on a limited array of contaminants, namely metals, pesticides, oxidants, and, to a lesser extent, other contaminants which are of widespread concern within Chesapeake Bay. We also narrowed the list of taxa to copepods, mysid shrimp, decapod larvae and bivalve larvae. This approach was used because of their dominance in the zooplankton community and the availability of extensive data.

The three most abundant species of holoplankters are the calanoid copepods, *Eurytemora affinis, Acartia tonsa* and *A. clausi.* These are also the most commonly used copepod species in toxicity studies, being readily cultured in the laboratory.[1,2,3,4] Copepods are the dominant holoplankton species in the Chesapeake Bay, and mysid shrimp represent a secondary dominant species. Several mysid species are known to occur in the Chesapeake Bay, notably *Neomysis americana* and *Mysidopsis bigelowi. N. americana* is not only numerically abundant, but also important as food for a variety of estuarine fishes.[5] While this species can be cultured, most toxicological research with mysids has centered on the subtropical species, *Mysidopsis bahia. M. bahia* seems comparable in sensitivity to *N. americana* for several toxicants;[6,7] therefore data for both species will be considered in this review.

Meroplankton species, a second important component of estuarine zooplankton communities, include larvae of a large proportion of benthic invertebrates as well as fish larvae. Two principal taxa in this group are larvae of molluscs and decapod crustaceans. Annelid larvae, while abundant, are less well studied within Chesapeake Bay. Data for fish larvae are discussed elsewhere in this volume.

Embryos and larvae of the oyster (*Crassostrea virginica*) and hardclam (*Mercenaria mercenaria*) are the most studied resident molluscan species in Chesapeake Bay. These species are of considerable interest because they are both commercially important and readily cultured in the laboratory.[8] Both species produce large numbers of gametes at each spawning, and hence are well represented in the plankton throughout the warm months of the year.

Larvae of decapod crustaceans are another major meroplanktonic component in Chesapeake Bay. A considerable diversity of species has been used in toxicity tests since many can be cultured using standard techniques.[9,10,11] Much of this work relates to species of no commercial interest. Relatively little work has been done with the commercially important blue crab (*Callinectes sapidus*) because the larvae are difficult to culture reliably. Many of the species most frequently used for toxicity tests are resident within Chesapeake Bay.

Since data for both Bay and non-Bay species are included, we have compared data for Bay and non-Bay species of a given genus or class (e.g. *C. virginica* and *C. gigas*) whenever both were available. These comparisons suggest that data for several non-Bay species of copepods, bivalve and decapod larvae are representative for Bay species.

EFFECTS OF HEAVY METALS

Considering mortality as the end point, zooplankters, regardless of taxon, exhibit a wide range in response to various metals with greatest sensitivity to mercury in the range 3-10 μg/L[4,12,13,14,15,16,17] and least sensitivity to lead (in the range 500-3000 μg/L for mysids;[13]) chromium, (over 10,000 μg/L in bivalve larvae[18]) or selenium (over 10,000 μg/L in bivalve larvae and over 1,000 μg/L in decapod larvae.[19]). The taxa differ in relative sensitivity to each metal only slightly (Table 1B).

There is no consistent trend in relative sensitivity to heavy metals among zooplankton. While bivalve larvae appear most sensitive to silver and mercury, mysids and copepods appear most sensitive to mercury and cadmium. These differences are also expressed to some degree in the different rank order of toxicities for the taxa; however, taxa seldom differ in sensitivity by more than an order of magnitude and generally by much less.

For some metals, notably cadmium, salinity has a dramatic effect on acute toxicity to various species. This has been interpreted to reflect complexation of metals by chloride ions, reducing their bioavailability; lethal concentrations calculated on the basis of free metal ion concentration seem unaffected by differences in exposure salinity.[20] Such an effect can be seen in data for *M. bahia* exposed to cadmium in different salinity regimes[6,13,21] and is substantiated by research in progress with some indication that the effect is not entirely the result of changes in free ion concentration.[22] A role of calcium in the observed uptake and acute toxicity of cadmium has been suggested.[23,24,25] An effect of salinity on cadmium toxicity has also been demonstrated in decapod larvae of several species.[26,27] Similar studies with copepods and bivalve larvae are lacking.

Studies of interactions between metals and both temperature and salinity are of special interest in estuaries such as Chesapeake Bay. Even in the absence of

chemical interactions between a metal and chloride (i.e. salinity), there is still a basis for interaction of these variables, especially near the thermal and salinity tolerance limits for any test species. Most research relating temperature and salinity interactions with heavy metals has been done with decapod larvae. It seems safe to conclude that at least for these zooplankters there are significant interactions between metals and temperature or salinity. Often, however, the effect of temperature or salinity alone on survival or some other endpoint is much greater than that of the metal alone.[27,28]

Evaluation of metal toxicity over a range of salinities and temperatures is important regardless of whether or not there is a direct effect of salinity and temperature on speciation of the toxicant. Organisms tend to be less tolerant to any added stress, such as a toxicant, when they are already challenged.

Results of laboratory studies are difficult to apply in the field because they are usually conducted under constant conditions with single chemicals. In contrast, organisms in estuaries are faced with complex mixtures of metals and other contaminants. Only two experimental studies were identified which tested interaction effects of two or more metals on zooplankton; one on decapod larvae[29] and one on a marine copepod.[30] For decapod larvae, time from hatch to megalopa exposed to mixtures of lead and zinc was extended, primarily resulting from lead. There was a significant interaction between lead and zinc, but zinc

TABLE 1

Summary of the acute toxicity data with respect to heavy metals. (All values are expressed as μg/L of the metal.)

A - LC50 data for Chesapeake Bay zooplankton species in four taxa.

Metal	Copepods	Mysids	Bivalve embryos		Decapod larvae	
Ag		249	5.8-21	(22)[a]		(55)
As		1740	750	(326)		(232)
Cd	90-130 (500-1,000)	11.3-113	3800	(611)	50-300	(247)
Cr	(5,000-8,000)	2030		(>10000)		(3440)
Cu	9-80 (80-1800)	181	16.4-103	(50-100)		(49)
Hg	10 (230)	3.5	5.6-4.8	(5.5-6.7)	9.9	(8.2)
Ni	(6000)	508	300 -1200	(349)		(1360)
Pb		3130	780 -2450	(758)		(575)
Se				(>10000)		(>1000)
Zn	(1450)	499	160 -3100	(119-310)	500-1000	(456)

[a]Values in parentheses were derived for non-Chesapeake Bay species; for bivalves, *Crassostrea gigas,* and for decapod larvae, *Cancer magister, Upogebia pugettensis* or *Callianassa californiensis.*

B - Rank order of metal toxicity to four taxa

Taxa	Rank Order
Copepods	Hg <· Cu <· Cd <· Cr <· Zn <· Ni
Mysids	Hg > Cd > Cu > Ag > Zn = Ni > As > Cr > Pb
Bivalve larvae	Hg > Ag > Cu > Zn > As = Ni > Cd ± Pb > Cr = Se
Decapod larvae	Hg > Ag = Cu > Cd ≥ As ≥ Zn > Pb > Ni > Cr ≥ Se

alone caused little or no increase in time to megalopa.[29] For copepods, copper, cadmium and chromium when presented individually or in mixtures caused mortality. There were clear interaction effects when presented in mixtures. Interestingly, the toxicity of the three metal mixture was higher than that of the metals tested separately but lower than that of any two metal mixtures.[30]

Death of individuals is only one criterion used to measure the response of zooplankters to heavy metals. Population extinction (an extension of the individual death concept) has also been used as an endpoint, especially for copepods for which tests often involve mixed age populations.[31] Various sublethal criteria are used including longevity, fecundity, respiration rate and feeding activity,[32] number of eggsacs and interval between eggsacs (broods),[33] swimming rate and development time.[34] Delay in development of larval stages is a recognized response to metals, especially for decapod larvae.[27,28]

Effective concentrations based on sublethal endpoints are sometimes lower than those based on mortality, but the significance of this is not always immediately clear. The physiological systems used to measure sublethal responses of larvae are, however, important in some way to the survival of the population of organisms. For example, an increase in duration of development affects the amount of predation pressure applied to a species during the vulnerable planktonic period. A prolonged larval period, even without other physiological consequences, could therefore reduce the numbers of larvae metamorphosing.

There is some evidence that copepod populations adjust genetically to chronic heavy metal stress. *Acartia tonsa* from polluted areas were determined to be resistant to sublethal copper stress,[32] with higher LC_{50} concentrations than observed for copepods from "clean" areas.[35] The genetic consequences may depend on the most sensitive stage and different developmental stages often differ markedly in sensitivity.[36]

PESTICIDES

Published information on the effects of pesticides on zooplankton species is relatively scarce given the many chemicals and formulations used for commercial pest control products. This becomes especially obvious when one considers the number of compounds in various classes of pesticides which have received attention.

Pesticides vary greatly in acute toxicity, ranging from 0.33 μg/L for *M. bahia* exposed for 96 h to phorate[37] to considerably over 10,000 μg/L for bivalve larvae exposed to several herbicides, bactericides, and monobutyltin.[38,39] In an attempt to simplify the comparison of available data for the several zooplankton taxa under consideration, the compounds were sorted into four classes: LC50s < 10 μg/L, 10-100 μg/L, 100-1000 μg/L, and > 1000 μg/L. Several points were immediately obvious from this summary of the data.

Many more compounds seem to have been tested with bivalve larvae than with copepods, mysids or decapod larvae; however, much of the information for bivalves is from a single study involving larvae of *C. virginica* and *M. mercenaria*.[38] In any case, copepods are clearly the least studied taxon with respect to mortality effects.

The crustacean taxa appear to be considerably more sensitive to pesticides than bivalve larvae. Data have been found for three or more taxonomic categories for only four compounds: atrazine, sevin, tributyltin (TBT) and toxaphene. Indeed, there are only two additional pesticides (malathion and dieldrin) for which data have been published for bivalve larvae and even one other zooplankton species. Thus the data used to compare relative sensitivities of species are limited. Atrazine is most toxic to copepods[40] and mysids, and least toxic to oyster larvae by several orders of magnitude. For sevin, mysids are most sensitive, and bivalve larvae least sensitive.[21,38,41] Data for toxaphene lead to the same conclusion.[21,38,42] One value for decapod larvae seems to belie this conclusion, but this value is questioned because of inconsistency in the reported data. The original data are no longer extant precluding a reevaluation. Only for TBT do bivalve larvae appear to be similar in sensitivity to crustaceans.[43,44,45]

Finally, mysids and decapod larvae seem quite similar in response to various pesticides (copepod data are too sparce to include in the comparison). Both taxa are very sensitive, with mysids slightly more sensitive than decapod larvae in most cases.

Relative toxicities based on laboratory tests must be interpreted in context with actual concentrations in the Bay. For example, tributyltin, used as an antimicrobial agent industrially or as an antifouling agent primarily in the marine and estuarine milieu, is highly toxic to the copepod *Acartia tonsa* (0.65 μg/L, 96 h LC50[45] and 1.1 μg/L, 48 h LC50),[46] to the copepod *Eurytemora affinis* (0.6 μg/L, 72 h LC50[46]) to mysids (0.61 μg/L)[43] and to bivalve larvae (1.1-1.6 μg/L)[44] and only slightly less toxic to decapod larvae (30-50 μg/L).[47] Field survey data in the Chesapeake Bay indicate that the ambient concentration in areas near marinas and shipbuilding/repair facilities average about 0.02 μg/L with periodic upward excursions to 0.9 μg/L; concentrations elsewhere are near the detection limit of <0.002 μg/L.[48] Over the greatest areal extent of the Bay and its tributaries, TBT concentrations are usually over 100-fold below an acutely toxic concentration. The absence of any chronic data at present reduces the reliability of further hazard assessment since no acute/chronic ratio is available for TBT. Application factors derived from laboratory acute and chronic test data for several pesticides vary over at least an order of magnitude. An application factor of <0.01 is not unknown for other compounds,[49] but there is no evidence that such a value is appropriate for TBT.

The effects of salinity and temperature on pesticide toxicity have not received attention comparable to that for metal toxicity. The limited data available[50,51] indicate that interactions do occur when testing decapod larvae. No data were

found for copepods, mysids or bivalve larvae. This lack of data represents a potentially significant gap when one attempts to evaluate pesticide hazard to estuarine zooplankton.

One response of decapod larvae to pesticides is prolonged larval duration even at concentrations which do not produce a marked reduction in survival.[52] This seems to be a response to every pesticide tested (as well as most other toxicants). The implications of this observation were discussed previously.

Underlying all lethal and sublethal effects is bioaccumulation which includes accumulation both from water and food. In some cases, accumulation through the food chain may dominate,[53] though in general uptake from water predominates. In some cases, biota may play a role in degrading the pesticide.[54] The effect of some pesticides taken up may be mitigated by elimination, which itself is influenced by feeding, egglaying and excretion.[55]

There is good evidence that decapod larvae accumulate dieldrin faster from water than from food.[52,56] The body burdens resulting from ingesting dieldrin contaminated food were sufficient to produce both lethal and sublethal effects, at high dietary concentrations.[57] Different rates of accumulation based on source are not unusual. It has recently been shown for a fish that when a pesticide is present in both water and food, uptake from the two sources will be additive.[58] The implication is that, if the food is at equilibrium with the water, uptake from water will always dominate.

OXIDANTS

The major sources of oxidant residuals, primarily chlorine, are disinfection of treated sewage and antifouling activities in industrial cooling systems (electric generating plants). Chlorination of treated sewage results in discharge principally of monochloramine (plus low concentrations of organochlorines, many of which do not contribute to the measured residual). In contrast, direct chlorination of a cooling water can result in primarily free chlorine plus a variety of combined chlorine residuals. In marine and estuarine waters, bromine analogs are produced through reaction with bromide.

After residuals are introduced into estuarine water, additional reactions will occur to modify the mixture of compounds in the total oxidant residual. Ultimately the residual will decay or be diluted, but in the process small yet potentially significant concentrations of various toxic materials may be formed. These include various "combined" residuals such as mono- and dichloramine, mono- and dibromamine, and bromate. In all cases, many haloorganic compounds may be formed by reaction with dissolved organics in saline water. The principal haloorganics formed in chlorinated sewage or surface saline waters and reported to be in surface waters of the Bay are small concentrations of the trihalomethanes, chloroform and bromoform.[59] Other oxidants, primarily

bromine chloride and ozone, have been proposed as alternatives to chlorine in the primary applications which affect receiving waters. In both cases, many of the same residual compounds may be formed.

Considerable research has been published regarding the toxicity of chlorine residuals to estuarine zooplankton including copepods, bivalve larvae and decapod larvae. Total residual chlorine is toxic to all of these taxa at concentrations of 20 to 100 μg/L in 48 or 96 h tests.[60,61,62,63,64,65,66,67] Toxicities of chlorine of around 400 μg/L were reported in two studies[68,69] although in 24 hour tests there was evidence of greater sensitivity in pre-adults. These estuarine zooplankters have been shown to be among the most sensitive organisms to oxidant residuals, rivaled in estuarine waters only by the eggs and larvae of some fishes.[70]

Oyster larvae seem to become more tolerant of oxidant residuals as they develop. The 48 h EC50 for oyster embryos is 26 μg/L,[60] whereas the 48 and 96 h EC50s are 300 and 60 μg/L, respectively.[65] There are somewhat conflicting data for pediveligers. In a study with chlorinated sewage added to estuarine water, settlement and subsequent metamorphosis were inhibited by total oxidant residual concentrations between 20 and 60 μg/L, quite similar to concentrations affecting embryos. In a contrasting study, more than 50% of recently attached oyster spat survived exposure to a total oxidant residual of 300 μg/L (no sewage effluent), perhaps reflecting the difference in methodology and the ability of the oyster spat, even newly set and metamorphosed, to close their valves under adverse conditions.[65]

Oxidant residuals of bromine chloride, expressed as molar equivalents of oxidant, are approximately as toxic to zooplankton and other estuarine organisms as chlorine produced oxidant residuals expressed on the same basis. However, these residuals appear less toxic on a mass concentration basis.[60,69] The similarity in toxicity based on molar equivalents can be interpreted to reflect a similarity in the mixture of oxidant residuals independent of the oxidant introduced.

Much less data have been published regarding ozone effects on estuarine zooplankton. Oyster larvae are extremely sensitive to ozone-produced oxidant residuals[72] which are presumably similar to those produced by chlorine or bromine chloride. Thus, though ozone decays rapidly, small barely measurable residuals could have adverse effects on estuarine zooplankton much as some think may now occur following chlorination.

The residuals of chlorination can be reduced to chloride by reaction with various reducing agents prior to release. When this is done, the acute lethal toxic effects associated with chlorination and oxidant residuals are reduced or eliminated.[63,71,73] Indeed, when secondary treated sewage is chlorinated and dechlorinated, the toxicity of the mixture when added to estuarine water may be reduced compared to the unchlorinated sewaged effluent.[71,73] Since some reducing agents are relatively inexpensive, it is realistic to consider dechlorination as one strategy for eliminating oxidant residuals in natural waters near

treated sewage effluent discharges as has been proposed under the Bay Cleanup Program. It should be noted, however, that chlorination-dechlorination with sodium thiosulfate caused reproductive failure in copepods which appeared otherwise unaffected.[68]

FIELD STUDIES OF POLLUTANT EFFECTS

Laboratory studies with single species exposed to one or several toxic substances can yield information on the effects of toxicants on the zooplankton community. However, one can only understand the extent of impact through carefully designed in-situ field studies. In Chesapeake Bay, there have been relatively few studies to evaluate effects of pollutants on zooplankton under complex field conditions. In these few studies, copepod distributions and bivalve larval settlement in the field have been used to provide direct information regarding the effects of pollutants on the zooplankton community.

No field studies were identified involving mysids, early stage larvae of bivalves, or decapod larvae to assess the impact of toxicants in natural surface waters on zooplankton communities. Generally, surveys of these components of the zooplankton community have been broad scale, and therefore cannot be used to focus on effects of a particular point source discharge or center of a non-point source discharge. In contrast, the number of field studies on copepods, in the Bay and elsewhere, seem to exceed the number of laboratory studies.

FIELD STUDIES WITH COPEPODS

One group of field studies with copepods dealt with uptake, body burden and bioaccumulation of contaminants, particularly heavy metals and pesticides. Another, involving a wide range of contaminants including hydrocarbons, oxidants and waste heat as well as heavy metals and pesticides, focused on toxic effects.

Mercury uptake is greater in microzooplankton and algae than in macrozooplankton and fish larvae.[74] Higher levels of mercury have been found in phytoplankton and detritus than in zooplankton.[75,76] One should not infer from these observations that there is no bioaccumulation from phytoplankton by herbivorous zooplankters. Differences in body burden may simply reflect differences in equilibrium kinetics among species, perhaps due to differences in lipid quality or quantity.

Several uptake studies involve complex mixtures of metals.[77,78,79,80,81,82] Both seasonal and geographic variation and in some cases interspecies variation in the body burdens of various metals were reported. No consistent patterns emerged.

While arsenic, copper, iron and zinc concentrations were higher in fish than in copepod prey, the opposite was observed for cadmium.[81] In contrast, the mercury contents in fish and zooplankton in another study were nearly equal.[83] These differences may reflect general responses to specific metals and how they are handled in fish as opposed to zooplankton.

The degree of heavy metal toxicity may depend on various physiological factors in addition to uptake; for example, whether the metal occurs in the enzyme pool or bound to a metallothionein,[84] the pH or cationic strength[85] and nutrition.[86] In at least one case, the availability of trace metals depended on naturally occurring organic matter.[87]

In a study of DDT uptake, retention by feral copepods depended on phytoplankton densities, with 60-70% of that ingested at low density being retained, and only 10% under bloom conditions.[88] DDT is also taken up directly from the water. Pesticides are bioaccumulated,[53] depending partly on biodegradability. Both pesticides and metabolic by-products have been measured in copepods.[54,89] Using experimental enclosures, biodegradation rates may have been limited by lack of inorganic nutrients. Biodegradation of pesticides may not be sufficient to reduce toxicity if other stable and toxic compounds are released by hydrolysis of the pesticides.

Rates of elimination of PCBs from a copepod increased with feeding and egg production.[55] In a study with euphausiids, high concentrations of PCB's were reported in fecal pellets.[90]

In contrast to heavy metals and pesticides, studies of oil and related hydrocarbons have been focused mainly on toxicity. In some cases the studies followed major oil spills. In studies of sublethal effects[91,92,93,94] effects on reproduction have been observed at concentrations of 50 mg/L crude oil[91] and on ingestion rates, viability and swimming behavior at concentrations as low as 80 μg/L.[92] Sublethal oil concentrations may interfere with chemoreception and food perception in copepods.[93] These indirect effects, however, may be mitigated by the ability of copepods to metabolize petroleum hydrocarbons.[94]

The effects of major oil spills on zooplankton and other biota appear to be short-term,[95,96,97,98,99] although studies of oil spills often lack controls.[99] In simulated spills, no significant effects were observed on sediments, bacteria, or copepods exposed for several days to concentrations up to 200 mg/L Prudhoe Bay crude oil.[100] However, in another simulation, chronic exposures of 190 μg/L No. 2 fuel oil reduced zooplankton populations.[101] This large difference may reflect differences in proportions of water-soluble toxic components in the different hydrocarbon mixtures. It appears that, if the concentrations of oil as a result of a spill do not result in the disappearance of phytoplankton or zooplankton, recovery may occur within months. However, it is obvious that studies on zooplankton, particularly short-term studies, tell us little of the effects on the ecosystem in general.

Steam electric power plants are a source of both waste heat and biocides,

(particularly chlorine) used to control bio-fouling. Some studies on heat and oxidants together have been done in Chesapeake Bay. Of the two contaminants, chlorine caused greater effects on copepod mortality than temperature.[102,103,104] A much greater impact has been suggested for larval zooplankton than phytoplankton;[104] so, in the environment, chlorine effects are most likely directly on the zooplankton.

In a short term assay for chlorine tolerance in individual copepods, genetic adaptation was observed in response to low concentrations of chlorine residuals (100 μg/L).[68] In addition, copepods collected from natural waters with measurable residual oxidant had significantly higher tolerances to chlorine than copepods from water with no detectable chlorine.[68] There is significant genetic variability in chlorine tolerance based on intra-class correlations within copepod families;[105] supporting the earlier observation of genetic changes in field and laboratory populations.

One possible alternative to chlorine as a biocide is bromine chloride.[106] There was little difference between toxicities of chlorinated and bromo-chlorinated water to copepods at various ages after inoculation.[69,107] Free chlorine appears more toxic than bromine chloride, but there is no difference when concentrations are expressed on a milliequivalent basis.[60]

Some studies on the effect of power plants on copepods have been concerned with entrainment effects due mainly to increased temperatures. The temperature increases which are tolerated depend on ambient temperatures.[108,109] Depending on the temperature differential, there may be genetic differences between progeny from intake and progeny from discharge samples of copepods.[110] These genetic differences may be delayed effects of entrainment[109,111] even if the immediate consequences measured by mortality differences are not significant.[112] Laboratory studies done in conjunction with some of these field studies have shown that genetic and physiological effects vary between the sexes and depend on rates and magnitudes of temperature changes.[113,114] The genetic expression of temperature tolerance, however, does not depend on ambient conditions, so laboratory and field trials can safely be compared.[115]

Power plants have an effect on non-entrained organisms in the surrounding receiving waters.[116,117,118] Mortalities of biota in receiving waters varied seasonally;[116] estuarine species were less sensitive than neritic forms to heated effluent[117] and, what may be a non-thermal effect, zooplankton seemed less able to avoid predators in the turbulent receiving waters.[118]

Dredging of contaminated sediments and sewage discharges are two additional human activities for which field studies of copepods have been made. Dredged material, which has some similarities in impact to drilling muds,[119] has been shown to affect biota least at a dredge site and most at a disposal site, with intermediate effects downstream from the dredge site.[120]

Impact assessment for dredging using zooplankton requires careful design and sampling.[121] Sediment toxicity testing is very difficult and perhaps more

subjective than toxicity testing in water.[122] The methodology for testing sediments for toxicity is still evolving with new species and endpoints being proposed.[123,124,125,126]

The effects of sewage outfalls on zooplankton populations are seen most clearly in the immediate vicinity of the outfalls.[127,128] In the absence of toxins the organic loading may actually result in an enrichment of biota beyond the immediate area of discharge.

CHLORINATION EFFECTS ON BIVALVE LARVAL SETTLEMENT

Settlement of oyster spat, and secondarily, barnacles, polychaetes, and tunicates was monitored in the James River, VA at an array of stations surrounding the discharge from the James River Sewage Treatment Plant (JRSTP).[129] This study focused on effects of a toxicant (chlorinated sewage) on naturally occurring late meroplanktonic stages of sessile species in the macrofouling community as well as the metamorphic phase of development.

As noted earlier, oyster pediveliger larvae exposed to chlorinated sewage in laboratory tests exhibit depressed settlement and metamorphosis at low concentrations such as might generally occur in the vicinity of sewage treatment plants. Near the JRSTP, total chlorine residual concentrations at or above concentrations observed to have an effect in the laboratory have been measured only in the boil from the discharge.[130] In the recent field study of settlement, no further data regarding residuals in the field were obtained. Residuals in the sewage effluent prior to discharge closely approximated 2.0 mg/L, which would produce a maximum residual concentration in the boil of less than 0.1 mg/L.

At no time during the two-year study was the number of oyster spat observed at stations within 30-40 m of the boil reduced compared to more remote locations, up to 1.6 km away. The same can be said for settlement of *Balanus* sp., *Polydora* sp., *Hydroides dianthus,* and *Molgula manhattensis.*

In New Haven Harbor, CT, spatfall was reduced whenever total chlorine residuals were high (0.33 mg/L to 0.27 mg/L) in a surface water near sewage outfalls, compared to areas with low chlorine residuals (0.03 mg/L to 0.19 mg/L).[67] These results suggest that the failure to observe an effect in the James River reflects the absence of sufficiently high concentrations to have an impact. However, the highest residual in the boil may have exceeded the laboratory estimates of inhibitory residuals.[71]

Total chlorine residual concentrations at the point of discharge in the James River may slightly exceed a concentration shown to inhibit settlement in the laboratory. However, these concentrations do not exceed 0.1 mg/L.[129] The following question must therefore be addressed: "why is no impact detectable?"

One explanation is that the expected concentration was never in fact realized, and therefore no inhibition of settlement occurred. This could be the case

if the dilution rate at JRSTP is greater than 95% or the decay rate for the chlorine residual is very high in the receiving water.

Alternatively, the effect of chlorine residuals may be so pervasive in the James River that the impact of chlorine residuals extends beyond the bounds of the present study. This is deemed unlikely since the results within this study area are comparable to those of a more extensive monitoring effort encompassing all major seed-rocks in the James River during the same period.[131]

A final alternative explanation rests on an important difference between the laboratory experiments and the field situation. Recruitment of pediveligers in the laboratory test is finite (5000 larvae added once at the start of the test) whereas in the field recruitment is continuous. Despite a low percent settlement and metamorphosis, the sheer number of pediveligers passing the setting substrate could lead to settlement indistinguishable from that elsewhere.

BIOLOGICAL WATER QUALITY TEST

As may be seen from the review, lethality is often used as a biological criterion for water quality. Sublethal criteria are also important and even essential if a range of water quality is to be tested. In this section we describe some lethal and sublethal tests not described earlier.

A biological test method with embryos of bivalves[132,133] in particular the Pacific oyster, was used in Puget Sound[133,134,135] to define areas of high, medium and low water quality. The test was based on larval survival and percentage abnormal larvae produced from embryos cultured in water collected from specific sites. This test was extremely sensitive; reliability of the data was enhanced by use of a reference toxicant control for condition of embryos used in different tests.[136]

The bivalve larval test[133] does not discriminate among a variety of anthropogenic toxicants nor between anthropogenic toxicants and naturally occurring toxicants such as phytotoxins. Excessive amounts of phytotoxins which would deteriorate overall water quality may reflect anthropogenic activities, and in that sense be anthropogenic. In any case, if the method were applied to locate the source of any toxicant, ancillary data regarding phytoplankton community structure and distribution should provide insight to identify the specific cause of decreased water quality.

This methodology has not been applied within the Chesapeake Bay although the technology exists to perform such evaluations using bivalve embryos. Such a test would provide a sensitive biological tool to help focus attention on those locations within the Bay and its tributaries at which there is decreased water quality. The oyster embryo test requires two days to complete and depends on availability of suitably conditioned broodstock which requires specialized equipment and considerable laboratory space. Oysters have the advantage for testing

estuarine water over many zooplankters of broad euryhalinity.

There is clearly a need for several measures of water quality based on sublethal criteria. Suitable endpoints might include development time and reproduction, or various physiological responses to stress.[137] Several of these endpoints are specific to the organism or the stressor. Among them are osmoregulation, ion regulation, taurine: glycine and other amino acid ratios, enzyme activity, energy availability (as energy charge ratio), oxygen consumption, serum constituents and finally measures of metabolism which integrate several biochemical and physiological responses.[137] In another review,[138] criteria for useful indicators of biological effects of pollution are listed, together with criteria for indicator organisms. Additional responses are described, based on growth and reproduction.

There are two cellular endpoints currently under investigation in the laboratory of the first author, which have not been suggested elsewhere. These are being investigated with the copepod *Eurytemora affinis* but are usable in principle with any indicator organism and, quite likely, with a wide spectrum of contaminants. The first of these is plasma membrane fluidity. It is a well-documented fact that membrane fluidity or viscosity changes as an adaptive response to temperature and other stressors. Such phase changes can be observed spectroscopically in whole organisms.[139] The other endpoint is synthesis of novel proteins under stress conditions.[140] These proteins are known as "heat shock proteins", but have been shown to be induced by a variety of stimuli in addition to heat shock. These proteins are now referred to as "stress proteins". All five stress proteins identified to date are synthesized following chronic exposure to contaminants or to environmental changes within normal ecological limits. To have practical applications, both endpoints will have to be linked to a simpler response. In the case of the stress proteins, an immunological method of detection would allow large-scale yet inexpensive assays.

Whatever laboratory biological test of water quality is used, coupling it with chemical analyses of the same water samples will facilitate interpretation of field observations which are possibly the result of pollutant effects. If a water sample from a given area tests high in water quality (e.g. embryo survival is high, abnormal development is infrequent, or stress proteins are not synthesized), whereas the zooplankton community is depauperate, one might shift attention to food availability or predation and away from anthropogenic chemicals as the basis for the community stress.

IN SEARCH OF A BALANCED ASSESSMENT

In developing this review of contaminant effects on the zooplankton community, a major objective was to assess the impact of each class of contaminant on this community at the present time. To do this, we examined both

laboratory and field data.

Laboratory studies, performed under controlled and reproducible conditions with single species and single compounds using death as the principal end-point, can identify certain compounds as extremely hazardous. One such compound is TBT since 1) it is highly toxic, 2) it is presently found within the Bay at concentrations which are possibly significant to the zooplankton communities in localized areas such as marinas, and 3) expanded use for macrofouling control on ship hulls or in cooling water systems is likely if no regulatory action is taken. Chlorine residuals are another pollutant identified since 1) they are highly toxic, 2) they are presently found at concentrations which may have an adverse effect, and 3) expanded or modified use for disinfection or macrofouling control could result in elevated concentrations. These types of laboratory data are generally considered sufficient to define a water quality criterion which is then used to regulate municipal and industrial discharges.

To demonstrate the existence of a real population effect in the field is a more complex task and is rarely attempted with zooplankton. It may, nevertheless, be an important step in the development and implementation of regulatory action. In the case of chlorine residuals, laboratory toxicity data were collected using valid methods. These methods could not include recruitment as would occur in the field. Using historical estimates of residuals at the point of discharge at one plant, it seemed that a real and present impact was highly likely at this and similar sites. Laboratory studies also demonstrated the benefit of dechlorination. On this basis, it would seem reasonable to propose dechlorination to eliminate the presumed present impact. However, a measurable impact of present chlorination practice could not be demonstrated in the field, either because of erroneous assumptions regarding chlorine residual concentrations near the discharge or because of real, albeit unavoidable, deficiencies in experimental design.

The important point is not to account for the precise reason(s) for the difference between conclusions based on laboratory tests and on actual field observations, but to recognize that any negative impact of chlorinated sewage discharge is difficult to demonstrate in the field. The field data thereby call into serious question the *necessity* for expensive dechlorination, although some general benefit of eliminating the release of a toxicant into receiving water must surely accrue.

Yet relevant field evaluations are difficult to design, time consuming and costly to implement, and results are often difficult to interpret. A balanced approach must be sought to prevent precipitous regulatory action based on laboratory data derived from standardized tests which were designed to define relative toxicity of substances rather than ecotoxicological effects. The answers may lie in two quite different directions. One is to make greater use of sublethal biological tests of water quality, particularly those based on the rapid responses discussed earlier. Parcels of ambient waters could be tested biologically and chemically

and the critical chemicals identified in cases of positive biological responses, using further tests on single chemicals if necessary. The other approach is to use community indices of low water quality, using field data augmented by laboratory microcosm tests.[141] Whatever methods are used, extrapolation from laboratory to field is no more straightforward in environmental toxicology than it is in any other area of environmental biology.

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