FILE Maxus Lib - Hudson/Raritan
REC'D Sed./Water Studies

CL4 300
Sed. Tox

TOXIC ASSESSMENT OF NEW YORK/NEW JERSEY HARBOR AMBIENT WATERS

Final Report

March 26, 1993

Submitted to:

Office of Water Enforcement and Compliance
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Submitted by:

Science Applications International Corporation
Environmental Testing Center
165 Dean Knauss Drive
Narragansett, Rhode Island 02882

MAXUS LIBRARY COPY

Contract No. 68-C8-0066, WA No. C-3-45
SAIC Project No. 01-0440-03-4018-010

Newark Bay Study Area
NBSA-00078

Table of Contents

Introduction 3

Methods 4
- Sample Collection 4
- Toxicity Tests with *Arbacia punctulata* 6
- Toxicity Tests with *Champia parvula* 7
- Chemistry 7
- Data Analysis 8

Results9
- Distribution of Toxicity by Station 9
- Distribution of Toxicity by Area 11
- Chemistry 11

Discussion 11

Conclusions and Recommendations 14

References 15

Table 1. Number of samples showing toxicity 16
Table 2. Range of values for each test method 17
Table 3. Response ranges from Weis, 1990 18
Table 4. Summary of toxicological results for each area 19
Table 5. Summary of data for selected chemicals 20

Figure 1. Map of station locations 21
Figure 2. Graph of June 1991 results for both species 22
Figure 3. Graphs of July and January results for *Arbacia* 23
Figure 4. Graph of July and January results for *Champia* 24
Figure 5. Power curves for both species 25
Figure 6. Map of effects by area for *Arbacia* 26
Figure 7. Map of effects by area for *Champia* 27
Figure 8. Copper bioassay curve for *Champia* 28
Figure 9. Graph of measured vs. estimated total copper 29

Appendix A. Summary of *Arbacia* toxicity data.
Appendix B. Summary of *Champia* toxicity data.
Appendix C. Raw toxicity data for *Arbacia*.
Appendix D. Raw toxicity data for *Champia*.
Appendix E. Data on salinity adjustment.
Appendix F. Summary of chemistry data.
Appendix G. Summary of reference toxicant data.
Appendix H. Plot of development to first cleavage vs. fertilization for *Arbacia*
Appendix I. Comments received on first draft of report.

Introduction

The U.S. Environmental Protection Agency (EPA) Region II is jointly sponsoring the New York and New Jersey Harbor Estuary Program (HEP) with the New York State Department of Environmental Conservation and the New Jersey Department of Environmental Protection and Energy. This study was one part of an HEP characterization plan whose main purpose is to document the nature and severity of environmental water quality problems in the Hudson/Raritan Estuary. A significant component of this plan is the spatial and temporal characterization of toxic contamination. To understand the potentials for ecological effects resulting from this contamination, toxicity tests of water samples collected throughout the estuary were conducted during the summer of 1991 and the winter of 1992. The main focus of the sampling events was to assess both small scale (spatial and temporal) and broad scale variability in toxicity. Sample stations avoided proximity to point sources of pollution. Thus results reflect general water quality and not that of areas of obvious potential impact. A water quality assessment of this nature never has been used on such a large scale for ambient waters.

An important consideration for this task was the choice of toxicity test methods. Embryo-larval and early juvenile life-stages of aquatic organisms are generally most sensitive to environmental perturbations. Assessing the extent of early-life impacts, primarily in sensitive species with standard ambient water toxicity tests, was the preferred technique chosen by HEP to determine ambient water toxicity distribution in the estuary. The test methods chosen were the sexual reproduction test with the marine red alga, *Champia parvula,* and the fertilization test with the sea urchin, *Arbacia punctulata.* There are several advantages to these test methods. They are both included in EPA's whole effluent toxicity testing program, and can test discrete samples. Other test procedures, such as those with mysid shrimp and fish, require media changes. Testing discrete samples gives a more detailed characterization of the variability of toxicity. Both test procedures (especially that for *Arbacia*) provide rapid screening of toxicity. Both of the species are sensitive to heavy metals (this is particularly true for *Champia* concerning copper), and heavy metals may be a significant causative agent for toxicity in the estuary. Finally, these two test methods are less expensive to perform than tests with either mysids or fish. This was a significant factor considering the large geographical area covered and the number of samples.

One hundred and thirty-eight water column samples were tested from three sampling events. The objectives of the first sampling event were to (1) estimate the variability in toxicity for four pre-determined locations that are representative of historical toxic gradients in the estuary and (2) select the most representative depth and time within a tidal cycle from which to collect future samples. These first four stations were at South Piermont Pier (Hudson River), Newark Bay Turning Basin, Raritan Bay and south of the North River Sewage Treatment Plant (Hudson River) by Grant's Tomb. Nine samples were collected at each of the four stations over an entire tidal cycle from three depths. The objective of the next two sampling events was to collect water column samples from throughout the HEP study area. Fifty-one locations were sampled during July 1991, and again in January 1992, in the Hudson River, Upper and Lower New York Bay, East River, Newark Bay, Jamaica Bay, Kill van Kull, Arthur Kill and Raritan Bay.

Toxicity tests were performed with both species on all samples. The EPA's Environmental Services Division (ESD) in Edison, NJ measured ammonia, total organic carbon, and various heavy metals. Many of the ambient samples were toxic to either one or both of the species tested. It also is significant that many of the other samples were not toxic to either species. However, much of the data for *Champia* is inconclusive for the January 1992 samples due to poor control response. The variability in toxicity among samples collected within a single area, or between samples collected during the two broad scale collections, was often less than that for the nine samples from a single station from the first sampling period. Since significant changes in water quality can occur on a relatively small scale, we cannot be certain that changes seen on a large scale were due solely to season or area sampled. The available data also suggest that the results observed could not be readily correlated with heavy metals or ammonia.

Methods

Sample Collection

Environmental Protection Agency Region II personnel aboard the EPA Ocean Survey Vessel *Peter W. Anderson* collected the samples. Field collections took place during June 19-25 and July 20-23, 1991 and January 8-11, 1992. Salinity was measured for each sample onboard the *Anderson* using a conductive salinometer. Water samples

were collected for both chemistry and toxicity tests with a rosette mounted 30 liter General Oceanics Teflon®-lined Go-Flo bottle. Go-Flo bottles and backups were filled with 0.1% HCl and allowed to stand for two hours before initiation of sampling to minimize contamination. The Go-Flo bottles were then rinsed with deionized water. Field blanks were collected at the beginning and end of each survey. Field duplicates were collected for each parameter every tenth station for chemistry. Due to the large size of the *OSV Anderson*, the EPA Region II Survey Vessel, *Clean Waters,* sampled Jamaica Bay and Upper Newark Bay. A 10 liter Teflon®-lined Niskin bottle was used for these stations following identical sampling techniques.

Figure 1 shows all sample stations. For the samples taken over the tidal cycle, each of four stations was sampled three times at slack tides (two high, one low) over the course of a single tidal cycle at three depths (1m below the surface, mid-depth below the pycnocline and 1m above the bottom). All samples for the broad scale sampling events were from 1m below the surface. To control against cross-contamination between stations, the Go-Flo was rinsed with 10% HCl followed by a deionized water rinse. In addition, before sample withdrawal, approximately 500 ml of sample water was released from the Go-Flo spigot. After this initial release, the sample was withdrawn into a one gallon labeled cubitainer (rinsed twice with sample water) and stored in the dark at 4 °C on board the vessel. No preservation was necessary for the toxicity samples, and chemistry samples were preserved according to the standard methods discussed in the chemistry section. All individuals collecting and handling samples wore disposable surgical gloves for all aspects of the cruise to minimize the introduction of contamination.

Upon arrival at the testing laboratory, coolers were opened and examined to insure each toxicity sample was intact and cool. Each sample was signed in onto a log-in sheet noting its condition and collection date. Initial salinities were measured using a hand-held refractometer, when field measurements were not provided. Samples with a salinity below 27 ppt were adjusted to 30 ppt using hypersaline brine (90 to 103 ppt) that was made by gently evaporating natural seawater. After adjustment, the salinity of the final sample was measured and recorded to verify that it was 30 ppt. All initial and final salinities, and the salinity of the brine used for adjustment are listed in Appendix E. .

Toxicity Tests With *Arbacia punctulata*

Male and female sea urchins, *Arbacia punctulata*, were held at EPA's Environmental Research Laboratory in Narragansett (ERL-N), Rhode Island. Each sex was held in a separate, 20-liter aerated tank with a seawater flow rate of 5 liters per minute. The temperature was maintained at approximately 15 °C. The sea urchins ate freshly collected kelp (*Laminaria* spp.) , *ad libitum.*

Test procedures followed EPA's protocol for complex effluents (EPA, 1988) with the exception of the development to first cleavage endpoint. Four female sea urchins were transferred to a large Carolina dish filled with enough seawater to just cover their shells. Eggs were obtained from the females by electrical stimulation. The electrodes from a 12-volt transformer were gently placed on the shell close to the gonadopores for about 30 seconds. The red eggs, which pool above the gonadopores, were collected with a 10 ml disposable syringe with a blunted large-gauge needle. Eggs were collected from all four females and kept at room temperature (about 20 °C) until use (no longer than two hours). The final egg concentration in the stock solution was 2,000 per ml. Sperm also were collected from four male sea urchins by electostimulation, using a 2 ml syringe, and kept on ice. The time of sperm collection was recorded; the sperm were exposed to the test material within one hour after collection. The sperm were diluted to a stock concentration of 50 million per ml, and the stock kept on ice until use.

A test began with the introduction of 100 µl of the well-mixed sperm stock to each test and control vial (which contained 5 ml of sample or control seawater) so that there were about 5 million sperm in each vial. The test vials were then covered, the time was recorded and the vials held at 21 to 23 °C for one hour. There were four replicates per sample, and both natural seawater and brine controls were used when the samples required salinity adjustments. After one hour, the egg stock was mixed by gentle aeration and 1 ml of stock added to each exposure vial. The vials were gently swirled to insure mixing and covered again. Seventy minutes after the addition of eggs, a small subsample was removed from a control vial to assure that cleavage had occurred. Usually cleavage was underway 70 minutes after fertilization and was completed to the two cell stage by 80 minutes. Once cleavage began, 1 ml of 10% buffered formalin in seawater was added, terminating the test. The fertilization and cleavage were evaluated by examining 100 individual embryos from each replicate with a compound microscope at 100X magnification. Reference toxicant tests were performed using sodium dodecyl sulfate.

Toxicity Tests with *Champia parvula*

Both male and female plants were maintained in natural seawater according to standard methods (EPA, 1988). Cultures were illuminated with ca. 75 $\mu E\ m^{-2}\ s^{-1}$ of cool-white fluorescent light on a 16:8, light:dark cycle. The temperature was held at 22 to 24 °C and the salinity at 28 to 30 ppt. Media were changed once a week.

As with *Arbacia*, test procedures for *Champia* followed EPA's protocol for whole effluents (EPA, 1988). Female branch tips, 7 to 10 mm in length, were cut to serve as inocula. Five tips were placed into each treatment cup along with one male branch (1.5 to 2.0 cm long) that was visibly producing spermatia. Tests were performed in 125 ml borosilicate Erlenmeyer flasks containing 100 ml of medium. Males and females were exposed together for 2 days followed by a recovery period for the females of up to 9 days for cystocarp development (evidence of sexual reproduction). Exposure chambers were hand swirled at least twice each day. Recovery bottles (250 ml with 150 to 200 ml of medium) were aerated. At the end of the recovery period, the numbers of cystocarps per female were counted. The results from each replicate flask (n=4) were reported as the mean number of cystocarps per plant. Reference toxicant tests were performed using sodium dodecyl sulfate.

Chemistry

Ambient water samples for chemical analyses were taken and stored in the same manner as toxicity samples. Total recoverable metal was analyzed for Ag, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Sb, Se, Tl and Zn. Total organic carbon (TOC) and total ammonia analyses also were conducted. These analyses were performed by the EPA's Environmental Services Division (ESD)--Edison, NJ. Silver, beryllium, cadmium, chromium, copper, nickel, antimony and zinc were analyzed by ICP using EPA standard methods SW846-3005 (preparation) and SW846-6010 (analytical). Chelation extraction was performed on all metals as described in section 9.2, *Methods for Chemical Analysis of Water and Wastes.* Arsenic, selenium, thallium, lead and mercury analyses were conducted by graphite furnace using EPA preparation method SW846-3020. EPA analytical methods used for these analyses were; arsenic (SW846-7060), selenium (SW846-7740), thallium (SW846-7841), lead (SW846-7421) and mercury (SW846-7470/cold vapor). Total organic carbon (EPA Method TSB's # C-31B) and total ammonia (EPA Method TSB's #C-39) analyses also were performed at ESD. Reporting

limits were determined by performing a one-day detection limit study. The detection limit was calculated using the standard deviation of seven replicate analyses of one typical harbor sample, spiked with all analytes of interest.

Data Analysis

Untransformed data met the test for normality (a probability plot), therefore data were analyzed without transformation. The *Champia* data were expressed as absolute counts. The *Arbacia* data were expressed as percent development to first cleavage. Natural seawater and brine controls were pooled before statistical analyses for each test series. To estimate the small scale variability of toxicity the results from the samples collected from each of the four stations during the first sampling event were compared against their respective performance controls using an unpaired, one-way *t*-test (alpha = 0.05) assuming unequal variance (Moser and Stevens, 1992). To determine if there was any relationship among toxicity and water depth and time within a tidal cycle, a two-way analysis of variance (ANOVA) for time and depth interactions was run for each of the four stations from the June, 1991 data set.

Statistically significant decreases relative to performance controls were determined for each of the samples from the two broad scale collection events using a one-way, un-paired *t*-test (alpha = 0.05) assuming unequal variances. Statistical significance was not tested between individual samples. Since no field replicates were taken, any differences detected between samples could not be attributed to either location of to sampling event. Differences between areas, however, could be examined. The 51 stations were divided among seven areas within the Hudson/Raritan Estuary (Figure 1). One-way, unpaired *t*-tests were used to compare data from each area with the two controls for the test series within which samples from that area were tested (e.g., natural seawater and brine controls). The comparisons were made to determine if the mean response for a given area was statistically less than the respective control samples. Data from each area for the two sampling events also were compared using a two-way, unpaired *t*-test.

The statistical hypothesis that is being tested in the above *t*-tests and ANOVAs is that two (ore more in the case of ANOVA) means are equal. The alpha level (0.05) tells us how often we may make a mistake by concluding that two means are statistically equal when in fact they are different. Statistical power gives an estimate of how

confident we can be in our conclusion when we say two sample means are different. Statistical power was determined empirically. For each *t*-test performed, the minimum significant difference (MSD) that could have been detected was computed. A cumulative frequency distribution was plotted for all of the MSDs from acceptable tests for each species against percent difference from the performance control. This plot can then be used to show the power to detect any given difference from the performance control.

Results

Distribution of Toxicity by Station

One-hundred and thirty eight ambient water column samples were collected for chemical analysis and toxicity testing. Thirty-six of these samples were during June 1991 (nine each from four stations), to estimate small scale variability in toxicity with both time and water depth. The remaining 102 samples were divided equally between two broad scale sampling events, July 1991 and January 1992, covering the entire estuary. Chemical measurements and toxicity tests using both *Arbacia* and *Champia* were performed on all 138 samples. Summary and raw data for the results are listed in the Appendices.

Figure 2 shows the toxicity results for the small scale sampling event. The data set represents four separate test runs. The results are normalized as a percentage of the performance control response. There is considerable variability for both test species with respect to time and water depth. There is no consistent relationship between either time of sampling (high or low slack tide) or water depth and the degree of toxicity observed relative to performance controls. There also is no consistent relationship between the responses of *Champia* and *Arbacia* to the toxicity of the samples. Appendices A , B, C and D list the raw data, as well as the summary of the means.

Figure 3 shows the results of both broad scale sampling events for *Arbacia*. The data are grouped by collection area from within the estuary and are expressed as a percentage of the performance control that went with a particular sample's toxicity test. An asterisk indicates a response that is statistically less than the performance control. In general, responses are lower from the July sampling event than from January. Appendices A and B list the raw and summary data are for the *Arbacia* results from both sampling events.

Figure 4 shows the results of both broad scale sampling events for *Champia*. As with *Arbacia*, the data are grouped by collection area and are expressed as a percentage of the performance controls. An asterisk indicates a response that is statistically less than the performance. In general, responses were greater from the July sampling event than from January. However, most of the January data are from tests for which the average number of cystocarps produced by the performance controls are less than the recommended 10 for an acceptable test. Appendices C and D list the raw and summary data for the *Champia* results from both sampling events.

Table 1 lists the number of samples from the broad scale events showing toxicity to the test species, expressed as statistical and detectable significance. Statistical significance reflects the results of the one-way, unpaired *t*-tests, while detectable significance reflects the ability of the toxicity tests to detect significant differences (i.e., the statistical power of the tests). Detectable significance was determine empirically for both species by plotting the cumulative frequency of the minimum detectable differences for each *t*-test. The resulting power curves are shown in Figure 5. The cut-off for detectable significance was set at that difference that could be detected at least 90% of the time. For *Arbacia* this resulted in a minimum detectable difference of 25%, for *Champia*, 35%. Detectable significance is a better indicator of significant differences than statistical significance. This is because the former incorporates into the decision process the magnitude of difference a the toxicity test was actually designed to detect.

The range of responses by both *Arbacia* and *Champia* varied depending on the area and collection event. Table 2 lists the ranges for both species for the four small scale sampling stations and the seven areas from each of the two broad scale sampling events. The variability in toxic response at a single station (the nine samples taken over a single tidal cycle from three depths) could be as great as or greater than that observed within a single area. For example, the percent development of *Arbacia* embryos to first cleavage ranged from 45 to 91 in June 1991, at station 11 in the Hudson River. The response for this species ranged from 62 to 90 percent among all eleven stations from the Hudson River during the July 1991, sampling event. Large variations in the toxicity of ambient water samples from the Hudson/Raritan Estuary to fertilization by *Arbacia* also were observed during a previous series of sampling events in 1990 (Table 3).

Distribution of Toxicity by Area

The response for each species, averaged for each area and sampling event, are listed in Table 4 and presented graphically in Figures 6 and 7. The data show that there were statistically significant improvements in water quality from the July 1991, to the January 1992, samples for *Arbacia*. This improvement occurred in four of the seven areas: Hudson River, East River, Kill van Kull, and Raritan Bay. The shifts in sensitivity for *Champia* were generally toward more toxicity in the January samples, but there were significant overall decreases in toxicity to this species in both the Hudson River and Raritan Bay in January relative to July samples.

Chemistry

Appendix F lists the raw data from EPA's Edison laboratory for the chemistry values measured for each sample. Table 5 lists the ranges for all samples for some of the metals (for which *Champia* and *Arbacia* data are available) and ammonia along with the expected 50% effect concentrations for both *Arbacia* and *Champia*. None of the chemicals measured occurred at concentrations higher than the expected EC50 concentrations for *Arbacia*. Only copper exceeded EPA's national ambient water quality standards based on total copper. Since copper was a potential toxicant of primary concern, it was singled out for a more detailed analysis than any of the other metals. Copper occurred at concentrations high enough to potentially be toxic to *Champia* in 86 of the 138 samples.

Data from past toxicity tests for copper were used to establish a relationship between total copper concentration and the number of cystocarps produced by *Champia* (Figure 8). The total copper value required to cause a given observed *Champia* response was estimated from this relationship. Thus for each sample with measured copper above 3 ppb, we plotted measured and estimated copper values (Figure 9). The estimated copper values were always below 3 ppb, independent of the measured value.

Discussion

Large scale ambient sampling studies such as the current project are rare. Field sampling for ambient water toxicity often consists of a series of single grab samples from various stations within a given area of concern, generally collected in proximity to point

sources of pollution. The main purpose of this study was not to determine regulatory compliance with existing chemical and toxicity standards, but rather to characterize the extent and pattern, if any, of ambient water column toxicity in the Hudson/Raritan Estuary. If compliance monitoring were the main focus, then all unacceptable *Champia* results would have to be excluded. In addition, more reliable reference toxicant tests for *Champia* would be needed. Most of the results from *Champia* tests on samples collected during the January 1992, sampling event were associated with unacceptable controls. These unacceptable controls may have been associated with the fact that the plants were recovered for the most part under continuous light. *Champia* needs a light:dark cycle for optimal growth and development. Because of these unacceptable controls, the magnitude of the *Champia* effects may be suspect. These data were included because regulatory decisions are not being made and they still shed light on the variability of the toxicity within the estuary.

There was an emphasis on heavy metal chemistry, rather than organics, because at present, the HEP is developing total maximum daily loads (TMDLs)/waste load allocations for toxic metals in the Estuary. EPA in conjunction with the States of New York and New Jersey has agreed that the states will modify discharge permits to include water quality-based effluent limits for copper, mercury and six other toxic metals, as necessary. Because of this, the magnitudes of potential toxic effects within the estuary are of great concern. The species chosen for toxicity testing are relatively sensitive to toxicants compared to some of the more commonly used test species, such as mysid shrimp and juvenile fish. It was important to use sensitive species since the goal was to be able to detect the presence of toxicity.

The objectives of the June 1991, sampling event were to not only address the magnitude of small scale variations in toxicity, but also to determine the best time within a tidal cycle and depth from which to sample during the broad scale sampling events. Although there were a number of June samples that were statistically less than their respective performance control, there was no consistent pattern of toxicity with respect to either time or depth. A two-way ANOVA also showed no significant interaction between depth and time. However, there were some statistical differences related to only time or only depth at specific stations. A good example of this is shown from the surface samples for station HR11, where the first high tide sample (A) had a frequency of development to first cleavage for *Arbacia* of 44.9% of the control, while the following low and high tides had frequencies of 91.4 and 89.3 % of the control, respectively.

Toxicity also could vary significantly with depth. The *Champia* results for station HR6 at low tide (B) showed a decrease in the number of cystocarps formed as the depth increased. Clearly, the part of the tidal cycle and the depth samples were taken could effect the toxicity of those samples. The lack of a consistent pattern, however, made it impossible to select the most representative time or depth for sampling during the other two events. To be consistent a decision was made to sample near high tide and at the surface.

Many of the ambient samples from the three collection events were toxic to the species tested. In fact, each station was toxic to either *Arbacia* or *Champia* from at least one of the sampling events. Neither *Arbacia* nor *Champia* was, overall, more sensitive to samples from the estuary. Depending on the event or the station, either species could be more sensitive than the other. The lack of a relationship between the sensitivity of these two species is consistent with earlier findings from their use in EPA's Complex Effluent Toxicity Testing Program (Schimmel et al., 1989). This underscores the importance of using more than one test species and having more than one collection event. Equally important in the characterization of toxicity is the fact that many of the samples tested showed no toxicity to either of the test species, even though these two test methods are generally considered to be among the more sensitive.

One of the original purposes in selecting July 1991 and January 1992 for the broad scale sampling events was to develop a baseline database for dry verses wet weather ambient water column toxicity distribution throughout the estuary. If only these two sampling events had been conducted, then we might have been tempted to draw conclusions about observed differences between the two events based on season alone. The variability of the data among stations within a single area from a single sampling event was in some cases less than that from a single station with multiple samples from the June 1991 sampling event. In other cases the reverse was true. Knowledge of the small scale variability in a characterization of toxicity is valuable when making decisions about the significance of the magnitude of differences among data sets from different areas or different sampling events. Even though we may observe statistically significant differences among samples within a given area or between the two main sampling events, if the magnitude of the differences is not greater than that measured at a single station with multiple samplings, then the observed differences cannot be unambiguously attributed to station or seasonal differences.

One of the assumptions made by the HEP at the beginning of the study was that heavy metals, especially copper, were major contributors to any toxicity that may be found in the Estuary. Because there was essentially no correlation between the measured total copper values and the number of cystocarps produced by *Champia*, the validity of that assumption is questionable. Copper was present in measured concentrations sufficient to potentially cause serious impacts on *Champia* reproduction. The lack of such impacts in the presence of high total copper strongly suggests that copper (and perhaps other metals) was not biologically available. Since the samples were not filtered, copper may have been bound to suspended solids.

As stated above, heavy metals, and copper in particular, were assumed to be a serious toxics problem in the estuary. Since measured total copper was below the 3 ppb detection limit in so many samples, there were concerns that there may have been analytical problems associated with the copper analyses. If there were any problems it is more likely that errors would have resulted in false high values not false low ones. No extraction was done to remove salts from samples. Therefore it's possible the build-up of salts throughout the ICP sample introduction system may have caused some imprecision resulting in varying background levels or "false hits". (K. Piest, ESD, personal communication to E. Stern, EPA, Region II).

Conclusions and Recommendations

1. Any toxicity evaluation for ambient waters clearly must include a characterization of the variability of the toxicity in both time and space. This characterization will aid in the final design of a sampling strategy. Resources for future sampling and testing should be allocated in direct proportion to the estimated variability (i.e., placing most of the effort in areas with the greatest environmental variability).

2. Many of the ambient samples were toxic to either *Arbacia* or *Champia,* but just as important, many of the samples were not. The patterns in toxicity could not be attributed to heavy metals, in particular copper, as had been previously assumed. Future work should include Toxicity Identification Evaluations (TIEs). The use of TIEs could help classify the type of toxicants that are present and perhaps relate that toxicity to potential point sources.

References

Burgess, R.M. 1990. Water column toxicity from contaminated sediments. M.S. Thesis. University of Rhode Island, Narragansett, RI.

Moser, B.K. and G.R. Stevens. 1992. Homogeneity of variance in the two-sample means test. American Statistician. 46:19-21.

Nacci, D., E. Jackim and R. Walsh. 1986. Comparative evaluation of three rapid marine toxicity tests: Sea urchin early embryo growth test, sea urchin sperm cell toxicity test, and Microtox. Environ. Toxicol. Chem. 5:521-525.

Schimmel, S.C., G.E. Morrison and M.A. Heber. 1989. Marine complex effluent toxicity program: Test sensitivity, repeatability and relevance to receiving water toxicity. Environ. Toxicol. Chem. 8:739-746.

Schimmel, S.C., G.B. Thursby, M.A. Heber and M.J. Chammas. 1989. Case study of a marine discharge: Comparison of effluent and receiving water toxicity. (in) Aquatic Toxicology and Environmental Fate: Eleventh Volume, ASTM STP 1007. G.W. Suter II and M.A. Lewis (eds). American Society for Testing and Materials, Philadelphia. pp. 159-173.

Thursby, G.B. and M. Tagliabue. 1988. Toxicity of thallium to saltwater plants. University of Rhode Island (Memorandum to D.J. Hansen, U.S. EPA ERL-Narragansett, and K.J. Scott, SAIC) May 23. 3 pp.

Thursby, G.B. and R.L. Steele. 1986. Comparison of short- and long-term sexual reproduction tests with the marine red alga *Champia parvula*. Environ. Toxicol. Chem. 5:1013-1018.

U.S. EPA. 1988. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA/600/4-87-028.

Weis, P. 1990. Assessment of ambient waters on early life stages: Preliminary draft. Report to New York-New Jersey Harbor Estuary Program.

Table 1. Summary of the number of samples showing toxicity to *Arbacia punctulata* development to first cleavage and cystocarp production by *Champia parvula*. Values in parentheses for the January samples represent the results when the *Champia* data from tests with unacceptable controls are omitted.

Species	July 1991 Samples	January 1992 Samples
Arbacia punctulata		
Statistical significance	38	15
Detectable significance	31	9
Champia parvula		
Statistical significance	19	32 (2)
Detectable significance	16	29 (0)
Combined		
Statistical significance	45	34 (15)
Detectable significance	37	29 (9)

Table 2. Range of values (expressed as a percent of the performance control) for each test method for each sampling event. The June, 1991, values represent the range of the nine samples from each station. The others represent the range of the values for all stations within each area.

Location	*Arbacia punctulata*	*Champia parvula*
June, 1991, Small scale variability		
HR-11	45-91	57-101
HR-6	77-97	46-91
NB-2	99-101	68-102
RB-6	58-84	48-83*
July, 1991, Broad scale variability		
Hudson River	62-90	47-111
East River	11-49	73-113
Newark Bay	52-83	62-117
Kill van Kull	18-77	62-103
Arthur Kill	57-96	51-94
Raritan Bay	14-41	30-96
Jamaica Bay	41-102	38-79
January, 1992, Broad scale variability		
Hudson River	94-102	88-128*
East River	53-95	26-89*
Newark Bay	63-70	18-25*
Kill van Kull	85-92	38-48*
Arthur Kill	23-83	8-57*
Raritan Bay	90-99	77-103
Jamaica Bay	31-92	0-45*

*Less than the required 10 cystocarps per female in the controls for tests with these samples.

Table 3. Response ranges for *Arbacia punctulata* fertilization from HEP 1990 sampling events (Weis, 1990). Values are expressed as percent of the performance control.

Location and Date	Range
Newark Bay & Kills	
March	81-100
May	46-81
August	60-90
Hudson River/Upper Bay	
April	26-41
June	18-47
August	0-83
East River	
May	81-98
June	70-86
August	31-71

Table 4. Summary of toxicological results for each area from the broad scale sampling events. Values are the means and standard deviations for all stations within each geographical area expressed as percent of the control.

	July, 1991		January, 1992		
Area	Mean	SD	Mean	SD	Significance
Arbacia punctulata					
Hudson River	79.2	10.3	99.3	2.2	a,c
East River	22.8	10.3	73.2	11.1	a,b,c
Newark Bay	72.6	14.1	75.5	3.2	a
Kill van Kull	50.6	24.4	98.5	3.8	a,c
Arthur Kill	76.0	15.6	77.0	25.9	a
Raritan Bay	28.4	9.0	108.0	3.9	a,c
Jamaica Bay	82.6	25.0	82.1	28.2	
Champia parvula					
Hudson River	83.2	17.4	103.6	10.5	a,c
East River	94.7	12.9	56.8	16.4	b,c
Newark Bay	80.9	24.9	21.2	3.0	b,c
Kill van Kull	83.4	17.8	41.3	6.0	b,c
Arthur Kill	80.1	15.6	27.0	18.5	b,c
Raritan Bay	64.2	18.1	88.6	8.8	b,c
Jamaica Bay	50.1	14.8	28.1	21.5	a,b

a = July, 1991, sample statistically significant from the two performance controls using raw means (not % of control). Determined by one-way, unpaired, *t*-test assuming unequal variance (alpha = 0.05).

b = January, 1992, sample statistically significant from the two performance controls using raw means (not % of control). Determined by one-way, unpaired, *t*-test assuming unequal variance (alpha = 0.05).

c = Statistically significant difference between July and January area means. Determined by two-way, unpaired, *t*-test assuming unequal variance (alpha = 0.05).

Table 5. Summary of data for selected chemicals. Measured column shows the range of values for that chemical. *Arbacia* and *Champia* values are the expected 50% effect concentrations.

Chemical	Measured Concentration (µg/L)	*Arbacia* EC50 (µg/L)	*Champia* IC50 (µg/L)
Cadmium	<4	3,800[a]	>100[b]
Copper	<3 - 14.4	12[a]	1.5[d]
Lead	<6	5,400[a]	11-20[c]
Silver	<7-10.9	51[a]	0.9[b]
Thallium	<8	-	6[e]
Zinc	<9-19	120[a]	-
Total ammonia	50-900	20,000[f]	1,200[g]

[a] from Nacci et al., 1986
[b] from Thursby & Steele, 1986
[c] Steele and Thursby, 1983 (based on two week exposure)
[d] from Figure 8
[e] from Thursby and Tabliabue, 1988
[f] from Burgess, 1990
[g] from Schimmel et al., 1989 (estimated from graph)

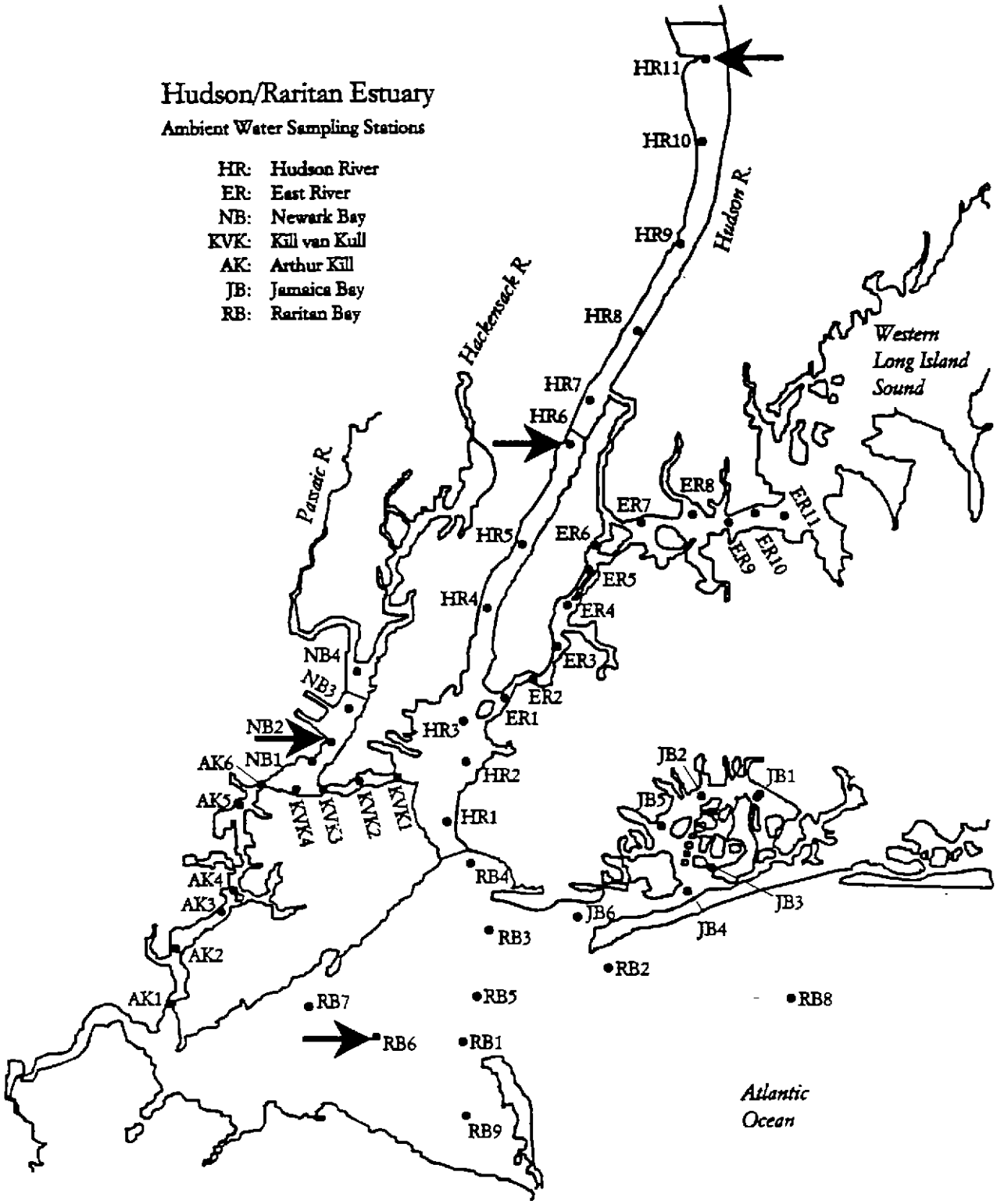


Figure 1. Map of the Hudson/Raritan Estuary showing locations of sampling stations. The four large arrows show the stations sampled during the June 1991 small scall sampling event.

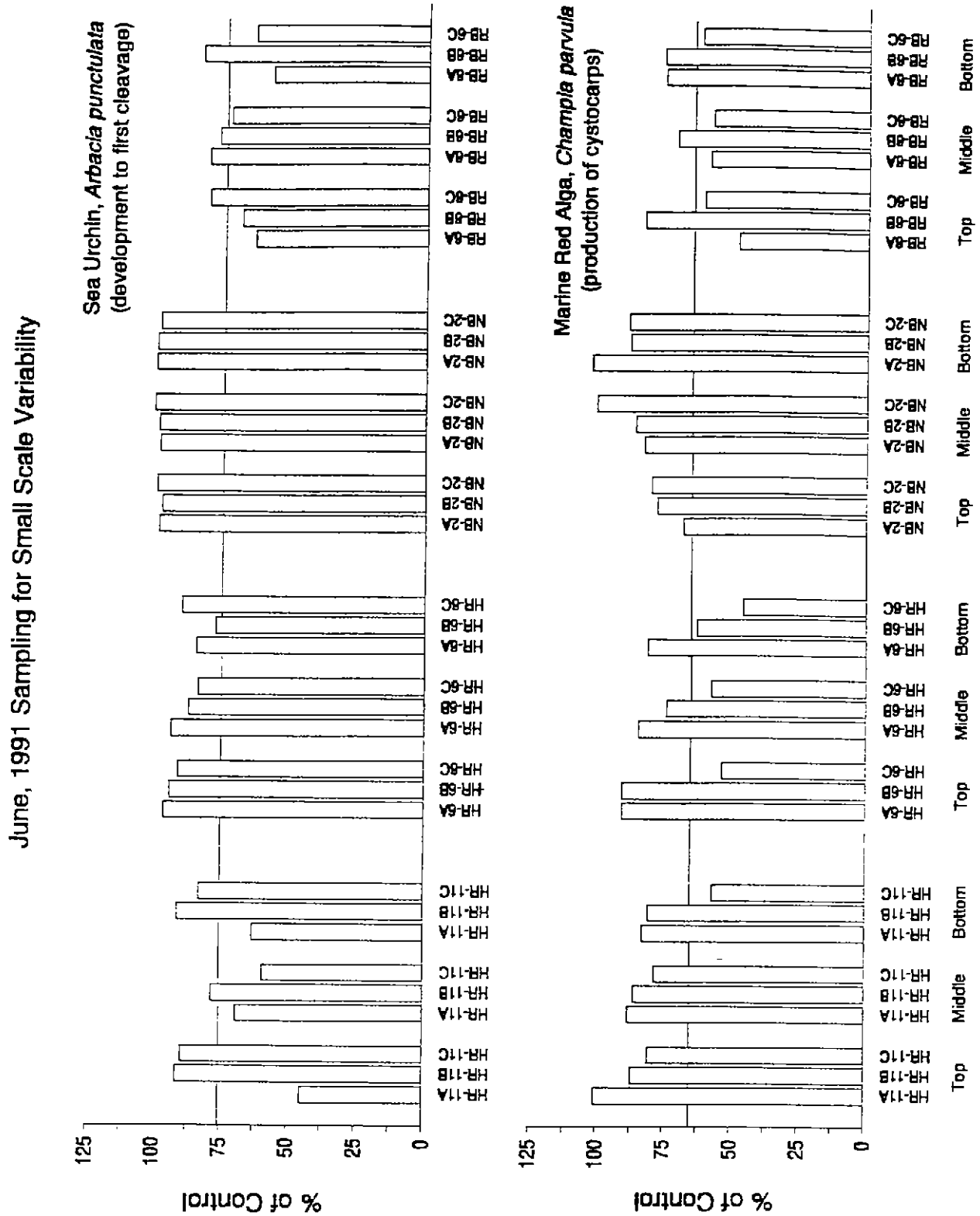


Figure 2. Results of toxicity for both *Arbacia* and *Champia* for samples collected during June 1991. Top, middle and bottom refer to sample depth. Samples with a station number ending in an A are from the first slack high tide, ending in B from the subsequent slack low tide, and ending in C from the second slack high tide. *Champia* results for station NB2 had a performance control that averaged less than 10 cystocarps per female.

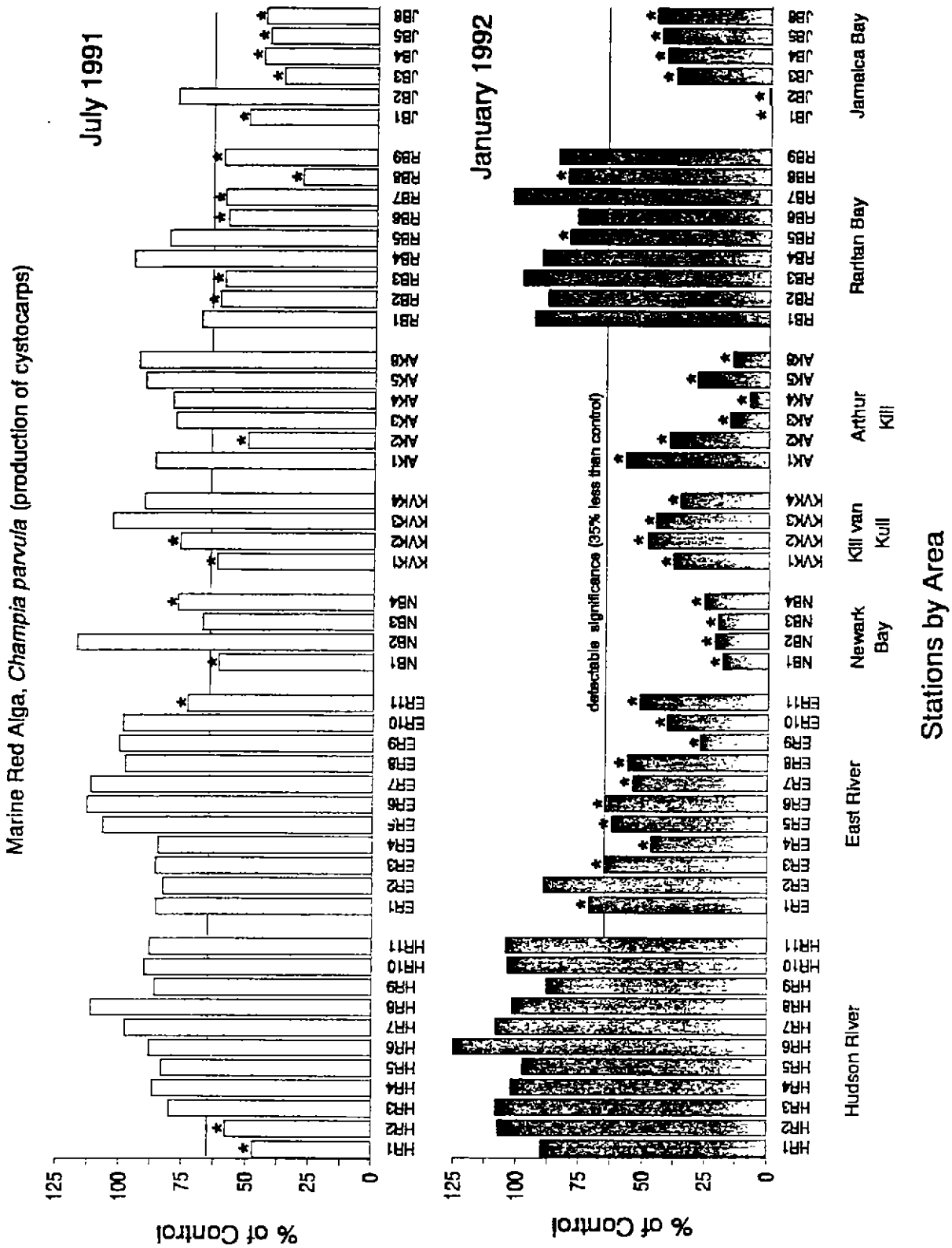


Figure 4. Results of toxicity tests for *Champia* from the July and January broad scale sampling events. An asterisk indicates results statistically less than the performance control. The horizontal line drawn at 65% is the cut off for detectable significance. All January samples except those from Raritan Bay were from tests with performance controls averaging less than 10 cystocarps per female.

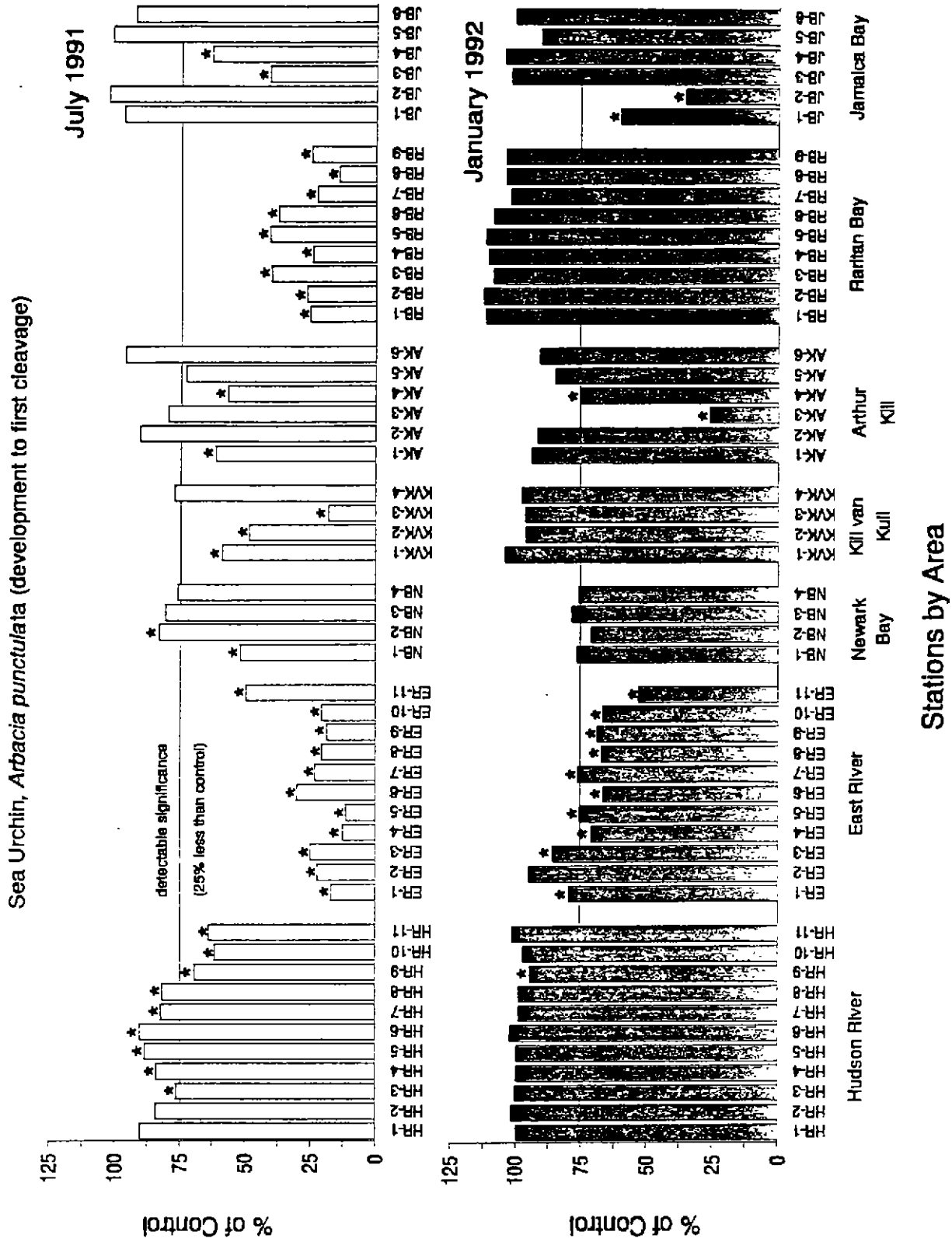


Figure 3 Results of toxicity for *Arbacia* from the July and January broad scale sampling events. An asterisk indicates results statistically less than the performance control. The horizontal line drawn at 75% is the cut off for detectable significance.

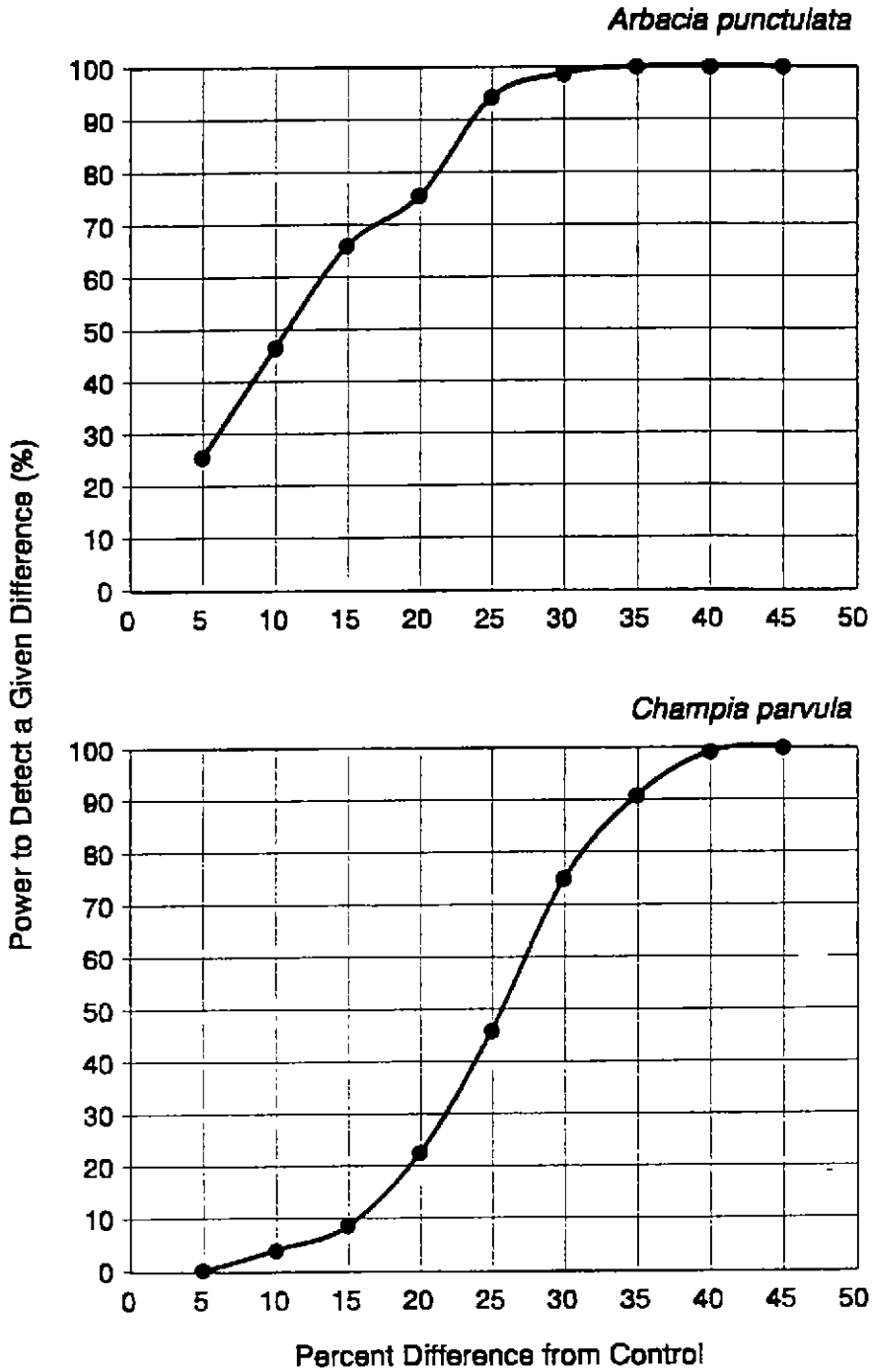


Figure 5. Cummulative frequency for minimum detectable significance (power curves) for *Arbacia* and *Champia*.

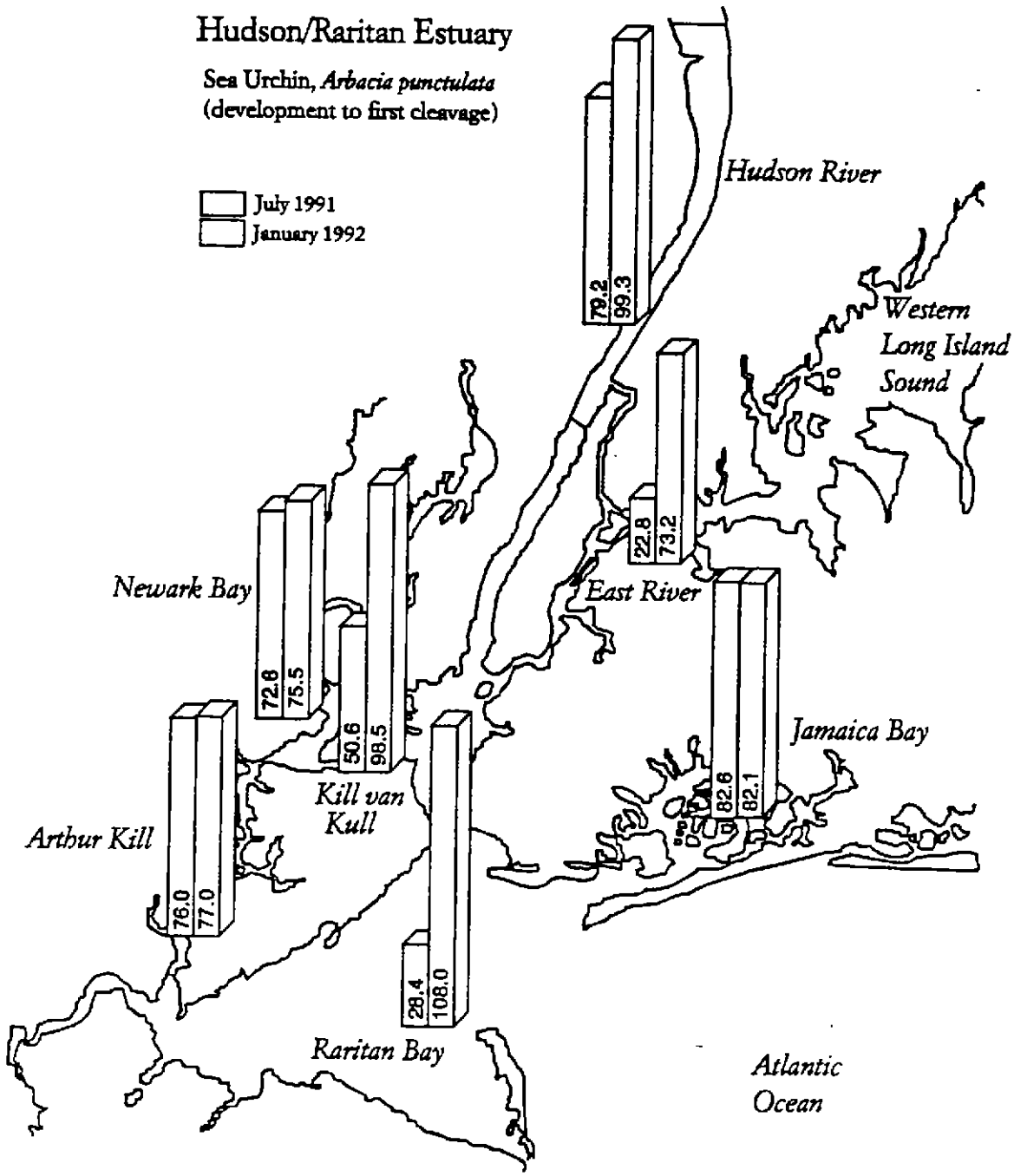


Figure 6. Map of the Hudson/Raritan Estuary showing average response for development to first cleavage by *Arbacia* for each area.

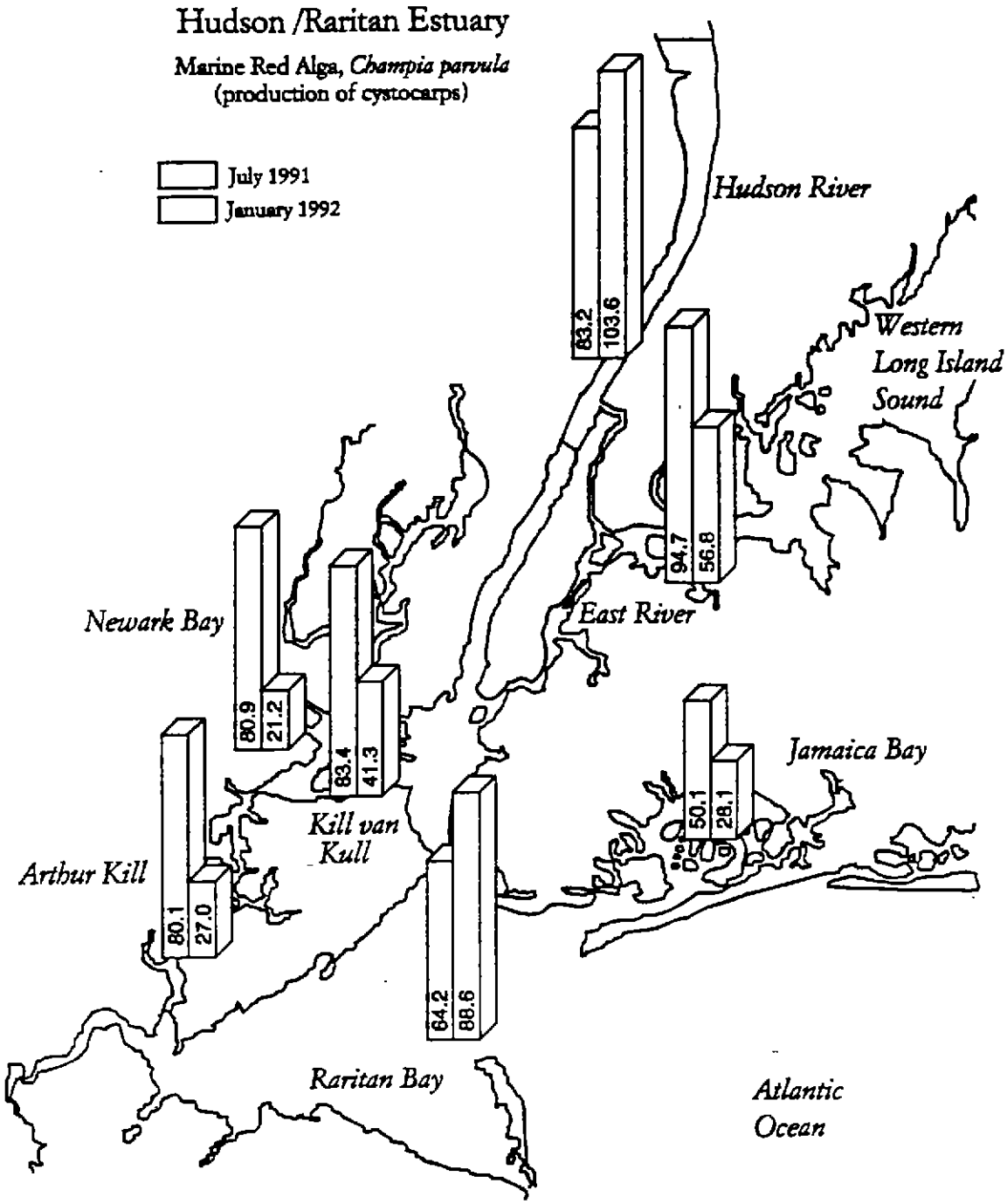


Figure 7. Map of Hudson/Raritan Estuary showing average response for production of cystocarps by *Champia* for each area.

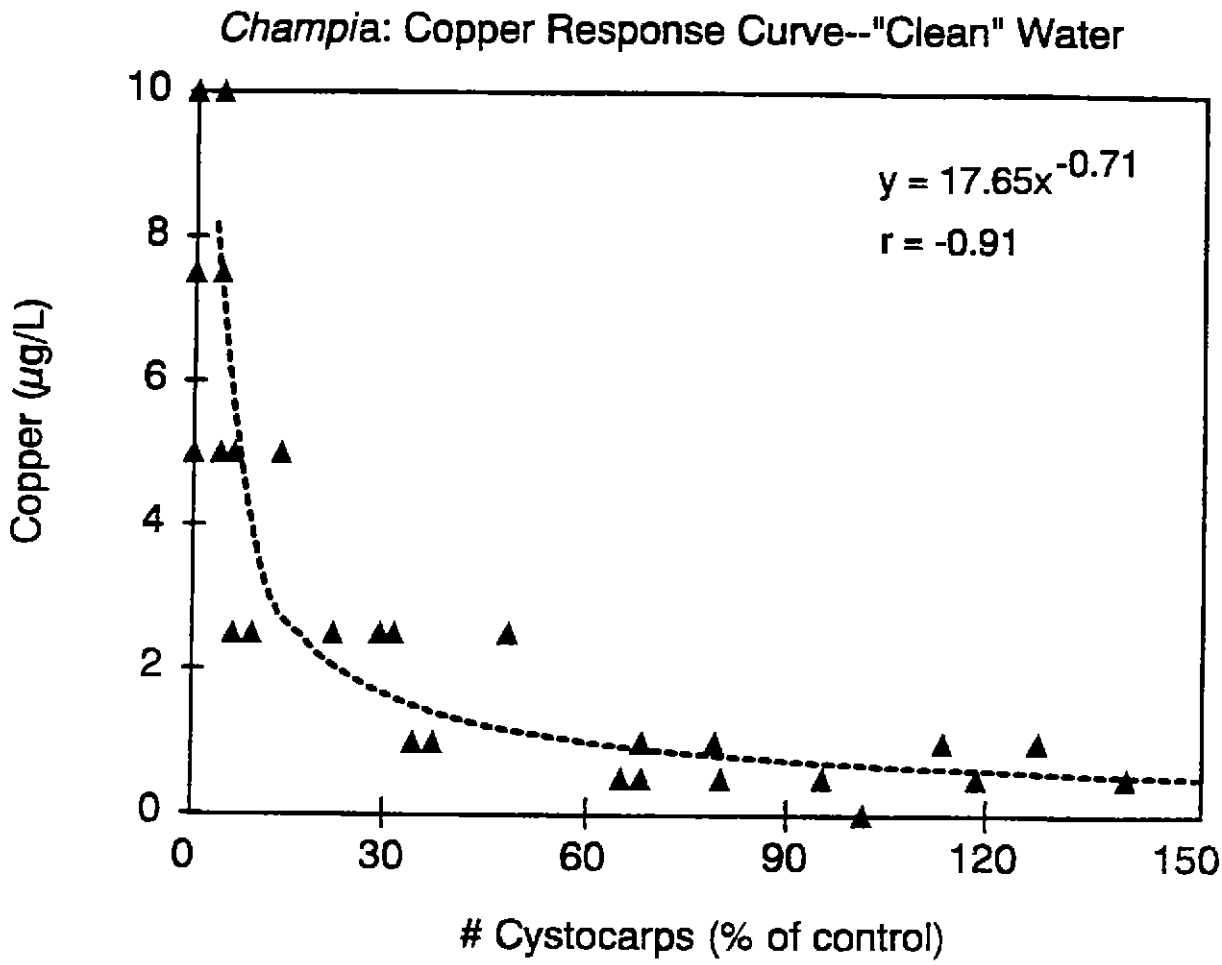


Figure 8. A bioassay curve for copper using production of cystocarps by *Champia*. Data are from a series of reference toxicant tests for copper conducted at EPA's Environmental Research Laboratory in Narragansett, RI.

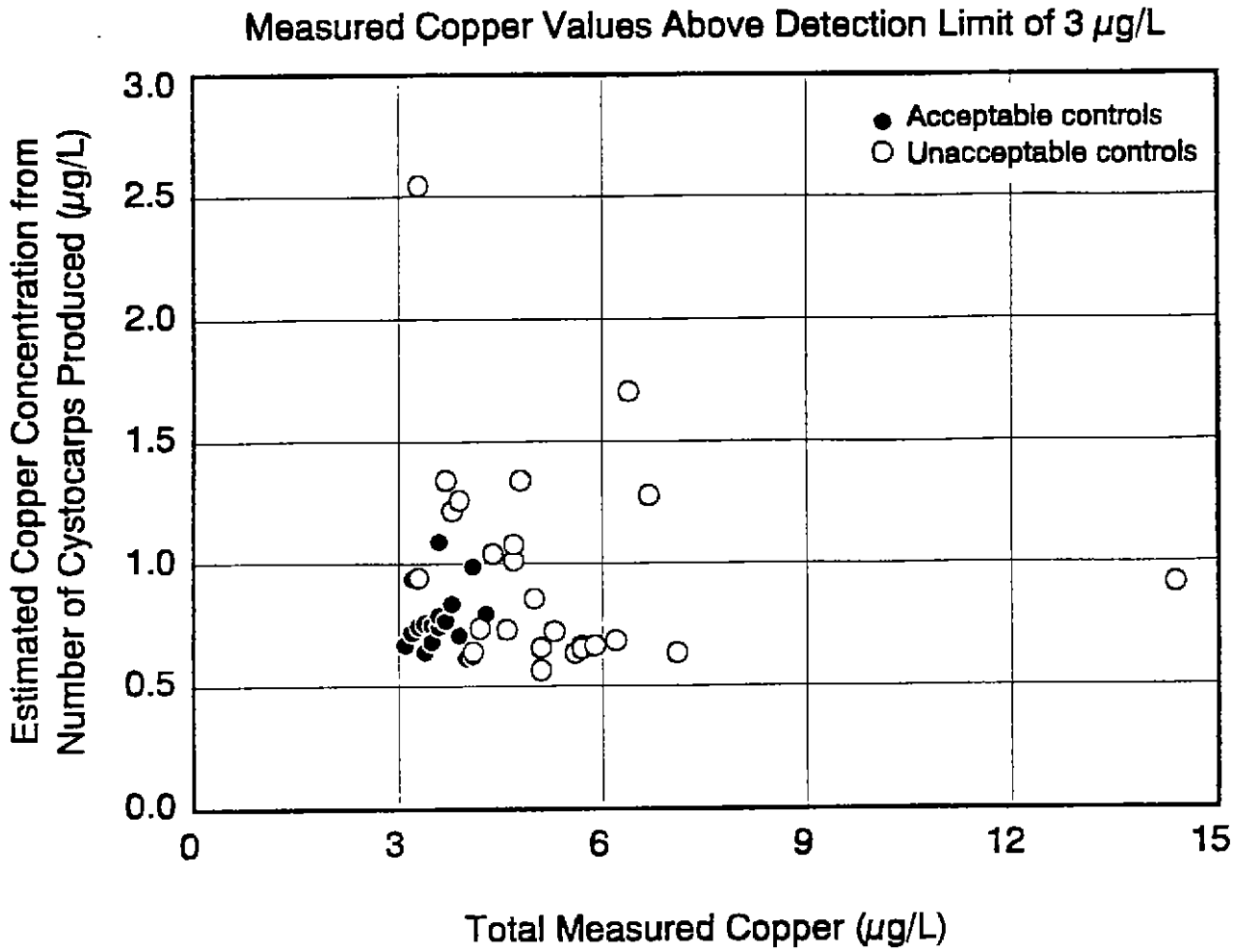


Figure 9. Total measured and estimated copper for each sample with measured copper values above the 3 ppb detection limit. Estimated values are based on the number of cystocarps produced by *Champia* for each sample using the equation in Figure 8.

Appendix A: Summary of toxicity data for *Arbacia.*

Table A-1 list the test dates and control values for the sperm cell test with *Arbacia punctulata* conducted with samples from the Hudson/Raritan Estuary. Table A-2 summarizes the means and standard deviations for samples from the small scale variability sampling event from June 1991. Table A-3 summarizes the data from the two broad scale sampling events in July 1991 and January 1992.

Table A-1. List of test dates and control values for the sea urchin sperm cell test with *Arbacia punctulata*. Controls were pooled for statistical analysis.

Test Series[a]	Test Start Date	Control (% Cleavage) Natural Seawater	Brine + DI[b]	Collection Date
June 1991 Samples				
1	6/22/91	96.5	98.3	6/20/91
2	6/23/91	97.5	96.3	6/21/91
3	6/24/91	93.5	94.5	6/22/91
4	6/26/91	91.7	92.2	6/24/91
July 1991 Samples				
5	7/21/91	88.3	92.2	7/21/91
6[c]	7/23/91	79.3	33.2	7/21/91
7	7/24/91	88.7	77.3	7/24/91
January 1992 Samples				
8	1/9/92	94.3	97.0	1/8/92
9	1/10/92	93.3	87.5	1/10/92
10[c]	1/11/92	27.5	6.2	1/11/92
11	1/13/92	90.7	74.0	1/11/92

[a]Refers to test series listed in Appendix C.
[b]Brine made from Narragansett Bay natural seawater was used when necessary to adjust salinity of samples. A brine control consisted of natural seawater diluted to the lowest salinity of a sample in a given test series and then reajusted to 30 ppt with the same brine used to adjust the samples.
[c]Results not used due to poor control response. Samples re-run with next test series.

Table A-2. Summary of the data for the sperm cell test from the June, 1991, event. Station numbers ending and A were from high tide, ending in B from the following low tide, and ending in C from the next high tide. Top, middle and bottom refer to water depths. Data are presented as actual counts, as well as percent of control. Test numbers refer to test series for raw data.

Test No.	Station	Location	Mean	SD	Signif.	%Control
1	Control		97.4	1.8		100.0
1	HR-11A	Top	43.8	27.6	*	44.9
1	HR-11B	Top	89.0	3.5	*	91.4
1	HR-11C	Top	87.0	2.6	*	89.3
1	HR-11A	Middle	67.3	21.7	*	69.1
1	HR-11B	Middle	76.3	9.6	*	78.3
1	HR-11C	Middle	57.8	23.0	*	59.3
1	HR-11A	Bottom	61.5	15.2	*	63.2
1	HR-11B	Bottom	88.8	4.1	*	91.1
1	HR-11C	Bottom	81.0	19.5		83.2
2	Control		96.8	1.7		100.0
2	NB-2A	Top	95.5	1.3		98.7
2	NB-2B	Top	94.8	2.2		97.9
2	NB-2C	Top	96.5	2.4		99.7
2	NB-2A	Middle	95.5	1.9		98.7
2	NB-2B	Middle	96.3	1.7		99.5
2	NB-2C	Middle	97.8	1.7		101.0
2	NB-2A	Bottom	97.3	1.3		100.5
2	NB-2B	Bottom	97.0	1.4		100.3
2	NB-2C	Bottom	96.0	1.4		99.2
3	Control		94.0	1.7		100.0
3	RB-6A	Top	60.3	28.1	*	64.1
3	RB-6B	Top	65.0	12.7	*	69.1
3	RB-6C	Top	76.5	5.2	*	81.4
3	RB-6A	Middle	76.7	6.7	*	81.6
3	RB-6B	Middle	73.3	7.0	*	77.9
3	RB-6C	Middle	69.3	9.8	*	73.7
3	RB-6A	Bottom	54.8	28.2	*	58.2
3	RB-6B	Bottom	79.3	5.5	*	84.3
3	RB-6C	Bottom	60.8	16.6	*	64.6
4	Control		92.0	1.7		100.0
4	HR-6A	Top	88.8	4.2		96.5
4	HR-6B	Top	86.8	3.1	*	94.3
4	HR-6C	Top	84.0	7.4		91.3
4	HR-6A	Middle	86.5	4.4	*	94.0
4	HR-6B	Middle	80.5	4.4	*	87.5
4	HR-6C	Middle	77.3	5.7	*	84.0
4	HR-6A	Bottom	78.0	2.9	*	84.8
4	HR-6B	Bottom	71.3	11.0	*	77.4
4	HR-6C	Bottom	82.8	1.7	*	89.9

*Statistically significant from performance control using a one-way, un-paired, t-test (alpha=0.05).

Table A-3. Summary of the data for the sperm cell test from both broad scale sampling events. Samples were collected by EPA in July, 1991, and January, 1992, respectively. Test number refers to test series number in for raw data in Appendix C.

	July 1991 Sampling Event				January 1992 Sampling Event				July	January
Sample ID	Test No.	Mean	SD	Signif.	Test No.	Mean	SD	Signif.	% Control	% Control
HR-1	5	81.5	9.8		8	95.3	1.3		90.3	99.6
HR-2	5	76.0	14.0		8	97.0	0.8		84.2	101.5
HR-3	5	68.8	6.1	*	8	95.8	2.1		76.1	100.2
HR-4	5	75.8	1.9	*	8	95.3	2.8		83.9	99.6
HR-5	5	79.8	2.5	*	8	95.3	3.8		88.3	99.6
HR-6	5	81.5	5.2	*	8	97.5	1.7		90.3	102.0
HR-7	5	74.0	7.5	*	8	94.3	3.0		81.9	98.6
HR-8	5	73.8	12.3	*	8	94.3	2.5		81.7	98.6
HR-9	5	62.5	6.6	*	8	90.3	2.4	*	69.2	94.4
HR-10	5	55.5	6.7	*	8	92.8	3.0		61.5	97.0
HR-11	5	57.5	17.5	*	8	96.8	3.3		63.7	101.2
ER-1	7	14.0	15.9	*	9	72.0	15.25	*	16.9	79.6
ER-2	7	18.5	15.8	*	9	85.8	10.72		22.3	94.9
ER-3	7	20.8	23.5	*	9	77.5	4.65	*	25.0	85.7
ER-4	7	10.5	11.1	*	9	64.3	8.42	*	12.7	71.1
ER-5	7	9.3	14.1	*	9	68.3	8.06	*	11.1	75.5
ER-6	7	25.0	9.3	*	9	60.0	7.26	*	30.1	66.4
ER-7	7	19.3	11.7	*	9	68.8	4.99	*	23.2	76.1
ER-8	7	17.0	12.0	*	9	60.8	11.03	*	20.5	67.2
ER-9	7	15.5	13.9	*	9	62.3	3.30	*	18.7	68.9
ER-10	7	17.0	13.8	*	9	60.3	4.35	*	20.5	66.6
ER-11	7	41.0	10.7	*	9	48.0	4.83	*	49.4	53.1
NB-1	7	43.0	15.8	*	11	63.0	8.5		51.8	76.5
NB-2	7	68.5	8.7	*	11	58.5	21.8		82.5	71.0
NB-3	7	66.7	15.3		11	64.7	23.2		80.4	78.5
NB-4	7	62.8	18.9		11	62.5	15.8		75.6	75.8
KVK-1	7	48.8	20.5	*	11	85.8	6.0		58.7	104.1
KVK-2	7	40.3	22.5	*	11	79.3	7.8		48.5	96.2
KVK-3	7	15.3	25.9	*	11	79.3	2.6		18.4	96.2
KVK-4	7	63.7	17.2		11	80.5	17.0		76.7	97.7
AK-1	7	50.8	24.7	*	11	77.3	3.1		61.1	93.8
AK-2	7	74.8	12.0		11	75.5	24.6		90.1	91.6
AK-3	7	66.0	19.5		11	21.3	8.5	*	79.5	25.8
AK-4	7	47.0	12.5	*	11	62.3	13.5	*	56.6	75.5
AK-5	7	60.3	25.3		11	69.8	17.5		72.6	84.6
AK-6	7	79.5	6.7		11	74.8	12.0		95.8	90.7
RB-1	7	21.0	12.6	*	11	91.8	2.9		25.3	111.3
RB-2	7	22.0	9.6	*	11	92.5	1.7		26.5	112.3
RB-3	7	33.3	23.2	*	11	89.5	6.2		40.1	108.6
RB-4	7	20.3	35.9	*	11	91.0	3.5		24.4	110.4
RB-5	7	33.8	12.6	*	11	91.8	1.5		40.7	111.3
RB-6	7	31.0	20.0	*	11	89.5	3.8		37.3	108.6
RB-7	7	18.8	6.0	*	11	84.0	5.9		22.6	101.9
RB-8	7	11.8	11.0	*	11	85.5	4.9		14.2	103.8
RB-9	7	20.5	8.3	*	11	85.5	2.6		24.7	103.8

Table A-3, Continued

JB-1	7	80.0	4.4		11	49.5	25.5	*	96.4	60.1
JB-2	7	85.0	3.5		11	29.0	22.5	*	102.4	35.2
JB-3	7	34.0	9.8	*	11	84.0	8.4		41.0	101.9
JB-4	7	52.3	16.8	*	11	86.0	4.1		63.0	104.4
JB-5	7	83.7	2.5		11	74.5	7.1		100.8	90.4
JB-6	7	76.5	14.8		11	82.8	5.3		92.2	100.4

*Statistically less than performance control using a one-way, un-paired, t-test (alpha=0.05).

Appendix B. Summary of toxicity data for *Champia*

Table B-1 list the test dates and control values for the sperm cell test with *Champia parvula* conducted with samples from the Hudson/Raritan Estuary. Table B-2 summarizes the means and standard deviations for samples from the small scale variability sampling event from June 1991. Table B-3 summarizes the data from the two broad scale sampling events in July 1991 and January 1992.

Table B-1. List of test parameters for the sexual reproduction test with *Champia parvula*. Controls were pooled for statistical analysis.

Test Series	Test Start Date	Control (no. of cystocarps per female) Natural Seawater	Brine + DI[b]	Collection Date	No. Plants per Replicate	Test Temp. (C)
Preliminary Run						
1	6/22/91	12.2	16.7	6/20/91	5	23
2	6/23/91	9.1	12.7	6/21/91	5	22
3	6/24/91	6.8	10.5	6/23/91	5	23
4	6/26/91	10.0	10.4	6/24/91	4	24
"Dry" Season Run						
5	7/22/91	13.7	14.0	7/20/91	5	21
6	7/23/91	16.1	14.3	7/21/91	5	23
7	7/24/91	12.1	9.9	7/21 & 24/91	5	22
"Wet" Season Run						
8	1/9/92	9.6	8.8	1/8/92	5	20
9	1/10/92	6.3	7.1	1/9/92	5	22
10	1/11/92	8.7	8.1	1/10/92	5	22
11	1/13/92	16.4	15.5	1/11/92	5	22

[a]Refers to test series listed in Appendix D.
[b]Brine made from Narragansett Bay natural seawater was used when nexessary to adjust salinity of samples. A brine control consisted of natural seawater diluted to the lowest salinity of a sample in a given test series and then readjusted to 30ppt with the same brine used to adjust the samples.
[c]There were four relicates per treatment.

Table B-2. Summary of the data for the algal sexual reproduction test from the June, 1991, event. Station numbers ending and A were from high tide, ending in B from the following low tide, and ending in C from the next high tide. Top, middle and bottom refer to water depths. Data are presented as actual counts, as well as percent of control. Test numbers refer to test series for raw data.

Test No.	Station	Location	Mean	SD	Signif.	% Control
1	Control		14.4	3.5		100.0
1	HR-11A	Top	14.5	2.8		100.7
1	HR-11B	Top	12.5	3.1		87.0
1	HR-11C	Top	11.7	2.3		80.9
1	HR-11A	Middle	12.7	3.8		88.2
1	HR-11B	Middle	12.4	3.0		86.1
1	HR-11C	Middle	11.3	3.7		78.5
1	HR-11A	Bottom	12.0	3.0		83.0
1	HR-11B	Bottom	11.7	2.8		80.9
1	HR-11C	Bottom	8.2	4.4	*	56.9
2	Control		10.9	3.8		100.0
2	NB-2A	Top	7.5	1.0	*	68.3
2	NB-2B	Top	8.5	1.8	*	78.0
2	NB-2C	Top	8.8	3.1		80.3
2	NB-2A	Middle	9.1	1.7	*	83.0
2	NB-2B	Middle	9.4	1.7	*	86.2
2	NB-2C	Middle	11.0	1.1		100.5
2	NB-2A	Bottom	11.2	3.1		102.3
2	NB-2B	Bottom	9.6	1.2		88.3
2	NB-2C	Bottom	9.7	1.6		89.0
3	Control		8.7	2.2		100.0
3	RB-6A	Top	4.2	1.3	*	48.3
3	RB-6B	Top	7.3	2.7	*	83.3
3	RB-6C	Top	5.3	1.0	*	61.2
3	RB-6A	Middle	5.2	0.2	*	59.2
3	RB-6B	Middle	6.2	1.1	*	71.6
3	RB-6C	Middle	5.1	0.9	*	58.0
3	RB-6A	Bottom	6.6	1.4	*	75.9
3	RB-6B	Bottom	6.7	0.7	*	76.4
3	RB-6C	Bottom	5.5	0 6	*	62.6
4	Control		10.2	2.0		100.0
4	HR-6A	Top	9.3	2.1		90.7
4	HR-6B	Top	9.3	1.1		90.7
4	HR-6C	Top	5.5	1.1	*	53.5
4	HR-6A	Middle	8.7	2.5		84.6
4	HR-6B	Middle	7.6	2.2		74.3
4	HR-6C	Middle	5.9	1.9	*	57.7
4	HR-6A	Bottom	8.4	2.6		81.6
4	HR-6B	Bottom	6.5	3.1	*	63.1
4	HR-6C	Bottom	4.7	2.4	*	45.9

*Statistically significant from performance control using a one-way, un-paired, t-test (alpha=0.05).

Table B-3. Summary of the data for the sexual reproduction test from both broad scale sampling events. Samples were collected by EPA in July, 1991, and January, 1992. Test number refers to test series number for raw data in Appendix D.

	July 1991 Sampling Event				January 1992 Sampling Event				July	January
Station	Test No.	Mean	SD	Signif.	Test No.	Mean	SD	Signif.	% Control	% Control
HR-1	5	6.6	2.5	*	8	8.3	1.3		47.1	90.2
HR-2	5	8.1	3.6	*	8	9.9	1.8		57.9	107.3
HR-3	5	11.1	2.1	*	8	10.0	1.6		79.9	108.4
HR-4	5	12.1	2.3		8	9.4	2.3		86.7	102.2
HR-5	5	11.6	3.6		8	9.0	0.7		83.1	97.6
HR-6	5	12.3	1.7		8	11.8	0.7		88.1	127.7
HR-7	5	13.6	1.8		8	10.0	1.7		97.5	108.4
HR-8	5	15.4	1.7		8	9.4	1.7		111.0	101.6
HR-9	5	12.0	4.0		8	8.1	0.8		86.0	88.0
HR-10	5	12.5	2.4		8	9.5	0.2		89.9	103.5
HR-11	5	12.2	1.3		8	9.6	0.8		87.9	104.3
ER-1	6	13.0	3.4		9	4.8	0.7	*	85.7	70.9
ER-2	6	12.6	2.0		9	6.0	1.2		82.9	88.8
ER-3	6	13.1	3.3		9	4.4	0.3	*	85.9	64.9
ER-4	6	12.9	4.6		9	3.1	0.9	*	84.9	46.3
ER-5	6	16.3	5.6		9	4.2	0.8	*	106.9	61.9
ER-6	6	17.2	2.1		9	4.3	2.5		113.2	64.2
ER-7	6	17.0	2.9		9	3.6	0.8	*	111.5	53.7
ER-8	6	15.0	3.4		9	3.8	1.1	*	98.4	56.0
ER-9	6	15.3	4.6		9	1.8	1.5	*	100.7	26.9
ER-10	6	15.1	5.5		9	2.7	1.1	*	99.0	40.3
ER-11	6	11.2	2.6	*	9	3.4	1.1	*	73.7	51.1
NB-1	7	6.8	2.8	*	10	1.6	1.1	*	61.6	18.2
NB-2	7	12.9	6.1		10	1.8	0.8	*	117.0	21.2
NB-3	7	7.5	3.3		10	1.7	0.3	*	67.7	20.0
NB-4	7	8.5	1.0	*	10	2.2	1.3	*	77.3	25.3
KVK-1	7	6.9	3.2	*	10	3.2	2.0	*	62.3	37.6
KVK-2	7	8.5	1.1	*	10	4.1	1.3	*	76.8	47.9
KVK-3	7	11.4	3.7		10	3.8	0.9	*	103.4	44.7
KVK-4	7	10.0	3.6		10	3.0	0.7	*	90.9	35.0
AK-1	7	9.5	3.5		10	4.8	1.5	*	86.7	56.8
AK-2	7	5.6	1.5	*	10	3.4	1.4	*	50.5	39.4
AK-3	7	8.7	3.0		10	1.3	1.1	*	78.8	15.3
AK-4	7	8.8	5.2		10	0.7	0.3	*	80.0	7.6
AK-5	7	10.0	2.2		10	2.4	1.4	*	90.9	28.2
AK-6	7	10.3	0.9		10	1.2	0.4	*	93.6	14.4
RB-1	7	7.6	3.8		11	15.0	3.5		69.1	93.9
RB-2	7	6.8	1.9	*	11	14.2	1.8		61.8	88.8
RB-3	7	6.6	2.9	*	11	15.8	2.0		60.0	98.4
RB-4	7	10.6	4.0		11	14.6	3.1		95.9	91.1
RB-5	7	9.0	5.2		11	12.8	2.3	*	81.8	79.8
RB-6	7	6.5	0.9	*	11	12.3	3.7		59.1	77.0
RB-7	7	6.6	1.6	*	11	16.5	2.5		60.0	102.8
RB-8	7	3.3	2.6	*	11	12.9	0.5	*	29.5	80.8
RB-9	7	6.7	3.0	*	11	13.5	2.7		60.9	84.5

Table B-3, Continued

JB-1	7	5.6	2.5	*	10	0.0	0.0	*	50.9	0.0
JB-2	7	8.7	3.5		10	0.1	0.2	*	79.1	1.2
JB-3	6	5.7	4.1	*	10	3.2	1.1	*	37.5	37.6
JB-4	7	5.0	1.4	*	10	3.5	1.7	*	45.5	41.2
JB-5	6	6.6	0.9	*	10	3.7	1.6	*	43.1	43.2
JB-6	6	6.8	1.6	*	10	3.9	0.4	*	44.7	45.3

*Statistically less than performance control using a one-way, un-paired, t-test (alpha=0.05).

Appendix C. Raw toxicity data for *Arbacia*

Data is listed for each replicate for the sperm cell tests conducted with the sea urchin, *Arbacia punctulata,* for all of the samples from all three Hudson/Raritan Estuary sampling events. Test series number refers to the test series in Table A-1. Experiment number refers to SAIC's Environmental Testing Center experiment number through which the original data can be traced. For test series number one there was no count for fertilization, only "not cleaved" and "cleaved". There also is no data for test series numbers 6 and 10. The samples for these two test series were rerun in series numbers 7 and 11, respectively.

June, 1991: Test Series 1
Experiment No. 910612

Sample ID	Not Cleaved	Cleaved
NSW Control	4	96
NSW Control	3	97
NSW Control	6	94
NSW Control	1	99
Brine Control	1	99
Brine Control	2	98
Brine Control	1	99
Brine Control	3	97
HR-11A,Top	21	79
HR-11A,Top	62	38
HR-11A,Top	88	12
HR-11A,Top	54	46
HR-11A,Middle	62	38
HR-11A,Middle	20	80
HR-11A,Middle	13	87
HR-11A,Middle	36	64
HR-11A,Bottom	61	39
HR-11A, Bottom	33	67
HR-11A,Bottom	28	72
HR-11A,Bottom	32	68
HR-11B,Top	10	90
HR-11B,Top	16	84
HR-11B,Top	8	92
HR-11B,Top	10	90
HR-11B,Middle	12	88
HR-11B,Middle	20	80
HR-11B,Middle	30	70
HR-11B,Middle	33	67
HR-11B,Bottom	10	90
HR-11B,Bottom	14	86
HR-11B,Bottom	15	85
HR-11B,Bottom	6	94
HR-11C,Top	14	86
HR-11C,Top	10	90
HR-11C,Top	12	88
HR-11C,Top	16	84
HR-11C,Middle	52	48
HR-11C,Middle	26	74
HR-11C,Middle	70	30
HR-11C,Middle	21	79
HR-11C,Bottom	12	88
HR-11C,Bottom	6	94
HR-11C,Bottom	48	52
HR-11C,Bottom	10	90

June, 1991: Test Series 2
Experiment No. 910614

Sample ID	Not cleaved	Not fertilized	Cleaved
NSW Control	1	0	99
NSW Control	2	0	98
NSW Control	4	1	95
NSW Control	3	0	97
Brine Control	4	0	96
Brine Control	6	0	94
Brine Control	3	0	97
Brine Control	2	0	98
NB-2A,Top	3	2	95
NB-2A,Top	2	1	97
NB-2A,Top	1	3	96
NB-2A,Top	4	2	94
NB-2A,Middle	3	1	96
NB-2A,Middle	2	0	98
NB-2A,Middle	4	2	94
NB-2A,Middle	6	0	94
NB-2A,Bottom	4	0	96
NB-2A,Bottom	3	0	97
NB-2A,Bottom	2	1	97
NB-2A,Bottom	1	0	99
NB-2B,Top	7	1	92
NB-2B,Top	6	0	94
NB-2B,Top	4	0	96
NB-2B,Top	3	0	97
NB-2B,Middle	2	0	98
NB-2B,Middle	1	3	96
NB-2B,Middle	1	2	97
NB-2B,Middle	2	4	94
NB-2B,Bottom	3	0	97
NB-2B,Bottom	2	0	98
NB-2B,Bottom	4	1	95
NB-2B,Bottom	0	2	98
NB-2C,Top	2	0	98
NB-2C,Top	2	0	98
NB-2C,Top	4	3	93
NB-2C,Top	3	0	97
NB-2C,Middle	4	0	96
NB-2C,Middle	2	0	98
NB-2C,Middle	0	0	100
NB-2C,Middle	1	2	97
NB-2C,Bottom	0	3	97
NB-2C,Bottom	1	2	97
NB-2C,Bottom	0	4	96
NB-2C,Bottom	1	5	94

June, 1991: Test Series 3
Experiment No. 910616

Sample ID	Not cleaved	Not fertilized	Cleaved
NSW Control	3	3	94
NSW Control	2	6	92
NSW Control	2	3	95
NSW Control	5	2	93
Brine Control	6	2	92
Brine Control	5	1	94
Brine Control	4	1	95
Brine Control	2	1	97
RB-6A,Top	1	52	47
RB-6A,Top	3	11	86
RB-6A,Top	4	69	27
RB-6A,Top	8	11	81
RB-6A,Middle	7	70	23
RB-6A,Middle	4	25	71
RB-6A,Middle	2	23	75
RB-6A,Middle	2	14	84
RB-6A,Bottom	0	64	36
RB-6A,Bottom	2	72	26
RB-6A,Bottom	3	25	72
RB-6A,Bottom	8	7	85
RB-6B,Top	8	38	54
RB-6B,Top	4	41	55
RB-6B,Top	9	20	71
RB-6B,Top	7	13	80
RB-6B,Middle	6	24	70
RB-6B,Middle	6	29	65
RB-6B,Middle	6	14	80
RB-6B,Middle	3	19	78
RB-6B,Bottom	4	22	74
RB-6B,Bottom	5	17	78
RB-6B,Bottom	7	15	78
RB-6B,Bottom	8	5	87
RB-6C,Top	5	25	70
RB-6C,Top	11	10	79
RB-6C,Top	5	13	82
RB-6C,Top	6	19	75
RB-6C,Middle	5	35	60
RB-6C,Middle	6	32	62
RB-6C,Middle	7	18	75
RB-6C,Middle	8	12	80
RB-6C,Bottom	9	21	70
RB-6C,Bottom	8	13	79
RB-6C,Bottom	13	44	43
RB-6C,Bottom	5	44	51

June, 1991: Test Series 4
Experiment No. 910619

Sample ID	Not cleaved	Not fertilized	Cleaved
NSW Control	8	2	90
NSW Control	7	1	92
NSW Control	2	5	93
NSW Control	2	6	92
Brine Control	2	5	93
Brine Control	4	1	95
Brine Control	4	6	90
Brine Control	5	4	91
HR-6A,Top	2	5	93
HR-6A,Top	4	13	83
HR-6A,Top	5	5	90
HR-6A,Top	1	10	89
HR-6A,Middle	5	12	83
HR-6A,Middle	1	11	88
HR-6A,Middle	6	11	83
HR-6A,Middle	1	7	92
HR-6A,Bottom	6	15	79
HR-6A,Bottom	6	13	81
HR-6A,Bottom	7	15	78
HR-6A,Bottom	8	18	74
HR-6B,Top	7	8	85
HR-6B,Top	8	8	84
HR-6B,Top	2	7	91
HR-6B,Top	5	8	87
HR-6B,Middle	1	14	85
HR-6B,Middle	3	14	83
HR-6B,Middle	5	16	79
HR-6B,Middle	4	21	75
HR-6B,Bottom	5	14	81
HR-6B,Bottom	8	12	80
HR-6B,Bottom	9	26	65
HR-6B,Bottom	10	31	59
HR-6C,Top	4	22	74
HR-6C,Top	5	12	83
HR-6C,Top	6	3	91
HR-6C,Top	4	8	88
HR-6C,Middle	3	28	69
HR-6C,Middle	8	14	78
HR-6C,Middle	11	9	80
HR-6C,Middle	9	9	82
HR-6C,Bottom	4	13	83
HR-6C,Bottom	7	11	82
HR-6C,Bottom	5	10	85
HR-6C,Bottom	9	10	81

July, 1991: Test Series 5
Experiment No. 910718

Sample ID	Not fertilized	Not cleaved	Cleaved
NSW Control	1	10	89
NSW Control	5	9	86
NSW Control	3	9	88
NSW Control	2	8	90
Brine Control	3	7	90
Brine Control	1	6	93
Brine Control	1	5	94
Brine Control	0	8	92
HR-1	1	5	94
HR-1	0	30	70
HR-1	0	20	80
HR-1	4	14	82
HR-2	4	13	83
HR-2	6	12	82
HR-2	4	12	84
HR-2	7	38	55
HR-3	7	17	76
HR-3	3	26	71
HR-3	4	30	66
HR-3	7	31	62
HR-4	2	25	73
HR-4	2	22	76
HR-4	2	21	77
HR-4	3	20	77
HR-5	2	15	83
HR-5	6	17	77
HR-5	5	15	80
HR-5	10	11	79
HR-6	6	17	77
HR-6	4	16	80
HR-6	7	13	80
HR-6	7	4	89
HR-7	9	23	68
HR-7	9	24	67
HR-7	1	19	80
HR-7	6	13	81
HR-8	15	29	56
HR-8	3	21	76
HR-8	5	16	79
HR-8	6	10	84
HR-9	5	25	70
HR-9	11	35	54
HR-9	6	30	64
HR-9	5	33	62

July, 1991: Test Series 5 Continued

HR-10	9	35	56
HR-10	5	34	61
HR-10	13	28	59
HR-10	6	48	46
HR-11	9	22	69
HR-11	21	47	32
HR-11	9	31	60
HR-11	7	24	69

July, 1991: Test Series 7
Experiment No. 910723

Sample ID	Not fertilized	Not cleaved	Cleaved	
NSW Control	5	5	90	
NSW Control	5	5	90	
NSW Control	4	10	86	
NSW Control	7	4	89	
Brine Control	34	1	65	
Brine Control	6	7	87	
Brine Control	16	7	77	
Brine Control	12	8	80	
NB-1	28	10	62	
NB-1	71	3	26	
NB-1	54	11	35	
NB-1	44	7	49	
NB-2	26	14	60	
NB-2	5	16	79	
NB-2	22	15	63	
NB-2	15	13	72	
NB-3	90	2	8	outlier
NB-3	12	8	80	
NB-3	40	10	50	
NB-3	16	14	70	
NB-4	12	24	64	
NB-4	6	7	87	
NB-4	32	9	59	
NB-4	40	19	41	
JB-1	7	10	83	
JB-1	93	3	4	outlier
JB-1	10	15	75	
JB-1	8	10	82	
JB-2	8	10	82	
JB-2	7	9	84	
JB-2	8	8	84	
JB-2	4	6	90	
JB-3	48	14	38	
JB-3	42	14	44	
JB-3	54	13	33	
JB-3	70	9	21	
JB-4	59	11	30	
JB-4	26	16	58	
JB-4	12	18	70	
JB-4	39	10	51	
JB-5	4	12	84	
JB-5	9	5	86	
JB-5	8	11	81	
JB-5	47	15	38	

July, 1991: Test Series 7 Continued

JB-6	27	13	60
JB-6	5	7	88
JB-6	21	11	68
JB-6	2	8	90
AK-1	7	13	80
AK-1	41	11	48
AK-1	66	14	20
AK-1	24	21	55
AK-2	37	5	58
AK-2	3	11	86
AK-2	10	10	80
AK-2	14	11	75
AK-3	41	19	40
AK-3	22	14	64
AK-3	14	12	74
AK-3	7	7	86
AK-4	50	9	41
AK-4	30	20	50
AK-4	16	21	63
AK-4	57	9	34
AK-5	12	12	76
AK-5	39	13	48
AK-5	53	16	31
AK-5	5	9	86
AK-6	4	11	85
AK-6	3	11	86
AK-6	7	13	80
AK-6	22	11	67
KVK-1	36	8	56
KVK-1	12	14	74
KVK-1	58	14	28
KVK-1	58	5	37
KVK-2	47	13	40
KVK-2	16	17	67
KVK-2	36	52	12
KVK-2	36	22	42
KVK-3	25	21	54
KVK-3	96	1	3
KVK-3	99	1	0
KVK-3	96	0	4
KVK-4	18	15	67
KVK-4	12	9	79
KVK-4	46	9	45
KVK-4	82	2	16

July, 1991: Test Series 7 Continued

RB-1	84	4	12	
RB-1	47	16	37	
RB-1	74	1	25	
RB-1	90	0	10	
RB-2	65	13	22	
RB-2	86	5	9	
RB-2	67	8	25	
RB-2	62	6	32	
RB-3	34	12	54	
RB-3	54	7	39	
RB-3	100	0	0	outlier
RB-3	54	6	40	
RB-4	93	3	4	
RB-4	95	2	3	
RB-4	100	0	0	
RB-4	19	7	74	outlier
RB-5	54	7	39	
RB-5	33	25	42	
RB-5	44	17	39	
RB-5	62	23	15	
RB-6	70	7	23	
RB-6	84	9	7	
RB-6	38	20	42	
RB-6	22	26	52	
RB-7	52	25	23	
RB-7	83	6	11	
RB-7	71	12	17	
RB-7	55	21	24	
RB-8	56	17	27	
RB-8	86	6	8	
RB-8	94	5	1	
RB-8	88	1	11	
RB-9	75	12	13	
RB-9	48	31	21	
RB-9	61	7	32	
RB-9	80	4	16	
ER-1	75	1	24	
ER-1	63	6	31	
ER-1	99	1	0	
ER-1	99	0	1	
ER-2	83	3	14	
ER-2	54	7	39	
ER-2	98	1	1	
ER-2	76	4	20	

July, 1991: Test Series 7 Continued

ER-3	72	8	20
ER-3	37	9	54
ER-3	86	6	8
ER-3	97	2	1
ER-4	77	5	18
ER-4	98	2	0
ER-4	95	3	2
ER-4	71	7	22
ER-5	98	2	0
ER-5	99	0	1
ER-5	58	36	6
ER-5	64	6	30
ER-6	83	3	14
ER-6	69	9	22
ER-6	65	7	28
ER-6	56	8	36
ER-7	82	6	12
ER-7	59	15	26
ER-7	93	0	7
ER-7	56	12	32
ER-8	76	6	18
ER-8	99	0	1
ER-8	64	6	30
ER-8	66	15	19
ER-9	100	0	0
ER-9	70	6	24
ER-9	60	10	30
ER-9	90	2	8
ER-10	100	0	0
ER-10	75	4	21
ER-10	79	7	14
ER-10	56	11	33
ER-11	47	12	41
ER-11	62	12	26
ER-11	39	14	47
ER-11	35	15	50

January, 1992: Test Series 8
Experiment No. 920102

Sample ID	Not Fertilized	Not Cleaved	Cleaved
NSW Control	3	6	91
NSW Control	1	3	96
NSW Control	2	3	95
NSW Control	2	3	95
Brine Control	0	1	99
Brine Control	0	3	97
Brine Control	3	0	97
Brine Control	3	2	95
HR-1	1	2	97
HR-1	2	4	94
HR-1	2	3	95
HR-1	4	1	95
HR-2	2	1	97
HR-2	2	1	97
HR-2	4	0	96
HR-2	0	2	98
HR-3	0	3	97
HR-3	1	5	94
HR-3	1	5	94
HR-3	2	0	98
HR-4	3	3	94
HR-4	1	0	99
HR-4	2	3	95
HR-4	3	4	93
HR-5	0	1	99
HR-5	5	5	90
HR-5	0	4	96
HR-5	1	3	96
HR-6	1	0	99
HR-6	0	1	99
HR-6	3	1	96
HR-6	2	2	96
HR-7	2	7	91
HR-7	1	1	98
HR-7	1	4	95
HR-7	1	6	93
HR-8	3	3	94
HR-8	5	4	91
HR-8	2	1	97
HR-8	2	3	95
HR-9	7	6	87
HR-9	4	4	92
HR-9	5	5	90
HR-9	3	5	92

January, 1992: Test Series 8 Continued

HR-10	4	4	92
HR-10	0	6	94
HR-10	3	8	89
HR-10	3	1	96
HR-11	0	0	100
HR-11	4	1	95
HR-11	0	1	99
HR-11	4	3	93

January, 1992: Test Series 9
Experiment No. 920104

Sample ID	Not Fertilized	Not Cleaved	Cleaved
NSW Control	2	2	96
NSW Control	3	0	97
NSW Control	0	3	97
NSW Control	0	17	83
Brine Control	2	11	87
Brine Control	3	12	85
Brine Control	2	8	90
Brine Control	5	7	88
ER-1	2	11	87
ER-1	26	20	54
ER-1	16	19	65
ER-1	5	13	82
ER-2	7	3	90
ER-2	4	2	94
ER-2	5	6	89
ER-2	16	14	70
ER-3	13	11	76
ER-3	7	20	73
ER-3	11	12	77
ER-3	6	10	84
ER-4	24	24	52
ER-4	15	14	71
ER-4	17	15	68
ER-4	14	20	66
ER-5	21	22	57
ER-5	14	15	71
ER-5	10	14	76
ER-5	17	14	69
ER-6	22	16	62
ER-6	25	23	52
ER-6	28	15	57
ER-6	16	15	69
ER-7	22	12	66
ER-7	22	10	68
ER-7	18	6	76
ER-7	23	12	65
ER-8	20	25	55
ER-8	17	6	77
ER-8	25	17	58
ER-8	22	25	53
ER-9	20	16	64
ER-9	29	11	60
ER-9	23	18	59
ER-9	21	13	66

January, 1992: Test Series 9 Continued

ER-10	24	15	61
ER-10	21	13	66
ER-10	33	11	56
ER-10	28	14	58
ER-11	36	20	44
ER-11	30	15	55
ER-11	38	16	46
ER-11	26	27	47

January, 1992: Test Series 11
Experiment No. 920108

Sample ID	Not Fertilized	Not Cleaved	Cleaved
NSW Control	0	12	88
NSW Control	2	11	87
NSW Control	0	10	90
NSW Control	0	2	98
Brine Control	2	4	94
Brine Control	1	2	97
Brine Control	63	6	31
Brine Control	only three replicates		
RB-1	2	4	94
RB-1	1	8	91
RB-1	4	8	88
RB-1	2	4	94
RB-2	0	5	95
RB-2	0	9	91
RB-2	4	4	92
RB-2	2	6	92
RB-3	0	5	95
RB-3	0	11	89
RB-3	1	6	93
RB-3	16	3	81
RB-4	6	8	86
RB-4	2	6	92
RB-4	0	6	94
RB-4	0	8	92
RB-5	1	9	90
RB-5	1	6	93
RB-5	1	6	93
RB-5	3	6	91
RB-6	1	4	95
RB-6	2	9	89
RB-6	0	13	87
RB-6	6	7	87
RB-7	3	11	86
RB-7	3	6	91
RB-7	8	15	77
RB-7	5	13	82
RB-8	5	12	83
RB-8	4	16	80
RB-8	1	8	91
RB-8	3	9	88
RB-9	0	14	86
RB-9	6	10	84
RB-9	4	7	89
RB-9	4	13	83

January, 1992: Test Series 11 Continued

JB-1	58	29	13	
JB-1	5	24	71	
JB-1	29	19	52	
JB-1	25	13	62	
JB-2	67	23	10	
JB-2	79	12	9	
JB-2	21	30	49	
JB-2	15	37	48	
JB-3	10	9	81	
JB-3	1	14	85	
JB-3	14	11	75	
JB-3	2	3	95	
JB-4	6	10	84	
JB-4	5	12	83	
JB-4	4	11	85	
JB-4	2	6	92	
JB-5	14	14	72	
JB-5	7	21	72	
JB-5	8	23	69	
JB-5	2	13	85	
JB-6	2	9	89	
JB-6	4	20	76	
JB-6	0	17	83	
JB-6	4	13	83	
NB-1	only three replicates			
NB-1	38	5	57	
NB-1	21	10	69	
NB-1	81	11	8	outlier
NB-2	4	15	81	
NB-2	50	16	34	
NB-2	46	7	47	
NB-2	16	12	72	
NB-3	11	13	76	
NB-3	56	6	38	
NB-3	6	14	80	
NB-3	81	10	9	outlier
NB-4	4	13	83	
NB-4	36	14	50	
NB-4	8	25	67	
NB-4	43	7	50	
KVK-1	3	8	89	
KVK-1	17	6	77	
KVK-1	4	6	90	
KVK-1	11	2	87	

January, 1992: Test Series 11 Continued

KVK-2	14	6	80
KVK-2	24	6	70
KVK-2	3	8	89
KVK-2	18	4	78
KVK-3	16	6	78
KVK-3	10	7	83
KVK-3	15	8	77
KVK-3	9	12	79
KVK-4	7	2	91
KVK-4	14	4	82
KVK-4	36	8	56
KVK-4	3	4	93
AK-1	14	9	77
AK-1	11	16	73
AK-1	11	10	79
AK-1	11	9	80
AK-2	5	9	86
AK-2	3	4	93
AK-2	7	9	84
AK-2	56	5	39
AK-3	66	6	28
AK-3	70	8	22
AK-3	86	5	9
AK-3	68	6	26
AK-4	8	12	80
AK-4	24	12	64
AK-4	41	11	48
AK-4	26	17	57
AK-5	12	12	76
AK-5	1	16	83
AK-5	17	7	76
AK-5	52	4	44
AK-6	24	9	67
AK-6	4	11	85
AK-6	7	8	85
AK-6	29	9	62

Appendix D. Raw toxicity data for *Champia*

Data is listed for each replicate for the sexual reproduction test using the marine red alga, *Champia parvula,* for all of the samples from all three Hudson/Raritan Estuary sampling events. Test series number refers to the test series in Table B-1. Experiment number refers to SAIC's Environmental Testing Center experiment number through which the original data can be traced.

June, 1991:Test Series 1
Experiment No. 910613

Station	No. Plants per Replicate	Mean Cystocarps per Replicate
NSW Control	5	7.0
NSW Control	5	15.6
NSW Control	5	13.6
NSW Control	5	12.4
Brine Control	5	15.8
Brine Control	5	17.8
Brine Control	5	18.0
Brine Control	5	15.0
HR-11A,Top	5	13.0
HR-11A,Top	5	11.4
HR-11A,Top	5	16.0
HR-11A,Top	5	17.6
HR-11A,Middle	5	8.2
HR-11A,Middle	5	12.8
HR-11A,Middle	5	12.4
HR-11A,Middle	5	17.4
HR-11A,Bottom	5	14.0
HR-11A,Bottom	4	11.0
HR-11A,Bottom	5	14.6
HR-11A,Bottom	5	8.2
HR-11B,Top	4	9.5
HR-11B,Top	5	12.4
HR-11B,Top	5	16.8
HR-11B,Top	5	11.4
HR-11B,Middle	5	13.4
HR-11B,Middle	5	13.6
HR-11B,Middle	5	14.6
HR-11B,Middle	5	8.0
HR-11B,Bottom	5	9.6
HR-11B,Bottom	5	9.2
HR-11B,Bottom	5	15.0
HR-11B,Bottom	5	12.8
HR-11C,Top	5	12.6
HR-11C,Top	5	8.4
HR-11C,Top	5	12.0
HR-11C,Top	5	13.6
HR-11C,Middle	5	12.2
HR-11C,Middle	5	10.4
HR-1C,Middle	5	6.8
HR-11C,Middle	5	15.8
HR-11C,Bottom	5	8.8
HR-11C,Bottom	5	3.2
HR-11C,Bottom	5	7.0
HR-11C,Bottom	5	13.8

June, 1991: Test Series 2
Experiment No. 910615

Station	No. Plants per Replicate	Mean Cystocarps per Replicate
NSW Control	5	12.4
NSW Control	5	9.4
NSW Control	5	5.6
NSW Control	5	5.0
Brine Control	5	12.4
Brine Control	5	11.6
Brine Control	5	16.6
Brine Control	5	10.2
NB-2A,Top	5	8.0
NB-2A,Top	5	7.2
NB-2A,Top	5	8.4
NB-2A,Top	5	6.2
NB-2A,Middle	5	9.0
NB-2A,Middle	5	8.0
NB-2A,Middle	4	7.8
NB-2A,Middle	5	11.4
NB-2A,Bottom	5	8.0
NB-2A,Bottom	5	15.4
NB-2A,Bottom	4	11.0
NB-2A,Bottom	5	10.2
NB-2B,Top	5	6.8
NB-2B,Top	5	7.6
NB-2B,Top	5	8.6
NB-2B,Top	5	11.0
NB-2B,Middle	5	9.6
NB-2B,Middle	5	11.6
NB-2B,Middle	5	8.8
NB-2B,Middle	5	7.6
NB-2B,Bottom	4	10.3
NB-2B,Bottom	5	8.4
NB-2B,Bottom	5	8.8
NB-2B,Bottom	5	11.0
NB-2C,Top	5	9.0
NB-2C,Top	5	11.4
NB-2C,Top	5	10.2
NB-2C,Top	5	4.4
NB-2C,Middle	5	12.4
NB-2C,Middle	5	10.4
NB-2C,Middle	5	11.2
NB-2C,Middle	5	9.8
NB-2C,Bottom	5	11.2
NB-2C,Bottom	4	11.0
NB-2C,Bottom	5	8.0
NB-2C,Bottom	5	8.6

June, 1991: Test Series 3
Experiment No. 910615

Station	No. Plants per Replicate	Mean Cystocarps per Replicate
NSW Control	5	6.4
NSW Control	5	6.8
NSW Control	5	5.6
NSW Control	5	8.2
Brine Control	5	9.4
Brine Control	5	10.8
Brine Control	5	11
Brine Control	5	10.6
RB-6A,Top	4	2.8
RB-6A,Top	5	4.4
RB-6A,Top	5	5.4
RB-6A,Top	5	no male added
RB-6A,Middle	5	5.2
RB-6A,Middle	4	5.0
RB-6A,Middle	5	5.4
RB-6A,Middle	5	5.0
RB-6A,Bottom	5	7.8
RB-6A,Bottom	4	5.5
RB-6A,Bottom	5	7.8
RB-6A,Bottom	4	5.3
RB-6B,Top	5	5.0
RB-6B,Top	5	8.8
RB-6B,Top	5	10.2
RB-6B,Top	5	5.0
RB-6B,Middle	4	7.5
RB-6B,Middle	5	5.6
RB-6B,Middle	5	replicate lost
RB-6B,Middle	5	5.6
RB-6B,Bottom	5	6.6
RB-6B,Bottom	5	6.4
RB-6B,Bottom	5	7.6
RB-6B,Bottom	4	6.0
RB-6C,Top	4	6.0
RB-6C,Top	5	4.4
RB-6C,Top	5	4.6
RB-6C,Top	4	6.3
RB-6C,Middle	4	4.0
RB-6C,Middle	5	6.0
RB-6C,Middle	5	4.8
RB-6C,Middle	5	5.4
RB-6C,Bottom	5	4.6
RB-6C,Bottom	4	5.8
RB-6C,Bottom	5	5.6
RB-6C,Bottom	5	5.8

June, 1991: Test Series 4
Experiment No. 910620

Station	No. Plants per Replicate	Mean Cystocarps per Replicate
NSW Control	4	6.3
NSW Control	4	11.8
NSW Control	4	11.8
NSW Control	4	plants fragmented and not counted
Brine Control	4	10.3
Brine Control	4	11.8
Brine Control	4	10.3
Brine Control	4	9.3
HR-6A,Top	4	10.3
HR-6A,Top	4	8.5
HR-6A,Top	4	11.5
HR-6A,Top	4	6.8
HR-6A,Middle	4	11.3
HR-6A,Middle	4	10.0
HR-6A,Middle	4	7.8
HR-6A,Middle	4	5.5
HR-6A,Bottom	4	5.3
HR-6A,Bottom	4	9.8
HR-6A,Bottom	4	7.3
HR-6A,Bottom	4	11.0
HR-6B,Top	4	9.0
HR-6B,Top	4	10.8
HR-6B,Top	4	8.3
HR-6B,Top	4	9.0
HR-6B,Middle	4	8.5
HR-6B,Middle	4	5.3
HR-6B,Middle	4	6.3
HR-6B,Middle	4	10.3
HR-6B,Bottom	4	6.3
HR-6B,Bottom	4	10.5
HR-6B,Bottom	4	3.0
HR-6B,Bottom	4	6.0
HR-6C,Top	4	4.5
HR-6C,Top	4	4.5
HR-6C,Top	5	6.4
HR-6C,Top	4	6.5
HR-6C,Middle	4	7.5
HR-6C,Middle	4	3.8
HR-6C,Middle	4	4.8
HR-6C,Middle	4	7.5
HR-6C,Bottom	4	3.5
HR-6C,Bottom	4	2.0
HR-6C,Bottom	4	7.3
HR-6C,Bottom	4	6.0

July, 1991: Test Series 5
Experiment No. 910719

Station	No. Plants per Replicate	Mean Cystocarps per Replicate
NSW Control	5	no male added
NSW Control	5	10.2
NSW Control	5	17.8
NSW Control	5	13.2
Brine Control	5	14.4
Brine Control	5	15.0
Brine Control	5	16.0
Brine Control	5	10.6
HR-1	5	4.4
HR-1	5	7.8
HR-1	5	4.6
HR-1	5	9.4
HR-2	5	3.4
HR-2	5	10.0
HR-2	5	11.6
HR-2	5	7.2
HR-3	5	8.2
HR-3	5	10.8
HR-3	5	12.6
HR-3	5	12.8
HR-4	5	11.0
HR-4	5	12.0
HR-4	5	10.0
HR-4	5	15.2
HR-5	5	11.4
HR-5	5	15.8
HR-5	5	12.0
HR-5	5	7.0
HR-6	5	10.2
HR-6	5	11.4
HR-6	5	13.6
HR-6	5	13.8
HR-7	5	13.0
HR-7	5	15.6
HR-7	5	11.4
HR-7	5	14.2
HR-8	5	18.0
HR-8	5	14.6
HR-8	5	14.4
HR-8	4	14.7

July, 1991: Test Series 5 Continued

HR-9	5	11.0
HR-9	5	7.8
HR-9	5	11.6
HR-9	5	17.4
HR-10	5	10.0
HR-10	5	14.2
HR-10	5	14.8
HR-10	5	11.0
HR-11	5	12.4
HR-11	5	11.0
HR-11	4	11.5
HR-11	5	14.0

July, 1991: Test Series 6
Experiment No. 910721

Station	No. Plants per Replicate	Mean Cystocarps per Replicate
NSW Control	5	18.6
NSW Control	5	16.6
NSW Control	5	15.8
NSW Control	5	13.6
Brine Control	5	12.6
Brine Control	5	14.0
Brine Control	5	18.0
Brine Control	5	12.6
ER-1	5	9.8
ER-1	5	16.6
ER-1	4	10.5
ER-1	5	15.2
ER-2	5	no male added
ER-2	5	10.4
ER-2	5	13.2
ER-2	5	14.2
ER-3	5	12.2
ER-3	5	8.8
ER-3	5	15.0
ER-3	5	16.2
ER-4	5	9.8
ER-4	5	8.6
ER-4	5	18.6
ER-4	5	14.6
ER-5	5	12.0
ER-5	5	20.0
ER-5	5	22.0
ER-5	4	11.0
ER-6	5	20.0
ER-6	5	17.2
ER-6	5	14.8
ER-6	5	16.8
ER-7	5	15.8
ER-7	5	17.2
ER-7	5	20.8
ER-7	5	14.0
ER-8	5	12.6
ER-8	4	20.0
ER-8	5	14.0
ER-8	5	13.2

July, 1991: Test Series 6 Continued

ER-9	5	19.6
ER-9	5	12.2
ER-9	5	18.8
ER-9	5	10.6
ER-10	5	13.2
ER-10	5	23.2
ER-10	5	12.4
ER-10	5	11.4
ER-11	5	11.2
ER-11	5	8.2
ER-11	5	14.6
ER-11	5	10.8
JB-3	5	9.6
JB-3	5	7.0
JB-3	5	6.2
JB-3	5	0.0
JB-5	5	6.8
JB-5	5	6.4
JB-5	5	5.4
JB-5	5	7.6
JB-6	5	6.8
JB-6	4	7.0
JB-6	5	4.8
JB-6	5	8.6

July, 1991: Test Series 7
Experiment No. 910724

Station	No. Plants per Replicate	Mean Cystocarps per Replicate
NSW Control	5	10.4
NSW Control	5	11.2
NSW Control	5	17.6
NSW Control	5	9.0
Brine Control	5	9.2
Brine Control	5	8.6
Brine Control	5	9.4
Brine Control	5	12.4
NB-1	5	10.0
NB-1	5	5.6
NB-1	5	8.0
NB-1	4	3.5
NB-2	5	21.6
NB-2	4	11.0
NB-2	5	7.4
NB-2	4	11.5
NB-3	5	5.0
NB-3	5	12.2
NB-3	5	5.4
NB-3	5	7.2
NB-4	4	8.0
NB-4	5	8.8
NB-4	5	9.8
NB-4	5	7.4
JB-1	5	6.8
JB-1	5	8.4
JB-1	5	2.6
JB-1	5	4.6
JB-2	5	12.6
JB-2	5	10.4
JB-2	5	4.8
JB-2	5	7.0
JB-4	5	5.0
JB-4	5	3.6
JB-4	5	6.4
JB-4	5	no male added
AK-1	5	6.6
AK-1	5	13.4
AK-1	5	replicate lost
AK-1	5	8.6

July, 1991: Test Series 7 Continued

AK-2	5	4.4
AK-2	5	5.8
AK-2	5	4.4
AK-2	5	7.6
AK-3	5	5.2
AK-3	5	10.8
AK-3	5	no male added
AK-3	5	10.0
AK-4	5	14.8
AK-4	5	11.0
AK-4	5	6.4
AK-4	5	3.0
AK-5	5	7.6
AK-5	5	9.4
AK-5	5	10.2
AK-5	5	12.8
AK-6	4	9.0
AK-6	5	11.2
AK-6	5	10.6
AK-6	5	10.4
KVK-1	5	2.4
KVK-1	5	7.0
KVK-1	5	8.0
KVK-1	5	10.0
KVK-2	5	9.6
KVK-2	5	7.8
KVK-2	5	9.2
KVK-2	5	7.2
KVK-3	5	14.0
KVK-3	5	15.0
KVK-3	3	7.3
KVK-3	5	9.2
KVK-4	5	6.8
KVK-4	5	10.2
KVK-4	4	8.0
KVK-4	5	15.0
RB-1	5	11.6
RB-1	5	2.4
RB-1	5	7.8
RB-1	5	8.6
RB-2	5	6.0
RB-2	5	9.6
RB-2	5	6.2
RB-2	5	5.4

July, 1991: Test Series 7 Continued

RB-3	4	10.0
RB-3	5	3.0
RB-3	4	6.0
RB-3	5	7.4
RB-4	5	11.0
RB-4	5	16.0
RB-4	5	7.0
RB-4	5	8.2
RB-5	5	9.8
RB-5	5	4.6
RB-5	5	16.0
RB-5	5	5.6
RB-6	5	7.4
RB-6	5	6.6
RB-6	5	5.2
RB-6	5	6.8
RB-7	5	5.2
RB-7	5	7.2
RB-7	5	5.4
RB-7	5	8.6
RB-8	5	2.4
RB-8	5	1.0
RB-8	5	7.0
RB-8	5	2.6
RB-9	5	10.0
RB-9	5	4.2
RB-9	5	4.2
RB-9	5	8.4

January, 1991: Test Series 8
Experiment No. 920103

Station	No. Plants per Replicate	Mean Cystocarps per Replicate (a)
NSW Control	5	11.8
NSW Control	4	10.5
NSW Control	5	7.0
NSW Control	5	9.2
Brine Control	5	6.6
Brine Control	5	10.4
Brine Control	5	7.6
Brine Control	4	10.6
HR-1	5	8.4
HR-1	5	8.4
HR-1	5	6.6
HR-1	4	9.8
HR-2	5	8.4
HR-2	5	12.0
HR-2	5	8.3
HR-2	5	10.8
HR-3	5	9.4
HR-3	3	10.3
HR-3	5	8.2
HR-3	4	12.0
HR-4	4	12.0
HR-4	5	8.4
HR-4	5	10.4
HR-4	5	6.8
HR-5	5	9.0
HR-5	4	8.5
HR-5	5	10.0
HR-5	5	8.4
HR-6	5	12.2
HR-6	5	10.8
HR-6	5	12.2
HR-6	4	11.8
HR-7	3	11.3
HR-7	5	8.0
HR-7	5	11.4
HR-7	5	9.2
HR-8	5	8.6
HR-8	5	11.8
HR-8	5	9.2
HR-8	5	7.8

January, 1992: Test Series 8 Continued

HR-9	5	8.6
HR-9	5	7.2
HR-9	5	9.0
HR-9	5	7.6
HR-10	4	9.3
HR-10	5	9.4
HR-10	5	9.8
HR-10	5	9.6
HR-11	5	9.8
HR-11	5	9.0
HR-11	5	10.6
HR-11	5	9.0

January, 1992: Test Series 9
Experiment No. 920105

Station	No. Plants per Replicate	Mean Cystocarps per Replicate
NSW Control	5	6.6
NSW Control	5	5.6
NSW Control	5	7.2
NSW Control	5	5.6
Brine Control	5	4.2
Brine Control	5	7.6
Brine Control	5	11.4
Brine Control	5	5.2
ER-1	5	3.8
ER-1	5	5.0
ER-1	5	4.8
ER-1	5	5.4
ER-2	5	6.8
ER-2	5	6.2
ER-2	5	4.2
ER-2	5	6.6
ER-3	5	4.4
ER-3	5	4.2
ER-3	5	4.8
ER-3	5	4.0
ER-4	5	2.8
ER-4	5	3.4
ER-4	5	4.2
ER-4	5	2.0
ER-5	5	3.4
ER-5	5	3.6
ER-5	5	5.0
ER-5	5	4.6
ER-6	5	7.6
ER-6	5	3.8
ER-6	5	1.6
ER-6	5	4.2
ER-7	5	3.8
ER-7	5	4.0
ER-7	5	4.2
ER-7	5	2.4
ER-8	5	3.4
ER-8	5	4.4
ER-8	5	2.4
ER-8	5	4.8

January, 1992: Test Series 9 Continued

ER-9	5	1.4
ER-9	5	0.0
ER-9	5	3.4
ER-9	5	2.4
ER-10	5	2.0
ER-10	5	4.2
ER-10	5	1.8
ER-10	5	2.8
ER-11	5	4.4
ER-11	4	2.3
ER-11	5	4.4
ER-11	5	2.6

January, 1992: Test Series 10
Experiment No. 920107

Station	No. Plants per Replicate	Mean Cystocarps per Replicate
NSW Control	5	6.4
NSW Control	5	9.6
NSW Control	5	11.6
NSW Control	5	7.4
Brine Control	5	7.4
Brine Control	5	8.0
Brine Control	5	9.6
Brine Control	5	7.6
JB-1	5	0.0
JB-1	3	0.0
JB-1	5	0.0
JB-1	5	0.0
JB-2	5	0.0
JB-2	5	0.0
JB-2	5	0.4
JB-2	5	0.0
JB-3	5	4.6
JB-3	5	3.0
JB-3	5	2.0
JB-3	5	3.2
JB-4	5	4.0
JB-4	5	5.6
JB-4	5	1.8
JB-4	5	2.6
JB-5	4	3.5
JB-5	5	3.4
JB-5	4	2.0
JB-5	5	5.8
JB-6	5	4.4
JB-6	5	3.6
JB-6	5	3.6
JB-6	5	3.8
NB-1	5	1.4
NB-1	5	1.6
NB-1	5	3.0
NB-1	5	0.2
NB-2	5	2.2
NB-2	5	1.6
NB-2	5	0.8
NB-2	5	2.6
NB-3	5	2.0
NB-3	5	1.4
NB-3	5	1.4
NB-3	5	2.0

January, 1992: Test Series 10 Continued

NB-4	5	3.4
NB-4	5	0.6
NB-4	5	3.0
NB-4	5	1.6
KVK-1	5	3.8
KVK-1	5	5.6
KVK-1	5	2.6
KVK-1	5	0.8
KVK-2	5	5.2
KVK-2	5	3.8
KVK-2	5	5.0
KVK-2	4	2.3
KVK-3	5	3.8
KVK-3	5	2.8
KVK-3	5	5.0
KVK-3	5	3.6
KVK-4	5	2.2
KVK-4	4	3.7
KVK-4	5	3.4
KVK-4	5	2.6
AK-1	4	4.3
AK-1	5	5.8
AK-1	5	6.2
AK-1	5	3.0
AK-2	5	2.0
AK-2	5	4.8
AK-2	5	2.4
AK-2	5	4.2
AK-3	5	2.8
AK-3	5	1.2
AK-3	5	0.0
AK-3	5	1.2
AK-4	5	0.6
AK-4	5	1.0
AK-4	5	0.4
AK-4	5	0.6
AK-5	5	3.0
AK-5	5	3.6
AK-5	5	2.6
AK-5	5	0.4
AK-6	4	1.7
AK-6	5	1.4
AK-6	5	0.8
AK-6	5	1.0

January, 1992: Test Series 11
Experiment No. 920109

Station	No. Plants per Replicate	Mean Cystocarps per Replicate
NSW Control	4	16.3
NSW Control	4	14.3
NSW Control	5	16.6
NSW Control	5	18.2
Brine Control	5	15.8
Brine Control	4	10.5
Brine Control	4	18.8
Brine Control	5	17.6
RB-1	5	18.0
RB-1	5	10.4
RB-1	4	14.3
RB-1	5	17.4
RB-2	5	13.8
RB-2	5	16.2
RB-2	5	14.8
RB-2	4	12.0
RB-3	5	15.4
RB-3	5	16.6
RB-3	5	13.2
RB-3	4	17.8
RB-4	4	16.3
RB-4	5	15.2
RB-4	5	16.8
RB-4	5	10.0
RB-5	5	10.0
RB-5	4	11.8
RB-5	5	15.0
RB-5	4	14.3
RB-6	5	13.8
RB-6	5	12.2
RB-6	4	7.3
RB-6	5	16.0
RB-7	4	18.8
RB-7	5	17.8
RB-7	5	13.0
RB-7	5	16.2
RB-8	5	13.0
RB-8	4	12.3
RB-8	5	13.6
RB-8	5	12.8
RB-9	5	15.6
RB-9	4	15.3
RB-9	5	13.4
RB-9	5	9.8

Appendix E. Salinity adjustment data

Data is listed for each sample for all three sampling events. Data include initial salinity, the salinity of the hypersaline brine used to adjust salinity, and the % of sample represented in the final volume.

Table E-1. Salinity adjustment data for June, 1991, sampling event.

Station	Initial Salinity	Brine Salintiy	% Sample in Final Volume
HR-11A,Top	9.9	102	78
HR-11A,Middle	12.8	102	81
HR-11A,Bottom	13.1	102	81
HR-11B,Top	8.0	102	77
HR-11B,Middle	10.8	102	79
HR-11B,Bottom	11.0	102	79
HR-11C,Top	13.6	102	81
HR-11C,Middle	13.4	102	81
HR-11C,Bottom	9.9	102	78
NB-2A,Top	22.5	103	91
NB-2A,Middle	22.5	103	91
NB-2A,Bottom	22.4	103	91
NB-2B,Top	25.2	103	94
NB-2B,Middle	23.4	103	92
NB-2B,Bottom	23.3	103	92
NB-2C,Top	22.6	103	91
NB-2C,Middle	22.8	103	91
NB-2C,Bottom	23.1	103	91
RB-6A,Top	26.1	103	95
RB-6A,Middle	26.5	103	95
RB-6A,Bottom	26.6	103	96
RB-6B,Top	26.2	103	95
RB-6B,Middle	26.2	103	95
RB-6B,Bottom	26.2	103	95
RB-6C,Top	26.0	103	95
RB-6C,Middle	26.0	103	95
RB-6C,Bottom	26.0	103	95
HR-6A,Top	23.1	103	91
HR-6A,Middle	24.6	103	93
HR-6A,Bottom	24.3	103	93
HR-6B,Top	15.5	103	83
HR-6B,Middle	20.0	103	88
HR-6B,Bottom	19.8	103	88
HR-6C,Top	19.8	103	88
HR-6C,Middle	23.3	103	92
HR-6C,Bottom	21.8	103	90

Table E-2. Salinity adjustment data for July, 1991, sampling event.

Station	Initial Salinity	Brine Salintiy	% Sample in Final Volume
HR-1	26.0	103	95
HR-2	24.5	103	93
HR-3	24.0	103	92
HR-4	18.0	103	86
HR-5	16.0	103	84
HR-6	15.0	103	83
HR-7	12.0	103	80
HR-8	11.5	103	80
HR-9	10.0	103	78
HR-10	10.0	103	78
HR-11	10.0	103	78
ER-1	24.0	103	92
ER-2	24.0	103	92
ER-3	24.0	103	92
ER-4	25.0	103	94
ER-5	25.0	103	94
ER-6	25.0	103	94
ER-7	25.0	103	94
ER-8	26.0	103	95
ER-9	26.0	103	95
ER-10	26.0	103	95
ER-11	26.0	103	95
NB-1	22.0	103	90
NB-2	22.0	103	90
NB-3	21.0	103	89
NB-4	21.0	103	89
KVK-1	22.0	103	90
KVK-2	24.0	103	92
KVK-3	24.0	103	92
KVK-4	25.0	103	94
AK-1	26.0	103	95
AK-2	24.0	103	92
AK-3	22.0	103	90
AK-4	21.0	103	89
AK-5	21.0	103	89
AK-6	21.0	103	89
RB-1	28.0	-	100
RB-2	29.0	-	100
RB-3	30.0	-	100
RB-4	26.0	103	95
RB-5	28.0	-	100
RB-6	26.0	103	95
RB-7	27.0	-	100
RB-8	30.0	-	100
RB-9	28.0	-	100
JB-1	25.0	103	94
JB-2	25.0	103	94
JB-3	28.0	-	100
JB-4	28.0	-	100
JB-5	28.0	-	100
JB-6	30.0	-	100

Table E-3. Salinity adjustment data for January, 1992, sampling event.

Station	Initial Salinity	Brine Salintiy	% Sample in Final Volume
HR-1	21.2	99	89
HR-2	21.2	99	89
HR-3	20.6	99	88
HR-4	16.7	99	84
HR-5	13.8	99	81
HR-6	13.3	99	81
HR-7	12.2	99	79
HR-8	10.9	99	78
HR-9	9.2	99	77
HR-10	8.1	99	76
HR-11	7.3	99	75
ER-1	23.9	99	92
ER-2	23.4	99	91
ER-3	23.3	99	91
ER-4	23.1	99	91
ER-5	23.1	99	91
ER-6	22.9	99	91
ER-7	23.1	99	91
ER-8	24.2	99	92
ER-9	24.7	99	93
ER-10	25.0	99	93
ER-11	25.7	99	94
NB-1	20.5	99	88
NB-2	18.8	99	86
NB-3	19.1	99	86
NB-4	18.9	99	86
KVK-1	22.3	99	90
KVK-2	21.3	99	89
KVK-3	22.3	99	90
KVK-4	22.7	99	90
AK-1	23.5	99	91
AK-2	23.2	99	91
AK-3	21.9	99	89
AK-4	20.3	99	88
AK-5	19.8	99	87
AK-6	19.9	99	87
RB-1	25.3	99	94
RB-2	26.8	99	96
RB-3	25.3	99	94
RB-4	23.8	99	92
RB-5	25.1	99	93
RB-6	25.2	99	93
RB-7	25.1	99	93
RB-8	28.0	99	97
RB-9	25.4	99	94
JB-1	27.0	-	100
JB-2	28.7	-	100
JB-3	29.7	-	100
JB-4	30.2	-	100
JB-5	30.2	-	100
JB-6	30.6	-	100

Sc

Appendix F. Raw chemistry data

Data are listed by sample for each of the heavy metals, total ammonia, and total organic carbon. EPA's Environmental Services Division in Edison, NJ conducted all of the chemical analyses. The detection limit for each chemical is listed in the first row of each table.

Table F-1. Summary of chemistry data from June, 1991, sampling event for small scale variability.

STATION ID		NH3	TOC	Ag	As	Be	Cd	Cr	Cu	Hg	Ni	Pb	Sb	Se	Tl	Zn
Detection Limit		0.05	5	1	1	1	3	7	3	0.2	8	6	11	1	4	9
RB6A	Top	0.09	5	9.3	1	1.2	3	7	3	0.2	8	6	11	1	4	9
RB6A	Middle	0.11	5	9.6	1	1.2	3	7	3	0.2	8	6	11	1	4	9
RB6A	Bottom	0.14	5	9.3	3.2	1.2	3	7	3	0.2	8	6	11	1	4	9
RB6B	Top	0.23	5	11	1	1.2	3	7	3	0.2	8	6	11	1	4	9
RB6B	Middle	0.23	5	9.6	4.3	1	3	7	3	0.2	8	6	11	1	4	9
RB6B	Bottom	0.23	5	9	1	1.2	3	7	3	0.2	8	6	11	1	4	9
RB6C	Top	0.16	5	8.3	5.8	1.2	3	7	3	0.2	8	6	11	1	4	9
RB6C	Middle	0.17	5	8.7	1	1.2	3	7	3	0.2	8	6	11	1	4	9
RB6C	Bottom	0.15	5	7.6	1	1	3	7	3	0.2	8	6	11	1	4	9
NB2A	Top	0.68	5	7.1	1	1	3	7	4.5	0.2	8	6	11	1	4	15.9
NB2A	Middle	0.67	5	7	1.2	1	3	7	3	0.2	8	6	11	1	4	12.2
NB2A	Bottom	0.68	5	8.5	1.7	1.2	3	7	3	0.2	8	6	11	1	4	11.6
NB2B	Top	0.64	5	7.2	1	1	3	7	3	0.2	8	6	11	1	4	9.6
NB2B	Middle	0.6	5	7.3	1.3	1.2	3	7	3.2	0.2	8	6	11	1	4	9.8
NB2B	Bottom	0.62	5	9.9	1	1	3	7	3.6	0.2	8	6	11	1	4	1.01
NB2C	Top	0.67	5	8.5	1	1.2	3	7	3.2	0.2	8	6	11	1	4	12.7
NB2C	Middle	0.65	5	9.9	1	1.2	4.8	7	3	0.2	8	6	11	1	4	10.6
NB2C	Bottom	0.65	5	10.9	1.5	1	3	7	4	0.2	8	6	11	1	4	11.1
HR6A	Top	0.36	5	7	1	1.1	3	7	3	0.2	8	6	11	2.6	4	9.2
HR6A	Middle	0.4	5	7	1	1.1	3	7	3	0.2	8	6	11	1	4	9
HR6A	Bottom	0.39	5	7.2	1	1.1	3	7	3	0.2	8	6	11	1	4	9
HR6B	Top	0.24	5	7	1	1	3	7	3.8	0.2	8	6	11	1	4	9.1
HR6B	Middle	0.37	5	7	1	1	3	7	3.5	0.2	8	6	11	1	4	9.1
HR6B	Bottom	0.36	5	7.7	1	1.1	3	7	4.7	0.2	8	6	11	1	4	9.6
HR6C	Top	0.34	5	7.3	1	1.1	3.7	7	3	0.2	8	6	11	1	4	9
HR6C	Middle	0.39	5	7	1	1.1	3	7	3	0.2	8	6	11	1	4	9
HR6C	Bottom	0.37	5	7	1	1.1	3	7	3	0.2	8	6	11	1	4	9
HR6A	Top	----	---	7	1	1.1	3	7	3	0.2	8	6	11	1	4	12.2
HR6A	Middle	----	---	7	1	1.1	3	7	3	0.2	8	6	11	1	4	9
HR6A	Bottom	----	---	7	1	1	3	7	3	0.2	8	6	11	1	4	9
HR11A	Top	0.17	5	7	1	1	3	7	3.6	0.2	8	6	11	1	4	9
HR11A	Middle	0.24	5	7	1	1	3	7	4.8	0.2	8	6	11	1	4	9
HR11A	Bottom	0.25	5	7	1	1	3	7	4.5	0.2	8	6	11	1	4	9
HR11B	Top	0.11	5	7	1	1	3	7	3.3	0.2	8	6	11	1	4	9
HR11B	Middle	0.19	5	7	1	1	3	7	4.8	0.2	8	6	11	1	4	9
HR11B	Bottom	0.19	5	7	1	1	3	7	5.1	0.2	8	6	11	1	4	20
HR11C	Top	0.14	5	7	1	1	3	7	3.8	0.2	8	6	11	1	4	9
HR11C	Middle	0.28	5	7	1	1	3	7	5.1	0.2	8	6	11	1	4	9
HR11C	Bottom	0.28	5	7	1	1	3	7	3.6	0.2	8	6	11	1	4	9

Table F-2. Summary of chemistry data from July, 1991, sampling event.

StationID	NH3	TOC	Ag	As	Be	Cd	Cr	Cu	Hg	Ni	Pb	Sb	Se	Tl	Zn
Detection Limit	0.05	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
HR01	0.15	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
HR02	0.41	5	7	1	3	4	7	4.1	0.2	8	6	13	1	8	9
HR03	0.38	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
HR04	0.27	5	7	1	3	4	7	3.3	0.2	8	6	13	1	8	9
HR05	0.20	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
HR06	0.16	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
HR07	0.14	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
HR08	0.12	5	7	1	3	4	7	3	0.2	8	6	13	2	8	9
HR09	0.05	5	7	1	3	4	7	3	0.2	8	6	13	2	8	9
HR10	0.05	5	7	1	3	4	7	3	0.2	8	6	13	2	8	9
HR11	0.05	5	7	1	3	4	7	3	0.2	8	6	13	2	8	9
ER01	0.51	5	7	1	3	4	7	3.5	0.34	8	6	13	1	8	9
ER02	0.55	5	7	1	3	4	7	3.7	0.2	8	6	13	1	8	9
ER03	0.58	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
ER04	0.52	5	7	1	3	4	7	3.4	0.2	8	6	13	1	8	9
ER05	0.52	5	7	1	3	4	7	3.4	0.2	8	6	13	1	8	9
ER06	0.49	5	7	1	3	4	7	4	0.2	8	6	13	4	8	9
ER07	0.42	5	7	1	3	4	7	4.1	0.2	8	6	13	1	8	9
ER08	0.40	5	7	1	3	4	7	3.5	0.2	8	6	13	1	8	9
ER09	0.33	5	7	1	3	4	7	3.1	0.2	8	6	13	1	8	9
ER10	0.32	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
ER11	0.32	5	7	1	3	4	7	3.8	0.2	8	6	13	1	8	9
NB01	0.46	5	7	1.3	3	4	7	3	0.2	8	6	13	1	8	9.1
NB02	0.46	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
NB03	0.48	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9.8
NB04	0.52	5	7	1	3	4	7	3	0.61	8	6	13	1	8	10
KVK01	0.49	5	7	1	3	4	7	3.2	0.2	8	6	13	1	8	9.4
KVK02	0.42	5	7	1.4	3	4	7	3	0.2	8	6	13	1	8	9
KVK03	0.42	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
KVK04	0.36	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
AK01	0.69	5	7	2.1	3	4	7	3.6	0.2	8	6	13	1	8	9
AK02	0.79	5	7	1.2	3	4	7	3.6	0.2	8	6	13	1	8	11
AK03	0.76	5	7	2.1	3	4	7	4.3	0.2	8	6	13	1	8	11
AK04	0.67	5	7	1	3	4	7	3.6	0.2	8	6	13	1	8	10
AK05	0.53	5	7	1	3	4	7	3.2	0.2	8	6	13	1	8	9.1
AK06	0.67	5	7	1	3	4	7	3.9	0.2	8	6	13	1	8	11
RB01	0.17	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
RB02	0.12	5	7	1	3	4	7	3	0.61	8	6	13	1	8	9
RB03	0.06	5	7	1	3	4	7	3	0.77	8	6	13	1	8	9
RB04	0.24	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
RB05	0.12	5	7	1	3	4	7	3	0.45	8	6	13	1	8	9
RB06	0.12	5	7	1	3	4	7	3	0.41	8	6	13	1	8	9
RB07	0.12	5	7	1	3	4	7	3	0.96	8	6	13	1	8	9
RB08	0.05	5	7	1	3	4	7	3	0.27	8	6	13	1	8	9
RB09	0.07	5	7	1	3	4	7	3	0.2	8	6	13	1	8	9
JB01	0.23	6.9	7	1	3	4	7	3	0.2	8	6	13	1	8	9
JB02	0.58	8.7	7	1	3	4	7	3	0.2	8	6	13	1	8	9
JB03	0.05	6.9	8.6	1	3	4	7	3	0.2	8	6	13	1	8	9
JB04	0.07	5	7.6	1	3	4	7	3	0.57	8	6	13	1	8	9
JB05	0.05	5.7	7.9	1	3	4	7	3	1.07	8	6	13	1	8	9
JB06	0.06	5	8.1	1	3	4	7	3	3.48	8	6	13	1	8	9

Table F-3. Summary of chemistry data from January, 1992, sampling event

StationID	NH3	TOC	Ag	As	Be	Cd	Cr	Cu	Hg	Ni	Pb	Sb	Se	Tl	Zn
Detection Limit	0.05	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
HR01	0.22	5	7	5	3	4	7	5.3	0.51	8	5	13	6	3	9
HR02	0.26	5	7	5	3	4	7	4.1	0.2	8	5.4	13	6	3	9
HR03	0.27	5	7	5	3	4	7	7.1	0.2	8	5	13	6	3	9
HR04	0.23	5	7	5	3	4	7	5.7	0.2	8	5	13	6	3	9
HR05	0.21	5	7	5	3	4	7	6.2	0.2	8	5	13	6	3	9
HR06	0.23	5	7	5	3	4	7	5.1	0.2	8	5	13	6	3	9
HR07	0.29	5	7	5	3	4	7	5.6	0.2	8	5	13	6	3	9
HR08	0.27	5	7	5	3	4	7	5.9	0.2	8	5	13	6	3	9
HR09	0.25	5	7	5	3	4	7	4.2	0.2	8	5	13	6	3	10.5
HR10	0.23	5	7	5	3	4	7	5.1	0.2	8	5	13	6	3	9
HR11	0.24	5	7	5	3	4	7	5.7	0.2	8	5	13	6	3	9
ER01	0.22	5	7.3	5	3	4	7	5	0.2	8	5	13	6	3	9
ER02	0.26	5	7	5	3	4	7	4.6	0.2	8	5	13	6	3	9
ER03	0.27	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
ER04	0.30	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
ER05	0.26	5	7	5	3	4	7	3.3	0.2	8	5	13	6	3	9
ER06	0.30	5	7	5	3	4	7	14.4	0.2	8	5	13	6	3	9
ER07	0.39	5	7	5	3	4	7	4.4	0.2	8	5	13	6	3	9
ER08	0.47	5	7	5	3	4	7	4.7	0.2	8	5	13	6	3	9
ER09	0.42	5	7	5	3	4	7	6.4	0.2	8	5	13	6	3	9
ER10	0.32	5	7	5	3	4	7	6.7	0.2	8	5	13	6	3	9
ER11	0.24	5	7	5	3	4	7	4.7	0.2	8	3	13	6	3	9
NB01	0.49	5	7	5	3	4	7	3	0.2	8	5	13	6	3	13.7
NB02	0.54	5	7	5	3	4	7	3	0.2	8	5	13	6	3	14
NB03	0.52	5	7	5	3	4	7	3	0.2	8	5	13	6	3	14
NB04	0.58	5	7	5	3	4	7	3	0.2	8	5	13	6	3	17.1
KVK01	0.38	5	8.3	5	3	4	7	3.7	0.2	8	5	13	6	3	9.2
KVK02	0.44	5	7.2	5	3	4	7	3	0.2	8	5	13	6	3	10.3
KVK03	0.35	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
KVK04	0.30	5	13.9	5	3	4	7	3	0.2	8	5	13	6	3	9
AK01	0.28	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
AK02	0.44	5	7	5	3	4	7	3	0.2	8	5	13	6	3	11.2
AK03	0.68	5	8.8	5	3	4	7	3.3	0.2	8	5	13	6	3	17.8
AK04	0.92	5	7	5	3	4	7	3	0.2	8	5	13	6	3	19.2
AK05	0.56	5	7	5	3	4	7	3	0.2	8	5	13	6	3	14.3
AK06	0.72	5	7	5	3	4	7	3	0.2	8	5	13	6	3	16.2
RB01	0.20	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
RB02	0.16	5	7	5	3	4	7	3	0.2	8	5	13	10	3	9
RB03	0.20	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
RB04	0.25	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
RB05	0.17	5	7	5	3	4	7	3	0.2	8	5	13	7.8	3	9
RB06	0.19	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
RB07	0.20	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
RB08	0.12	5	7	5	3	4	7	3	0.2	8	5	13	10	3	9
RB09	0.20	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
JB01	0.80	5	7	5	3	4	7	3	0.2	8	5	13	10.5	3	9
JB02	0.51	5	7	5	3	4	7	3	0.2	8	5	13	6	3	9
JB03	0.16	5	7.9	5	3	4	7	4.8	0.2	8	5	13	6	3	9
JB04	0.16	5	7	5	3	4	7	3.9	0.2	8	5	13	6	3	9
JB05	0.20	5	7	5	3	4	7	3.8	0.2	8	5	13	6	3	9
JB06	0.15	5	8.2	5	3	4	7	3	0.2	8	5	13	10	3	9

Appendix G. Reference toxicant data for *Arbacia* and *Champia*.

Table G-1. Fertilization data from reference toxicity tests with sea urchins exposed to sodium dodecyl sulfate. For each replicate 100 eggs were randomly counted. EC50s were calculated using trimmed Spearman-Karber with Abbott's correction for contorl mortality.

Raw Data: Number fertilized eggs

Conc. ug/l	6/13/91		6/18/91		6/19/91		7/15/91		1/14/92		1/22/92	
	Unfert	Fertilized	Unfert	Fertilized	Unfert	Fertilized	Unfert	Fertilized	Unfert	Fertilized	Unfert	Fertilized
NSW Control	4	96	2	96	1	99	10	90	23	77	2	98
	2	98	3	97	1	99	12	88	25	75	3	97
	1	99	5	95	2	98	9	91	25	75	2	98
	4	96	6	94	0	100	12	88	31	69	5	95
Brine + D.I.	-	-	1	99	-	-	14	86	25	75	3	97
	-	-	2	98	-	-	26	74	20	80	2	98
	-	-	3	97	-	-	13	87	9	91	2	98
	-	-	4	96	-	-	2	98	6	94	0	100
0.8	3	97	-	-	-	-	12	88	36	64	0	100
	4	96	-	-	-	-	23	77	51	49	1	99
	3	97	-	-	-	-	12	88	45	55	0	100
	3	97	-	-	-	-	8	92	40	60	1	99
1.2	3	97	6	94	3	97	40	60	49	51	3	97
	5	95	13	87	4	96	20	80	48	52	2	98
	6	94	5	95	6	94	15	85	45	55	0	100
	4	96	10	90	6	94	12	88	34	66	3	97
2.5	3	97	59	41	11	89	4	96	60	40	0	100
	3	97	52	48	10	90	20	80	55	45	1	99
	3	97	62	38	30	70	57	43	42	58	2	98
	8	92	57	43	20	80	56	44	50	50	2	98
5.0	9	91	83	17	56	44	100	0	30	70	5	95
	6	94	85	15	62	38	100	0	49	51	2	98
	14	86	68	32	53	47	99	1	56	44	3	97
	9	91	85	15	59	41	100	0	66	34	4	96
10.0	38	62	100	0	99	1	98	2	41	59	88	12
	49	51	100	0	96	4	99	1	63	37	79	21
	60	40	100	0	97	3	99	1	73	27	82	18
	70	30	100	0	93	7	99	1	87	13	89	11
EC50	9.56		2.66		4.3		2.79		2.38		9.12	

(-) These concentrations were not tested.

Table G-2. Development to first cleavage data from reference toxicity tests with sea urchins exposed to sodium dodecyl sulfate. For each replicate 100 eggs were randomly counted. The total unfertilized and uncleaved subtracted from 100 equals the percent cleaved. EC50s were calculated using trimmed Spearman-Karber with Abbott's correction for control mortality.

Raw Data: Number Unfertilized and Uncleaved eggs

Conc. ug/l	6/13/91		6/18/91		6/19/91		7/15/91		1/14/92		1/22/92	
	Unfert *	Uncleaved	Unfert *	Uncleaved	Unfert *	Uncleaved	Unfert	Uncleaved	Unfert	Uncleaved	Unfert	Uncleaved
NSW Control	7		4		17		2	1	5	6	0	9
	3		3		51		1	2	8	3	0	17
	6		3		27		3	7	1	3	1	27
	9		4		9		2	2	2	4	1	14
Brine + D.I.	-		3		-		2	7	6	6	4	20
	-		4		-		2	4	3	6	2	21
	-		3		-		4	6	6	10	0	33
	-		4		-		7	6	4	5	16	55
0.8	8		-		-		12	34	2	6	1	17
	9		-		-		6	34	1	2	1	15
	9		-		-		15	40	7	5	2	20
	6		-		-		5	25	0	2	0	11
1.2	6		20		14		5	29	5	3	1	19
	22		10		21		11	58	6	4	1	22
	20		11		26		8	33	7	6	3	12
	27		13		11		9	43	5	7	2	12
2.5	27		63		61		28	15	3	12	0	18
	37		77		40		41	14	3	9	0	18
	37		47		61		11	21	5	10	0	23
	33		61		54		37	20	5	3	2	18
5.0	64		70		81		98	0	5	49	5	56
	53		75		80		96	2	4	54	4	67
	63		84		92		100	0	5	64	6	69
	64		72		83		100	0	8	58	1	68
10.0	87		99		98		100	0	47	52	53	40
	89		100		99		100	0	40	60	61	37
	97		100		96		100	0	75	25	66	31
	97		99		98		100	0	58	42	55	42
EC50	3.81		2.54		2.31			2.05		4.87		4.45

* Unfertilized not counted for these tests; only uncleaved.

(-) These concentrations were not tested.

Table G-3. Data from reference toxicity tests with Champia exposed to sodium dodecyl sulfate.

	Test Start Date	
Conc. (mg/L)	6/13/91	11/5/91
Raw Data: Mean Cystocaarps per replicate		
Control	9.8	10.0
Control	11.0	7.6
Control	7.6	13.4
0.03	-	13.6
0.03	-	11.8
0.03	-	9.4
0.05	-	9.4
0.05	-	13.5
0.05	-	10.2
0.08	-	12.0
0.08	-	10.6
0.08	-	11.6
0.13	11.0	13.0
0.13	10.0	16.4
0.13	15.0	11.8
0.22	12.8	7.0
0.22	9.2	20.2
0.22	10.0	11.4
0.36	14.0	0.0
0.36	9.2	0.0
0.36	11.8	0.0
0.60	9.2	-
0.60	10.0	-
0.60	10.2	-
1.00	11.0	-
1.00	6.0	-
1.00	9.0	-
IC50 (mg/L)	>1.0	< 0.36

(-) These concentrations were not tested.

Appendix H. Plot of the relationship between % fertilization and development to first cleavage for *Arbacia punctulata.*

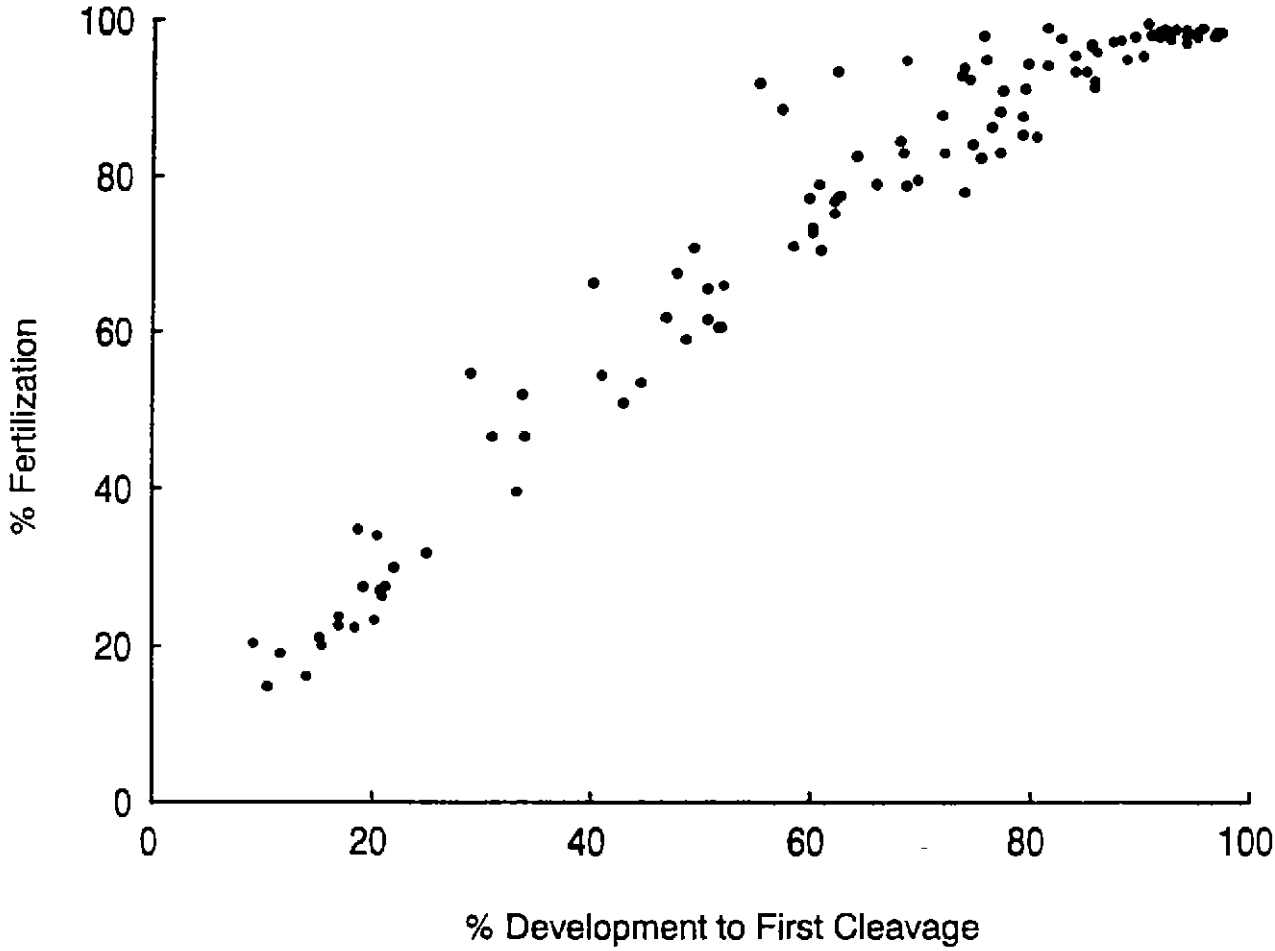


Figure H-1. A comparison between end points for development to first cleavage and fertilization for the sea urchin, *Arbacia punctulata*. Samples were from both the small and large scale sampling events from the Hudson/Raritan Estuary. Both values came from the same set of data. Percent fertilization is the sum of both fertilized eggs and embryos that developed to first cleavage.

Appendix I. Response to comments on draft report dated March 25, 1992.

New York State Department of Environmental Conservation
50 Wolf Road, Albany, New York 12233

Thomas C. Jorling
Commissioner

May 1, 1992

Mr. Kevin Bricke
Assistant Manager
Water Management Division
USEPA - Region II
26 Federal Plaza
New York, New York 10278

Dear Kevin:

As requested by Seth Ausubel in his April 1, 1992 transmittal memorandum, attached are our comments on the draft report "Toxic Assessment on New York-New Jersey Harbor Ambient Waters." As you are aware, New York State has advocated bio-effects toxicity testing in the Harbor Estuary Program, and we are hopeful that this study will begin to supply some of the answers. Although the report finds toxicity to the two species tested, you will see from our comments that additional information about the study is needed before New York State can understand the test results and, more importantly, assess toxicity in Harbor waters.

The attached comments were prepared by Drs. Peter Jones and Edward Kuzia. If your staff or SAIC have questions about these comments, Dr. Jones can be reached at (518) 457-3651. We believe that experts at EPA should take a critical look at the study's quality assurance, as well as perform an interpretation of the study. Do you plan such a review, and if so, who will review QA and perform the interpretation.

The study that produced this report was planned early in the Harbor/Estuary Program, and as a result, the report is not now the responsibility of a Program Module Work Group. Because the report contains potentially important information with implications for work plans throughout the Work Group structure, we recommend that the Toxic Work Group take the responsibility of discussing and managing the report and that the Standards Work Group review and discuss the report as well. We believe it is necessary that these discussions precede the Manhattan College Conference and the finalization of work plans for FFY91 and FFY92, which are being developed.

Sincerely,

Phil

Philip DeGaetano, P.E.
Director
Bureau of Water Quality Management

Attachments

cc: Mr. Eric Stern
Dr. Shing-Fu Hsueh
Mr. Thomas Brosnan

92

INITIAL REVIEW AND COMMENTS ON THE DRAFT REPORT ON AMBIENT TOXICITY TESTS AND TOXIC ASSESSMENT NY/NJ HARBOR (SAIC, 1992)

The Draft Report entitled **Toxic Assessment on New York-New Jersey Harbor Ambient Waters** submitted by Science Applications International Corporation represents a large amount of information of potential importance, both to the NY/NJ Harbor Estuary Program and other investigations of toxic contaminants in marine coastal waters nationwide. In offering comments, we acknowledge that this is a draft report and appreciate the expediency with which it was prepared. First our comments address the draft report presentation and overall study; specific comments on the tests results follow.

General Comments on Report:

1. It appears from a reading of the summary that the report limited itself to determining the toxicity of the harbor waters to certain life stages of two species of aquatic organisms. The introduction, however, refers to the undefined term of "toxicity." It is not clear from the report whether the tests were to be viewed as toxicity screening evaluations or well defined assessments of toxicity to the Harbor/Estuary aquatic community. We recommend that the report contain a clearer description of the purpose and scope of the study in order to understand the objectives and evaluate whether the report has met them. This draft presentation could, perhaps, be included as an appendix to a larger document that contains an interpretation of the results. If the authors are aware, they should also identify the intended audience, reasons for generating the report and explain how the final report will be used. For example, can the report be used to evaluate the validity of the National Criterion or the New York State Standard for Copper in the Harbor?

2. If the authors are aware, the introduction should also provide a clearer picture of the parties and individuals responsible for the development and design of the study. In order to fully appreciate the study results, it is important to understand the selection of the tests that were used, and the rationale behind the study design. Accountable staff can provide greater detail of explanation for those whom are not intimately familiar with the tests and final field study design.

3. The report summary presents findings on the toxicity of Harbor waters to Arbacia and Champia. We would like to see the report address the broader issue of toxicity and present conclusions on such. For example, can the authors offer an ecological diagnosis for the aquatic community inhabiting the estuary and harbor based on conclusions from this report? Can the authors provide an understanding of the health of the harbor/estuary ecosystem based on the findings in this report?

Specific Comments on Tests and Study:

1. Experiments with live aquatic organisms very often require acclimation to the natural constituents of experimental waters, such as salinity composition, in order to avoid false positive responses in tests that can be attributed to shock or stress to organisms due to environmental change. Both the Arbacia and Champia tests appear highly sensitive. Arbacia appears especially sensitive to the surfactant reference toxicant, sodium dodecyl sulfate, which could indicate an osmotic sensitivity as readily as a sensitivity to anthropogenic toxicants. Has it been demonstrated that acclimation to clean marine waters, in which the organisms were not reared or maintained, is not necessary or useful? Acclimation of test organisms to receiving waters is routine in conventional effluent tests when the receiving waters are used as dilution and control waters. The test organisms in this study were not acclimated to the test waters, test organisms or gametes were placed directly into the test water. Reproductive success was then measured against control water to which control organisms were well acclimated. Can the authors account for potential effects from differences in the ionic composition of natural marine waters from different locations? Are data available to demonstrate that exposure of the test organisms to near shore non-toxic ambient waters, without acclimation, yields a "non-toxic" response? If acclimation is not necessary, it should be stated along with an explanation of why acclimation is not necessary.

2. Re: Appendix D. - Reference Toxicant Data. According to EPA (1988), the purpose of the reference toxicant test, that is run in the same time frame as the ambient tests, is to provide a measure of the sensitivity of the brood of organisms to be used in a test relative to the sensitivity of previously used broods of the same species. It is not clear from the data listed on page D-2, however, just how this judgment of comparable sensitivities is rendered. The Arbacia broods tested in 7/15/91 and 1/14/92 yielded EC50 values that were very close (2.8 and 2.4 ug/L, respectively). Are these two broods judged to be of similar sensitivity, even though the 7/15/91 brood exhibited an apparent threshold in sensitivity between reference toxicant concentrations of 2.5 and 5.0 ug/L, while the 1/14/92 brood did not. Perhaps an explanation of the basis for judging comparable sensitivities among broods would aid in understanding the meaning of this quality assurance procedure. Moreover, could the precipitous increase in adverse effects between 2.5 and 5.0 ug/L in the 7/15/91 control brood explain the increased sensitivity of the 7/15/91 run?

   For the sake of clarification, the Method 1008, Sea Urchin (Arbacia punctulata) Fertilization Test (page 247, EPA, 1988) calls for a reference toxicant test using copper sulfate. Was there a significant reason for using sodium dodecyl sulfate as the reference toxicant in this study? Why does Champia yield so variable and insensitive a response and Arbacia exhibit such a strict sensitivity (i.e. about 100 times more sensitive than Champia) to the reference toxicant when both species are very sensitive and within 10 ug/L of each other in sensitivity to the routine reference

toxicant copper sulfate? Is there a biological explanation of why the surfactant reference toxicant acts so intensely on the urchin gametes but is resisted with opposite intensity by the red macroalgal cells?

3. Reference toxicant tests (Appendix D) performed on Arbacia and Champia indicate a high degree of variability. In the Arbacia fertilization test there is as much as a four fold difference in response in 6 tests. In the two Champia reference toxicant tests, the IC 50 was reported to be greater than 1 mg/l in the 6/91 test and <0.36 mg/l in the 11/91 test. In the 6/91 test the IC 50 could not be established, apparently because the highest concentration tested was 1 mg/l. In the November test the highest concentration tested, 0.36 mg/l, completely inhibited cystocarp production in Champia. These reference tests demonstrate substantial variability in sensitivity among broods and groups of test organisms. Can the authors explain this apparent inherent variability in sensitivity of test broods or groups? Are the responses to ambient waters just as inherently variable as they are to the reference toxicants? Which reference toxicant response, 6/13 or 11/5, characterizes Champia's sensitivity?

4. The report mentions that the Arbacia test followed the EPA (1988) protocol with the exception of the development to first cleavage end point. An explanation is needed for the departure from the standard protocol to include developmental background for the new end point. Does this departure from the standard protocol represent research? If the toxic effect is a lag in the time necessary for initial cleavage, is it known that this effect persists throughout the development of the embryo? Is it known whether the embryo cannot continue to develop properly after initial cleavage has been delayed? It wouldn't take very much fundamental information to provide a large measure of assistance in understanding the meaning of the new end point.

   The sensitivity of the end point of fertilization in the Arbacia test has been defined in Nacci, et al. (1985) (mean EC 50 = 12.1 ± 2.7 ug/L; Range = 6.9 to 17.6 ug/L) based on 26 sperm cell toxicity tests with the reference toxicant copper sulfate: The mean EC 50 for the fertilization end point in the Arbacia test is within the range of sensitivities for species used to develop the National Criterion for Copper (EPA, 1985). The data for the fertilization end point in the Arbacia test from this study are available. An analysis of the fertilization end point data would be useful, particularly to lend perspective to the time to cleavage response from the same test.

5. Page 8: Station Differences. The last two sentences of the second paragraph indicate that the response of Champia in the wet season samples should be accepted with caution. What does "accepted with caution" mean? Does it mean that the Champia responses in the wet season samples are ambiguous? Can you explain how and why environmental conditions should vary (i.e., light level too low, page 6) in a laboratory controlled test. Is there a problem with consistent results in the Champia test? Would ambient samples with poor water clarity exhibit a "toxic response" due to the influence of turbidity on light attenuation? Perhaps unclear results from a test

indicate that a conclusion cannot be drawn and that the tests should be rerun. The phrase "accepted with caution" should either be clarified or replaced.

6. If the results of water chemistry analyses performed on ambient test waters are available, the information can be integrated into the reported test findings for correlation of biological response with water chemical constituents. Presentation of chemical analysis would be useful because historical chemical information has lead to an expected geographic distribution of potential bio-effects that is not apparent in the findings of this report. For example, the most recent Battelle (1988-1991) water chemistry analyses have shown the waters of the Arthur Kill to contain greater concentrations of metal pollutants than the waters of the Bight Apex. Yet at high tide (Test Series 7), an expected clean-water station (RB-8 in the Bight Apex) yielded a much greater bio-effect than did the waters of the Arthur Kill. Without water chemistry findings this bio-effect result seems anomalous. Toxicity identification procedures are needed in this study and subsequent studies to link contaminant analyses with biological response before conclusions can be drawn about toxicity.

7. The Arbacia test is considered acceptable (page 250, EPA, 1988) if 70%-90% fertilization of eggs occurs in control chambers. According to the Guidance Manual for Conducting Sperm Cell Tests with the Sea urchin, Arbacia Punctulata, For Use In Testing Complex Effluents (Nacci, et al, 1985; page 17), greater than 90% fertilization may result in masking of toxic response. Can it then be concluded that raw data test results from Test Series 8 (Appendix A) listed on page A-12 represent unacceptable tests because fertilization exceeded 90% in controls and in 10 site water tests? Did the authors reject this acceptability constraint?

8. The principles for acceptance of controls in the Arbacia test and the definition of biological effect seem to allow contradictory results. For example, if the performance control exhibits 90% fertilization and the site-water experimental treatment exhibits 80% of the control fertilization (i.e., a count of 72 fertilized from a total of 100 observed), the site-water response falls within the acceptable response limits of a control (i.e., 70%-90% fertilization). Although the site-water could be judged an acceptable control, it can also be judged a biological response to toxic substances. Have results, as in this example, ever arisen? Shouldn't the definition of biological response allow for at least as much variability in response as the acceptance criteria for controls?

9. Although the tests that were selected were developed for EPA's Complex Effluent Toxicity Testing Program, the tests appear to have a limited field validations record for ambient waters toxicity assessments. The report should contain greater explanation of the history of development and use of the tests, as well as documentation of the test strengths, weaknesses, and record of success in the identification of ambient toxicity and lack of ambient toxicity. Information on the natural history and ecology of the species of aquatic organisms used in the tests would also aid in

understanding the meaning of the tests. For example, can you provide a description of the habitat in which Arbacia punctulata optimally reproduces and grows. Are broods of Arbacia punctulata reared in laboratories that routinely perform the toxicity tests?

10. The EPA National Water Quality Criteria are developed to protect 95% of the tested species (Stephan, et al., 1985; Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses), unless a recreationally or commercially important species is very sensitive. It is reasoned that protecting all of the species all of the time is unreasonable, impractical and unnecessary. Can the author give some sense of where in the spectrum of species sensitivities, where within the 95% of species to be protected, does Champia parvula fit especially with respect to species of metal that have been found to exceed water quality criteria in the Harbor?

Bio-effects toxicity testing with sensitive species is useful and needed in pollution abatement programs nationwide. Large scale broadcast testing in a complex system of water bodies, however, has not been commonly performed. Expert interpretation is required in order to understand and appreciate the ecological implications for the NY/NJ Harbor ecosystem and should be presented in detail in this toxic assessment report.

References

EPA, 1985. Ambient Water Quality Criteria for Copper - 1984. Office of Water Regulations and Standards, U.S. Environmental Protection Agency, Washington, D.C. 20460.

EPA, 1988. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. (C.I. Weber, et al., Eds.). Environmental Monitoring and Support Laboratory Cincinnati, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268. EPA/600/4-87/028, May, 1988.

Nacci, D.E., R. Walsh, and E. Jackini. 1985. Guidance Manual for Conducting Sperm Cell Tests With The Sea Urchin, Arbacia punctulate, For Use In Testing Complex Effluents. In Aquatic Toxicity Testing Seminar Manual, prepared by EPA Environmental Research Laboratory, Narragansett, RI, and Office of Water Enforcement and Permits, Environmental Protection Agency, Washington, D.C.

Stephen, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman and W.A. Brungs (1985). Guidance for Deriving National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. Office of Research and Development, Environmental Research Laboratories, Duluth, MN, Narragansett, R.I., Carvalles, OR.

Philip DeGaetano. P.E.
New York State Department of Environmental Conservation
May 1, 1992

Response to "General" Comments

1. The purpose and scope of the study was made clearer in the introduction.

2. A more detailed explanation behind the reason for selecting *Arbacia* and *Champia* as test species was included.

3. The request for an "ecological diagnosis for the aquatic community inhabiting the estuary and harbor" is beyond the scope of the project.

Response to "Specific" comments

1. Acclimation: The test organisms were reared in the Narragansett Bay control water. There are no significant ionic balance differences in natural marine waters. After our meeting at the Environmental Testing Center last year with EPA and State personnel it was apparent that this question arose out of experience with freshwater testing. Natural freshwaters vary much more in there relative ionic composition than do saltwaters.

2. and 3. Reference toxicant results address more than just species sensitivity. Even if sensitivity is the major cause of the variability, one is only measuring sensitivity to a single compound. The mechanism of toxicity to the reference toxicant may have nothing to do with the unknown toxicants in the ambient waters.

4. An explanation for the "departure from the standard protocol" was added. A plot of first cleavage vs fertilization also was added in the appendices.

5. The phrase "accepted with caution" was omitted. Additional text was added to caution the reader that these tests were not done for the purpose of compliance, but only to characterize the extent and variability of toxicity. Even if the *Champia* data were eliminated, the *Arbacia* data tells essentially the same story.

6. Water chemistry analyses were included.

7. The new version of the *Arbacia* protocol uses >50% as the acceptability requirement for controls (George Morrison, ERL-Narragansett). The primary concern is not to avoid 100% fertilization by manipulation of the sperm to egg ratio. If sperm are too abundant (i.e., more than is required for 100% fertilization), then toxic effects could be masked.

8. The question is asked, "Shouldn't the definition of biological response allow for at least as much variability in response as the acceptable criteria for controls"? On the surface this appears like a logical argument, however, the test protocol artificially attempts to lower the fertilization rate by manipulation of the sperm to egg ratio. All

"toxic" responses must be relative to a control for a given test, not an overall concept of acceptable control.

9 and 10.The statements appears to want to determine whether or not the test species are ecologically relevant to the Hudson/Raritan Estuary. This is not the issue. The purpose was to detect toxicity. Therefore we used the most sensitive measure for toxicity that we have available. The relative position of *Champia* among the "95%" protection for Water Quality Criteria is not relevant.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION II

DATE: MAY 13 1992

SUBJECT: Comments on SAIC Draft Report (Mar 25, 1992), Toxic Assessment on NY-NJ Harbor Waters

FROM: Joel O'Connor

TO: Eric Stern

Overall, this is a rigorous and exceptionally valuable report. If this work is followed up as it should be, it may well be the most socially consequential insight into the Harbor for years. I am not able to give the draft a complete review, but have a few comments on statistical issues.

The argument is made (p 4, para 1) that four replicates do not permit much faith in tests of homogeneity. Precisely right, nor in the t-tests themselves. The (appropriately) few replicates per station make the individual station comparisons less credible than the regional (aggregate station and agggregate control) tests I suggest below. Each aggregate comparison I suggest has enough measurements to provide robust estimates of means and variances, so the assumptions of specific tests <u>can</u> themselves be tested. The legitimacy of statistical tests hinges on these assumptions, particularly the normal distributions of variates in the normal-curve statistics used. That a few percentages exceed 100 (p 4, para 1) is not a reason for testing untransformed percentages. If the arc-sin transform is helpful, it is only necessary to conclude that the 100+% measurements reflect natural variability, <u>not</u> response to waters <u>known a priori</u> to be more toxic than control waters (although not necessarily measurably so). Hence, all 100+% values are most appropriately considered to be 100%. This seems more reasonable than confounding tests with some data values which, superficially, imply that Harbor waters are less toxic than control waters. If you don't like this rationale, there are a wide variety of effective normalizing transformations which don't go wild with values > 100%. Since normal-curve tests are quite sensitive to their data distributions, there is compelling reason to transform distinctly non-normal distributions.

The conclusion that "it did not make any difference" at what depth or when water samples were taken (p 6, para 1; p 7, para 2) should be illustrated by simply including the relevant means from Table 3. As it is, the assertions lack evidence.

On page 6, para 2, l 9: ... less toxic [<u>to Arbacia</u>] overall than ... (Similarly for parallel sentence <u>re Champia</u> on p 7, para 3, l 9.)

What is the rationale for saying that "biological significance" requires that a response be both less than the control ($P < .05$) and <80% of the control response? A rationale does exist for an acceptable alpha error in significance tests (e.g., managers don't want to overlook real effects at more than 1 out of 20 stations). That small differences (5-10%) can be statistically significant (p 7, para 4) does not necessarily make these differences any less significant in other senses. What we are assessing is the toxicity of natural waters. Statistical significance is reliable evidence of toxicity. The issue is no more complicated. Finding "biological significance" in any percentage of control response is (1) arbitrary and (2) implies knowledge of some undefined "biological significance." We neither have such knowledge, nor have forseeable expectations of getting it. That an "80% criterion" (p 7, last para; p 8) was selected, arbitrarily, in two particular programs is very selective reference to authority. Biologists have alleged without evidence that all sorts of percentages have "biological significance," but many acknowledge that, for all we know, statistical significance is biological significance. This conclusion that statistical significance in field observations generally constitutes "biological significance" was argued convincingly some time ago by Buffington, Sharma and McFadden (1980. Assessment of Ecological Damage: Consensus. pp. 25-32, In: Council on Environmental Quality and U.S. Fish & Wildlife Service. Biological Evaluation of Environmental Impacts. Biological Sciences Program, U.S.F&WS, Washington, D.C. (FWS/OBS-80/26).

Perhaps because toxicity tests are generally affordable at only a few points in space, a convention seems to have arisen wherein statistical comparisons are made between the control (reference?) and each test station. Tests of the several hypotheses ('Station Differences,' p 8) are interesting (<u>if</u> they are powerful enough), but they do not test the hypotheses of greatest interest: 'water <u>in each region</u> (e.g., the Hudson R.) is not more toxic than control waters.' This is a distinctly different hypothesis than those tested (in the report) by determining statistical significance of individual 'station differences,' followed by qualitative assessment of these differences within the Hudson R. My suggestion would test for differences between mean response within each region and <u>its</u> mean control value (mean of control values within each region). Also, the bottom line test is that between all station values for the Harbor vs their mean control. Based upon Tables 4-8, I expect nearly all such tests to be significant ($P < .05$). These tests seem to address the most obvious and significant issue: are these waters toxic?

Note that these (and other) tests should use the original variates (# cystocarps/female and freq. devel. to 1st cleavage per 100 indiv.), not percentages of control. Use of the % of control as the test variate (as seems to have been done generally) confounds the tests with unnecessary variance components (variance among control values and covariance of station vs control values), thereby reducing the likelihood of detecting true differences which actually exist. Similarly, the use of original variates is also appropriate for data summaries (e.g., Fig. 6).

The report does not estimate the power (1 - Beta error) of any statistical tests. These tests are easy to do (see any good stat text) and are essential here. Without knowledge of Beta error we have no idea of whether tests not found to be significant result from (1) true absence of measurable toxicity or (2) insufficient sampling effort. These results are important enough to be interpreted rigorously, including probabilistic evidence that sampling was adequate for the purpose intended (as I believe it was). Further, cost effective sampling for the next study requires knowledge of statistical power in the work described.

I doubt that these suggestions for analysis will change the conclusions, although the degree of toxic effects will probably be even more evident. Broadly, the authors have done an outstanding and very difficult job. Few investigators are able to even do this sort of valuable work. We are very fortunate to have the experts working with us.

I presume the final report will be published as an EPA report, and strongly encourage an article in a good journal.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION II

DATE: MAY 1 3 1992

SUBJECT: Additional Comments on SAIC Draft Report (mar 25, 1992), Toxic Assessment on NY-NJ Harbor Waters

FROM: Joel O'Connor

TO: Eric Stern

In my May 13, 1992 memorandum on this draft report, I forgot to make a very important suggestion: the field sampling strategy should be fully described under METHODS. As I understand it, sampling locations were selected probabilistically. This is very important to document because it is the probabilistic nature of sampling which permits statistical tests with minimal concern about selection bias. Without such probability sampling, any statistical tests are suspect and there is no evidence that mean values are unbiased.

Joel O'Connor
U.S. EPA Region II
May 13, 1992 and supplemental comments

1. The issues of normality and homogeneity of variance were more completely dealt with in the methods.

2. The reference to "biological significance" was omitted and replaced with a discussion of statistical power.

3. Statistical analyses for "area" differences were included in Table 4.

4. Statistical analyses were repeated using original data instead of % of control.

5. A paragraph was added to the methods on station selection through "systematic sampling".

DEP

May 12, 1992

FILE

Mr. Eric Stern
U.S. EPA
Water Management Division
26 Federal Plaza
New York, New York 10278

New York City
Department of
Environmental
Protection

Re. NYNJHEP draft report entitled "Toxicity Assessment of NYNJ Harbor Ambient Water

Bureau of
Clean Water

Dear Mr. Stern:

96-05 Horace Harding
Expressway
Corona, New York
11368
(718) 595-5066

As part of our continuing participation in the Estuary program committee, my staff and the NYCDEP consultant (Lawler Matusky and Skelly Engineers, and Cosper Environmental Services) have reviewed the NYNJHEP draft report entitled "Toxicity Assessment of NYNJ Harbor Ambient Water" dated March 25, 1992. As Mr. Tom Brosnan discussed with you, we requested that a copy of the raw data and summary data tables be provided to NYCDEP on diskette, so that we could do some of our own analyses, and perhaps glean additional information from the data. Unfortunately we have not received these data yet. Our comments on the draft report and appendices are as follows.

ALBERT F. APPLETON
Commissioner

Specific

EDWARD O. WAGNER, P.E.
Deputy Commissioner

1) The *Arbacia Punctulata*(sea urchin) short-term toxicity test endpoint as recommended by EPA for use in NPDES testing is percentage of fertilized eggs (EPA/600/4-87/028). To some degree, field validation of the relationship between the results observed with this endpoint to community effects has been conducted. Why was a new endpoint(identification of a first cleavage of the fertilized cell) introduced in this test? What documentation between an observed difference of this endpoint to ecological significance has been established in regulatory or scientific literature? What types of experimental conditions affect this endpoint and what is the variability of this endpoint to several standard reference toxicants with regard to test acceptability?

When using the percentage of fertilized eggs as the endpoint, several of the conclusions drawn about "hits" in Appendix A are not valid(eg. "Dry" Season HR-3, HR-4, HR-5, NB-2, etc.).

Printed on recycled paper

2) As stated in Table 2, five out of 11 *Champia parvula* control tests did not meet the 10 cystocarps per female criteria for accepting the test results. Although such a statement is made within the text, no statement is made in the summary of conclusions with regard to the caution that should be applied in the "hits" observed for these particular tests. Why were sites in runs with poor control results not tested again as in the case of *Arbacia Punctulata* where control percentage of cleavage was considered below a critical level(i.e. Run 6 and 10). Tables 5 and 6 should have a notation for those runs where control response was below acceptable levels but were used anyway.

3) The Reference Toxicant Data as given in Appendix D does not exhibit consistent results(95% C.I. for *Arbacia Punctulata* unfertilized egg endpoint EC50 is 1.6 to 8.6 ug/l which almost covers the span of exposure concentrations[0.8 -10.0 ug/l]).

The *Champia parvula* was tested only twice with the standard reference and neither result agreed(6/13/91, >1.0 mg/l; 11/5/91, ,0.36 mg/l). Reference toxicants are tested to provide precision and validity of toxicity data. How can the validity of the ambient tests be accepted when the standard reference results are not acceptable.

As chronic results are being examined, an IC25, NOEC, and LOEC would be more appropriate to monitor for quality of chronic result rather than EC50.

4) The summary and recommendations made in page 12 of the report do not have the appropriate caution statements included. Before other follow-up studies to this one can be made (Statement 6 of Summary and Recommendations) for the purpose of comparing results to this study, and evaluating "potential for ecological effects" and the "spatial and temporal characterization of toxic contamination", problems noted above (ie. comments 1, 2, and 3) should be resolved (ie. retesting at sites where controls and/or reference results failed). Care should be taken in interpreting this data and making conclusions in regard to "hits" and adverse ecological effects.

5) *Champia parvula* is an alga which is extremely sensitive to copper (NOEC 0.5 to 1.0 ug/l, LOEC 1.0 to 2.5 ug/l of dissolved copper; Chronic value 0.71 to 1.58 ug/l of dissolved copper, EPA/600/4-87/028). The New York State and Federal chronic criteria for dissolved copper is 2.9 ug/l. In the NY/NJ Harbor area studies have found that in general dissolved copper is approximately 2 ug/l or higher (EPA Status Report).

As reviewed in the standard development "a reasonable level of protection will probably be provided if all except a small fraction of the taxa are protected (0.05 of data set used in criteria development, PB85-227049). Several saltwater algae other than *Champia parvula* were tested and the response observed was within the range observed for saltwater fish and invertebrates used to derive the criterion (EPA 440/5-84/031). In comparison to most saltwater organisms *Champia parvula* is more sensitive than most saltwater organisms tested to copper and use of such a sensitive organism could result in too stringent of a criterion for a "hit" for "potential ecological effects".

6) The report should indicate the source and condition of the tested organisms(ie. whether imported from other laboratories or cultured in-house). This may affect the test results.

7) The report should state the condition of the *Arbacia Punctulata*, such as how many individuals had to be stimulated before sufficient quantity of eggs were found. The quality of eggs can be affected by this condition.

8) The figure 2 on page 15 shows that 2 species have an opposite response(distribution) to the dry and wet event samples. Is any associated chemical characterization data available which can explain this behavior?

9) Since brine was used to adjust salinity for samples with low initial salinity(ranging from Tappan Zee to Atlantic Ocean), not all tested samples were 100% ambient water. Results for different stations may not be compared to each other quantitatively. A comparable endpoint(ie. IC10 or IC25) should be adopted and additional discussion on the relationship between observed toxicity and salinity adjustment should be provided.

10) There is no clear picture of toxicity spatially, temporally, and by species presented in this report. Page 11 states that "Based on the results of this study, a toxic evaluation for ambient waters clearly must include a characterization of the variability of the toxicity in both time and space. This characterization will aid in the final design of a sampling strategy." Is this study an ambient toxicity scanning or an early stage task of the final study? What is the purpose of this study?

General

1) Other species (ie. Mysid, etc.), which are routinely used in NY, NJ, CT marine NPDES studies, would be more appropriate to use in the ambient toxicity testing due to large NPDES/SPDES database existing for these species and the resultant ability to compare ambient toxicity versus discharge toxicity. At a minimum, a couple of these species should be included in any harbor testing program.

2) Some of the grouping of stations together seems artificial(e.g. Hudson River = 11 stations from Battery to Tappan Zee). Grouping of stations could be refined using cluster analysis.

3) Evidence of toxicity all over, including some outer harbor sites(e.g. H11 = Tappan Zee, RB8 = Atlantic Ocean) is difficult to believe. The reliability of this data should be checked again.

It is our opinion that the data presented in this report should be carefully re-examined before making conclusions and applying the results for future studies.

This Department is carrying out an ambitious biomonitoring program on its treatment plant effluents at this time. We can appreciate the concern over ambient water toxicity in the harbor. We feel strongly, however, that water quality improvement programs are best served through sound scientific investigation which is well documented.

At this time, much of this work is still in the developmental stage and new to the NY/NJ estuary. Therefore, this data should be thoroughly reviewed and discussed before official release or use by any other programs.

We look forward to seeing a revised draft and if any of these comments require further clarification, please feel free to call me at (718) 595-4974 or Tom Brosnan at (212) 860-9388.

Sincerely yours,

Angelika B. Forndran, P.E.
Chief
Division of Scientific Services

cc: Wagner, Heckler, Brosnan, Tipping, Sartoris, Stubin, Kuang
Mr. Ausubel(USEPA)
Mr. New man, Mr. Kuzia(NYSDEC)
Mr. Apicella(LMS)
Mr. Cosper(Cosper)

WK/BIO/ambient.epa

Angelika B. Forndran, P.E.
New York City Department of Environmental Protection
May 12, 1991

Response to "Specific" Comments

1. A plot of fertilization verses cleavage was added to the appendices. In addition, a better explanation for the purpose of using cleavage as a surrogate for fertilization was added.

2. Unacceptable *Champia* tests were flagged better.

3. Reference toxicant results address more than just species sensitivity. Even if sensitivity is the major cause of the variability, one is only measuring sensitivity to a single compound. The mechanism of toxicity to the reference toxicant may have nothing to do with the unknown toxicants in the ambient waters. An explanation on the limitations and problems with reference toxicant tests has been added in the discussion.

4. While we cannot draw too many conclusions regarding the ecological significance of the toxicity results from this study, we also cannot rule them out because of the emphasis on reproductive endpoints.

5. Although it is true that *Champia* is more sensitive to copper than most marine organisms that have been tested, *Champia* is not universally more sensitive to all chemicals. Copper may not be a major problem in the estuary. A fact that is made all the more clear by using a species very sensitive to copper.

6. Source and condition of organisms noted in methods.

7. Only one spawn, using four animals for *Arbacia*. This has been noted more clearly in the methods.

8. The data is insufficient to explain the opposite response of the two organisms to many of the samples. However, this should not be considered unusual given the expected diversity of chemicals that may be found in the harbor/estuary.

9. IC25s, IC50s, etc. can only be calculated for data from dose response exposures. There is no correlation between initial salinity (i.e., the amount of brine used) and the distribution of toxicity.

10. Maps showing mean responses were added.

Response to "General" Comments

1. More detail for the rationale for *Champia* and *Arbacia* has been added to the introduction. In addition, mysids and fish are not sensitive enough to detect the expected levels of heavy metals that may have been present in the samples.

2. The grouping of the stations was pre-determined by EPA.

3. The text make more clear the fact that many stations were "not toxic" even though very sensitive species were used.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION II

DATE: MAY 13 1992

SUBJECT: COMMENTS ON DRAFT REPORT-TOXIC ASSESSMENT ON NY-NJ HARBOR WATERS

FROM: Richard Spear, Chief
Surveillance and Monitoring Branch

TO: Mario DelVicario, Chief
Marine & Wetlands Protection Branch

A draft report entitled, "Toxic Assessment of New York - New Jersey Harbor Ambient Waters" was prepared by Science Applications International Corporation. Grab samples from 51 stations throughout the New York - New Jersey Harbor area were collected during July 1991 and January 1992. Arabacia punculata (sea urchin) and Champia parvula (macroalga) were used to determine baseline toxicity of the samples. Chronic endpoints, cleavage (cell division after fertilization) in the sea urchin test and reproduction (formation of cystocarps) in the macroalga were measured in the test samples against laboratory controls of natural seawater and diluted brine. Testing was performed under guidelines from the EPA manual, "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms (EPA/600/4-87/028). Statistical differences relative to performance controls were determined for each of the samples using a one-way, un-paired t-test (alpha 0.05). Variability from spatial and seasonal sampling considerations was also considered.

The objectives outlined by the project are commendable. Much needed baseline toxicity data for the New York - New Jersey Harbor Area were collected. However, there were a number of concerns regarding the collection, evaluation, and interpretation of the data.

1. The test endpoint measured and statistically evaluated in the sea urchin fertilization test was cleavage. Cleavage is the initial mitotic division following fertilization. EPA sea urchin test method 1008 defines fertilization as the formation of a fertilization membrane around the single celled zygote. A 20 minute exposure of gametes is prescribed. This exposure period allows for an adequate exposure for fertilization to occur. The laboratory observed a 70 minute or greater exposure of gametes to allow for cleavage to occur. The tests were terminated when cleavage was observed to have occurred in the controls only. The report indicated that inherent variability of time between the fertilization stage and cleavage stage occurred. Test results may be misleading because we can not predict if additional time would have resulted in greater cleavage of test samples. The 20 minute exposure of gametes prescribed in the EPA methods was selected in part because fertilization, if it would occur, would happen within this time period in any sample. Due to the obvious variability of time to first cleavage, ending the test only when the controls had sufficient time to cleave may have affected test results.

2. The data, expressed as percent of eggs which cleaved, were not transformed. Interpretation of percentage data is typically arc-sine square root transformed to stabilize the data variance and normalize the results. A total of 100 eggs was examined for fertilization and cleavage for each test sample and expressed in terms of control fertilization success (ie, 74% of control cleavage, 95% of control cleavage). For test samples which supported fertilization and cleavage greater than the control (>100% of control), arc-sine square root transformation could not be defined. The data could have been transformed. This data transformation could have been accomplished by evaluating the raw percentage data (not expressed in terms of control results). This method of data evaluation is recommended in the EPA guidance manual.

3. Typical control fertilization success is between 70-90% for the sea urchin test. The natural seawater control for the successful tests ranged between 88.3 and 97.5%. The laboratory should be commended for such successful organism and control responses. There were situations where fertilization in the test samples was over 90%, but a significant difference was measured. In an effort to qualify these situations, the laboratory distinguished between statistical significance and biological response. A significant biological response is defined as a test sample response which differed from the control response by more than 20 percent. The 20 percent criterion for biological significance is proposed in the EPA/ACE dredged material guidance manual (Green Book) which considers sediments toxic if survival relative to a reference sediment in less than 80 percent. Based on the situation described above (test samples with over 90 percent fertilization success showing a statistical significance), the use of this type of criteria is useful. However, the strict adaptation of the criteria from another test type may be not be appropriate. The dredged material guidelines use acute toxicity as an endpoint, a different test matrix (sediment), and different test organisms. Also, the EPA/ACE criteria are based on a reference sample. The role of a reference sample versus a performance control may be very different. In future studies, if an appropriate site can be located, the use of a reference station may yield valuable information for toxicity tests of samples collected in the NY/NJ harbor area. The EPA test method for the sea urchin fertilization test allows up to a 30 percent failure in control fertilization without invalidating the test results. The use of a biological significance criteria is a helpful tool for this type of data interpretation. A modification or relaxing of the criteria for the sea urchin and macro alga test may be needed to allow for a more appropriate biological significance criteria.

4. There were a number of occasions where inadequate control response would limit the usefulness of the data. For the marine macroalga tests, the natural saltwater control did not produce the minimum test acceptability response for cystocarp formation in 6 of the ten test series conducted (on three occasions, the brine control data were substituted for statistical analyses).

Also, insufficient control response occurred in one test series using the sea urchin. These issues could directly affect the quality of the data and the degree of confidence in the test results.

a. Over one half of the test series from the macroalga testing (approximately one-half of the test samples) had controls which did not produce the required number of cystocarps. EPA requires an average of 10 cystocarps in the control. In the affected tests, cystocarp formations ranged between 6.3 and 9.9. Interpretation of results of these tests is difficult to properly evaluate. Especially when you consider that following the 48 hour exposure period, all organisms (including the organisms exposed to the test samples) were incubated for up to 9 days in the control water (EPA has found a 5 - 7 day recovery period as adequate). Also, for test series 2, 3, and 4 control data from the brine control were substituted for t-test comparisons. This substitution is not scientifically valid. The major problem with attempting to qualify these types of single concentration tests are that there is only one test concentration per station with no dilutions. Therefore, dose-response relationships can not be determined. The laboratory indicated insufficient lighting contributed to the poor control response. This situation alone can be a source of great variability of results.

b. When control cystocarp production is less than 10, the statistical capabilities are compromised. There is a much greater chance of measuring a statistical difference for these types of data.

c. In test series 11 from the sea urchin testing and test series 7, 8, 9, and 10 from the macroalga testing, insufficient control response in the brine control was documented. Brine was used to adjust the salinity of most of the test samples. The amount of brine added was dependent on the ambient salinity. These occurrences could affect test results and should be noted in the report.

5. There was high variability among the test replicate response throughout many test series which resulted in exclusion of these data points from statistical analyses. The reporting laboratory did not specify if formal statistical analyses were employed to determine the outliers or if the data were excluded based on qualitative judgements. There appeared to be a number of additional data points which had high variability among the treatment replicates which were not excluded from statistical analyses. In general, all treatments from the sea urchin test with a standard deviation of 15 or above or 4.0 and above in the macroalga testing may possibly contain additional outliers.

There were other items related to variability. On at least two occasions, a significant effect was indicated for macroalga test samples which had a greater number of cystocarps than non-statistically different test samples which had fewer number of cystocarps (Test series 7, NB-1, NB-3, NB-4; and Test series 9, ER-3, ER-6). These occurrences may be transcription errors or in the case of Test series 7 results for the NB station, related to response variability among the replicate treatments.

6. In the Results and Discussion Section of the report, it was stated that there was a 20% change in response in Hudson River samples collected in the "Dry" versus the "Wet" season sampling events. It was concluded that the time of sampling may highlight variability of toxicity. This general statement can not be disputed. However, the variability observed, either all or in part, could be attributed to variability of organism response.

7. Quality assurance data for Champia parvula consisted of two reference toxicant tests. The IC50 (median inhibition concentration) value was not bracketed in one test. Trend deviations (more cystocarps in higher toxicant concentrations) and poor dose-response relationships were evident in both tests. The ability of the laboratory to obtain precise and consistent results for the test method was not demonstrated with these data.

Overall, the report yielded some valuable insights. There appear to be some limitations of the data which preclude definitive assessments of toxic effects. Continued toxicity characterization of this system is strongly supported. Please call me (FTS 340-6686) or Jim Ferretti of my staff (FTS 340-6728) if you have any questions or comments.

cc: E. Stern, WMD-MWPB
R. Braun, ESD-SMB
E. Kuzia, NYSDEC

Richard Spear
US EPA Region II
May 13, 1992

1. First cleavage was essentially used as a surrogate for fertilization in order to make the endpoint clearer. A plot show the close relationship between fertilization and first cleavage has been added to the appendices.

2. Statistics were run on the original data. Data were not transformed because the original data met the test for normality and *t*-tests assuming unequal variances were used.

3. "Biological significance" has been replaced with "detectable significance" which is another name for statistical power.

4.A better explanation of the limitations due to control variability has been added. In addition, all statistical analyses used pooled controls. There was no correlation between the amount of brine added and the toxicity of samples.

5. Very few data were designated as outliers. The power curves used for detectable difference incorporate the variability of the data in the decision for hits.

6. Reference to the limitations in data interpretation due to organism response has been added, particularly with regards to "detectable significance". Detectable significance incorporates the variability of the organism's response among different controls as well as among different replicates.

7. There were obvious problems with the *Champia* reference toxicant tests. Since they were not run simultaneously with the toxicity tests we can't draw any conclusions about the variability in sensitivity (or lack there of) in *Champia* over the time period covered by the study.

FAX Communication
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
FAX: (508)457-2172

29 May 1992

Mr. Eric Stern
U.S. Environmental Protection Agency
Region II
Jacob K. Javits Federal Bldg.
New York, NY 10278
FAX (212)264-4690

Dear Mr. Stern:

I apologize for my late review of the draft report entitled "Toxic Assessment on New York - New Jersey Harbor Ambient Waters". I have been overwhelmed with review requests during the past two months (over 50 at last count) and I've tried to give each a thorough review rather than a quick or cursory one. If you have any questions, please do not hesitate to contact me.

Sincerely,

Judith McDowell Capuzzo

Judith McDowell Capuzzo
Senior Scientist
Biology

Review: "Toxic Assessment on New York - New Jersey Harbor Ambient Waters", submitted by SAIC, Narragansett, RI

This document is a fairly straight-forward summary of bioassay experiments assessing water quality for the New York - New Jersey Harbor area. There are some editorial or style changes that are needed but the data presentation and analysis are appropriate. The conclusions are sound and reflect a careful analysis of the data.

Specific corrections are as follows:

page 1, line 24 - "...signed in onto a log-in sheet..." - Awkward phrasing.

page 1, line 27 - "...hypersaline natural-seawater brine" - How was this prepared? How did salinity adjustment affect toxicity in preliminary experiments?

page 2, line 29 - delete "also".

page 4, first para - I question the use of the word "faith" as a synonym for "power" in line 4; line 7, should be "data were". If arc-sin transformations presented a problem for values greater than 100%, were log transformations performed?

page 4, second para - The first sentence is confusing.

page 5, first para - This paragraph appears to be misplaced in this section and should be included in the methods section. Line 9 - what is "hide tide", does the author mean high tide, in which case the sentence is confusing?

page 6, line 1 - insert %.; line 5 "consist" should be "consistent".

page 7, line 15 - Should be "... 50% or less".

page 8, line 19 - delete "in" in phrase "... were in found".

Judith McDowell Capuzzo
Woods Hole Oceanographic Institution
May 29, 1992

Only addressed typographical errors.

M E M O R A N D U M

To: Eric Stern

From: Sarah Clark SC

Date: May 4, 1992

Subject: Peer Review of HEP Ambient Toxicity Report

cc: Eugenia Flatow
Seth Ausubel
Kit Kennedy

VIA FAX

Attached is a review of the HEP Ambient Toxicity Report done by Doug Rader, Ph.D. of EDF's North Carolina office. This review is submitted on behalf of the CAC. Doug is a marine biologist and was formerly head of the Tar-Pamlico Estuary Progam. I hope it is useful in your evaluation of the report and its findings.

Post-it™ brand fax transmittal memo 7671 | # of pages ▸ 3

To Eric Stern / Seth Ausubel	From Sarah Clark
Co.	Co.
Dept.	Phone #
Fax #	Fax #

MEMORANDUM

To: Sarah

From: Doug

Date: May 4, 1992

Subject: NY/NJ Toxicant Assessment Review

Here goes, quick and dirty:

1) This data set has an amazingly high frequency of toxicity, for both organisms at both sampling times. Given the hit and miss nature of such tests, it is absolutely remarkable that 90% of samples taken from a waterbody are shown by biological testing to be toxic.

2) The data really aren't presented in a way that allows assessment of "wet" versus "dry" effects. I grant the winter is, on average, higher flow than the summer, but local conditions and weather conditions during the time immediately preceding the sampling period can have much greater effects (particularly on toxicants that are normally particle-associated or flocculate in marine environments). The specific rainfall history for the period immediately preceding sampling should be presented, along with information on seasonal rainfall data. It is impossible to compare effects based on putative differences in rainfall without some information related to rainfall (much less compare interspecific differences in response to "wet" and "dry" effects.) Also, sampling only twice necessarily compounds the wet versus dry effects with spatial differences -- "slugs" of contaminated water could be at any point in the dispersal process.

3) The choice of organisms is interesting: an alga and an herbivore, both with reproductive stages relatively sensitive to pollution. But, such tests may well underestimate certain toxic impacts of NPS-mediated pollutants, particularly for particle-associated/adsorbed metals and their effects on benthic suspension and deposit feeders. I haven't really looked at your NPS metals data, but urban runoff commonly has much of the total toxic metal in particulate form or adsorbed to sediment particles. Such material, of course, is still available to deposit feeders and suspension feeders when suspended, and can be released with changing dissolved oxygen concentrations at the sediment/water interface. Thus, your astounding results probably underestimate the toxic effects of NPS.

4) An assessment of the normalization of salinity should be presented: volumetric adjustments, total salinity changes required, etc. I would be surprised to learn that changing salinities does not effect the toxicity of the metals involved. (Certainly, hardness does; LC50's used to derive water quality standards for metals are different for marine and fresh waters.) Also, changing salinity may well affect the chemical behavior of some of the materials (bioavailability, etc.), either through pH changes or some other mechanism. Analysis?

5) I would have prefered to see a brackish water organism used in the tests to complement the marine ones (e.g., mysid shrimp). Tests should include both

acute and chronic toxicity tests where the protocols exist. I would also like to see test conducted on benthic deposit feeders!

6) The paper should include enough background about the toxic consitituents of urban runoff and which materials affect the tests used. That is, are the tests used sensitive to the toxicants present, and if so, which ones? Are there materials likely to be present that are not active in these tests?

7) Some of the statistics leave a bit to be desired. It is dangerous to equate lack of statistical significance in variables or in interaction terms in parametric ANOVA's with lack of importance of the actual effects themselves (p. 6, p. 7). It is not necessarily the case that it "would make no difference when or where to sample at a given station." It is absolutely the case that changing near-bottom d.o. regimens affect the solubility of metals and nutrients; when such events were underway, very significant effects would be expected with respect to sample position (in time and space). I also find the difference drawn between statistic significance and "significant toxicity" a little troubling (and, in fact, a strange reversal of the application of statistical significance used above). I would like to see the biological basis for the 80% figure (as opposed to the bureaucratic basis presented in the paper).

8) Does one expect that the two species should react similarly to all toxicants? (p. 8) Most toxicants? Some toxicants? Which toxicants? Frankly, an r-squared of 58% is high -- that means that roughly 60% of the variability in toxicity to urchins during the wet season is "explained" by variability in toxicity to seaweed. That sounds interesting to me, unless there is some reason to expect that the correspondence should be higher. (The reason given for "only" 0.58 is plausible.)

9) I don't know what to make of the relatively reduced toxicity in some portions of the Hudson River (the middle section, overall). I suspect some more complicated things are going on. For instance, Champia showed an almost monotonic decrease in toxicity down the river during the preliminary sampling -- a strange result, unless something related to salinity adjustment is also going on. I suspect that the sampling regimen is not nearly complicated enough to assess spatial or temporal effects.

Thanks for sharing this interesting data!

Doug Rader, through Sarah Clark
EDF in North Carolina
May 4, 1992

1. The text make more clear the fact that many stations were "not toxic" even though very sensitive species were used.

2. Wet verses dry designations have been replaced with dates.

3. The possibility of underestimating toxicity due to particle bound materials was not addressed.

4. There were no patterns in toxicity relative to initial salinity.

5. The rationale for selecting *Champia* and *Arbacia* was made more clear.

6. The text makes more clear the fact that heavy metals were initially presumed to be the major cause of toxicity in the estuary.

7. The phrase "if makes no difference" was changed to be more specific. Also, "biological significance" was replaced with statistical power.

8. The part of the report was modified, the direct correlation analyses were removed as not relevant.

9. Sampling regime was not complicated enough to tests for all possible issues that may have arose. That is one of the main problems with having to cover such a large area with limited funds. Definitive answers to small scale differences suffer.

Daniel Salvito with comments from Ben Parkhurst
Public Service Electric and Gas Company
April 30, 1992

1. The "confusing style" should be less so in the current version.

2. "Toxicity for toxicity sake" was precisely the purpose of the study. The purpose was not to determine compliance with any current or future standards, but to characterize the extent and variability of toxicity. Therefore, one uses the best measurement available to determine that toxicity.

3. The "acceptance" of the *Champia* data in this case adds additional information to the variability of the toxicity data. It would not be accepted if the purpose was to determine compliance.

Western Aquatics, Inc.

ENVIRONMENTAL SCIENTISTS

P.O. Box 846
Laramie, Wyoming 82070
Telephone (307) 742-7624
FAX 307-742-0818

Executive Park, Suite 220
1990 Highway 54
Durham, North Carolina 27713
Telephone: (919) 544-9404
FAX. 919-544-9453

VIA FAX
April 30, 1992

Mr. Dan Salvito
PSE&G
P.O. Box 570
80 Park Plaza
Newark, NJ 07101

RE: Comments on "Toxic Assessment on New York-New Jersey Harbor Ambient Waters"

Dear Dan:

Although I whole-heartedly support the use of ambient toxicity tests to monitor ambient water quality, my major comment on this study is that these data by themselves have little ecological or toxicological significance. That is because (1) we do not know the relationship between these toxicity test results and effects to the biological communities that inhabit these waters, and (2) we do not know the chemical causes of the toxicity.

To remedy this situation, I would recommend (1) that an attempt be made to correlate these data with existing ecological data for the various sites for benthos, plankton, fish, etc., and chemistry data to see if there are any significant relationships among toxicological, chemical, and biological data, and (2) that, for sites where significant toxicity was found, toxicity identification evaluations (TIEs) be conducted to identify probable chemical causes of toxicity.

In contrast to EPA's freshwater toxicity tests, EPA's Complex Effluent Toxicity Testing Program has not conducted any studies to determine the relationship between saltwater ambient toxicity test results and effects to resident biota. Although I am not familiar with the other components of this study, possibly such data already exist or are being collected that would allow such comparisons. Without knowledge of such relationships these data have little practical use. If this study is continued, long-term trends in water quality also could be established that could be related to trends in biological communities. A problem for doing TIEs with these species, however, is that TIE methods do not yet exist.

It would be helpful to know the cause of the toxicity so that probable sources could be identified. Such data would be useful to the EPA in evaluating plans to reduce toxicity and improve aquatic life. For example, are the chemicals causing toxicity present in contaminated sediments, present in municipal or industrial wastewaters, or were they derived from non-point sources? Such knowledge would be extremely useful for evaluating

the likely effectiveness of harbor clean-up plans.

Control variability was quite high. The copy I reviewed did not have the appendices with reference toxicant data. Possibly, reference toxicant control data could be compared to control data to see if some of the variability could be explained by variations in sensitivity or health of the animals.

Reasons for accepting *Champia* data when acceptance criteria were not attained appeared to be subjective. Why have the criteria if you are not going to apply them objectively?

Finally, the objectives of the study were not clearly stated and need to be so stated. The objective appears to have been to measure toxicity for toxicity's sake. Hopefully, there is more to it.

Sincerely,

Ben Parkhurst

Benjamin R. Parkhurst, Ph.D.

PSE&G Public Service Electric and Gas Company

80 Park Plaza, Newark, NJ 07101 / 201 430-7000 MAILING ADDRESS / P.O. Box 570, Newark, NJ 07101

To: Eric Stern

CC: Eugenia Flatow
Anne Galli
Ben Parkhurst

From: Daniel Salvito

Subject: Review of "Toxics Assessment on New York-New Jersey Harbor Ambient Waters"

Date: April 30, 1992

As requested I have completed my review of the draft paper. I had also sent a copy of this paper to Dr. Benjamin Parkhurst. Dr. Parkhurst is an aquatic toxicologist with Western Aquatics, Inc. of Laramie, Wyoming and has many years of experience and publications in this field. I hope that you find both of sets of our comments useful.

Overall I find the author's style to be rather confusing. For instance, in the introduction the author refers to an earlier "screening" biomontoring. Is this the same preliminary run referred to on page five? On page seven, the author makes reference to an "80% criterion for biological significance". This is derived from a species different than those used in the study and used for sediment tests not for water column tests. Is it appropriate to apply this criterion to the toxicity tests performed in this study? And if it is appropriate, it should be presented more clearly with an explanation as to why it is appropriate.

It is also unclear, from reading the text, as to what conclusions the author is actually trying to draw. Upon the author's review of the data, he concludes that there is observed toxicity. This is true; however, the author attempts to make correlations between different sampling points and different sampling times (both seasonally and throughout tide cycles). These correlations do not appear to be conclusive nor well drawn. For example, on page nine, after discussing a variety of station differences, the author combines all the data for both species and both seasons and arrives at the conclusion that "approximately 90% are biologically toxic". This conclusion is made after going through some explanation as to the inconsistent relationship between "which stations were toxic in the dry season and which were toxic in the wet season" (page 8). Why is there an attempt to mingle all the data? This needs to be better explained.

As Dr. Parkhurst states in the attached comments, it is important not to "measure toxicity for toxicity's sake" but, to attempt to define trends in the data and sources of the toxicity. By following the seventh recommendation made in the paper, it should be possible to draw these sorts of conclusions.

Thank you for the opportunity to contribute to this process. Should you have any questions, please call me at (201) 430-8223.