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SURVEY OF TOXICITY IN AMBIENT WATERS OF THE HUDSON/RARITAN ESTUARY, USA: IMPORTANCE OF SMALL-SCALE VARIATIONS

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Abstract—This study was part of a characterization of the nature and severity of water-quality problems in the Hudson/Raritan Estuary in New York State and New Jersey, USA. The toxicity of ambient water was measured at 51 stations in the estuary by using standard tests with the sea urchin *Arbacia punctulata* and the marine red alga *Champia parvula*. Toxicity identification evaluations on samples from two stations suggested that cationic metals were the source of the observed toxicity. Overall results showed that toxicity could vary as much on the small scale, i.e., with depth and tide at a single site, as over several stations within a given subarea of the estuary. Thus, knowing about small-scale variations in toxicity is essential to understanding the significance of the variations from different areas or different sampling events.

Keywords—Ambient water toxicity *Arbacia* *Champia* Hudson Valley, USA

INTRODUCTION

This study was part of a plan to document the nature and severity of water-quality problems in the Hudson/Raritan Estuary (New York State and New Jersey, USA). A significant component of this plan was characterizing the spatial and temporal variations of toxic contamination. The toxicity of water samples collected throughout the estuary was measured during summer 1991 and winter 1992. Follow-up samples were taken on smaller spatial scales in summer 1993 and spring/summer 1994. The main focus of the sampling and testing was to compare small-scale and broad-scale variability in toxicity. Sampling stations were selected to avoid known point sources of pollution. Thus, the results reflected general water quality.

One important consideration was choosing the toxicity tests. The preferred technique for determining how the toxicity was distributed in the estuary was to assess effects on early life stages, primarily in sensitive species, with standard ambient-water toxicity tests. The tests selected were sexual reproduction of the marine red alga *Champia parvula* and fertilization of the sea urchin *Arbacia punctulata*. These tests offer several advantages, and both are part of the U.S. Environmental Protection Agency's whole-effluent toxicity-testing program and use discrete samples [1]. (Other marine test procedures, such as those with mysid shrimp and fish, require media changes.) Testing discrete samples characterizes the variability of toxicity in more detail; both tests (especially that for *A. punctulata*) are rapid, and both species are sensitive to heavy metals, which were suspected of being significant causes of toxicity in the estuary; finally, these two tests are less expensive than tests with mysids or fish. This factor was significant because of the large number of samples required to deal with the broad temporal and spatial scales of this study.

Water samples were taken in seven sampling events. The first event had two objectives, which were to estimate the small-scale variability of toxicity at four locations that represented historical maxima in the estuary and to select the most representative depth and time within a tidal cycle (if any) during which to collect all future samples. The objective of the next two sampling events was to collect water-column samples throughout the Hudson/Raritan area. The last four sampling events were part of a follow-up toxicity identification evaluation (TIE). The TIE data confirmed that, at least for the two stations tested, heavy metals probably caused some of the toxicity. Data from the earlier events showed the importance of characterizing small-scale variability when interpreting broad-scale variations in toxicity.

METHODS

Sampling

Water was sampled at the sites shown in Figure 1. Preliminary samples were collected at South Piermont Pier (Station HR11), Newark Bay Turning Basin (Station NB2), Raritan Bay (Station RB6), and south of the George Washington Bridge (Station HR6). Nine samples were collected at each of these stations from June 19 through 25, 1991, three times at slack tides (two high, one low) in a single tidal cycle at three depths (1 m below the surface, middepth below the pycnocline, and 1 m above the bottom). The remaining samples were taken only at 1 m below the surface during high tide. All 51 sites were sampled from July 20 through 23, 1991, and again during January 8 through 11, 1992. Limited samples were taken (via helicopter) at selected stations on August 23, 1993 (15 stations), September 7, 1993 (nine stations), and March 30 and July 19, 1994 (two stations each date, 10 samples for each station during 1 h). Multiple samples from each station were

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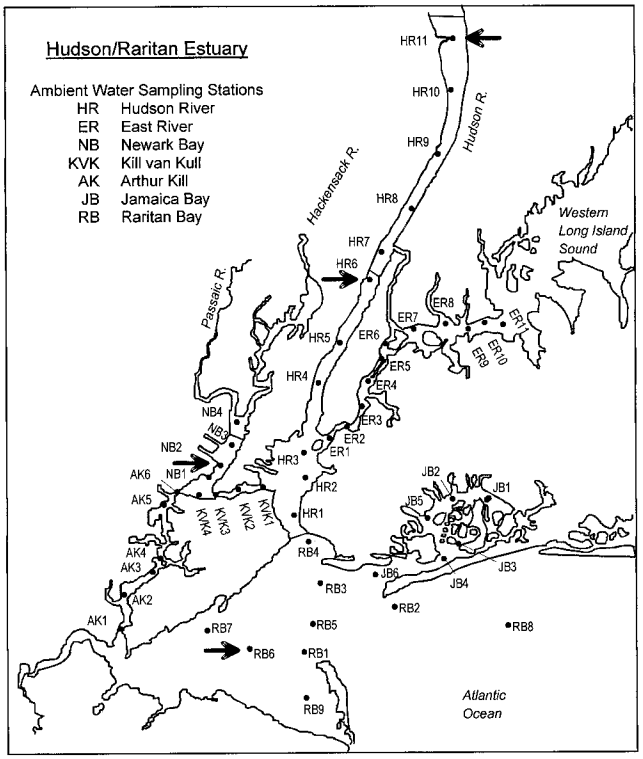

Fig. 1. Hudson/Raritan Estuary (New York State and New Jersey, USA) and sampling stations. Arrows show the four stations sampled during the initial small-scale sampling event of June 1991.

taken to maximize the probability of finding toxic samples on which to conduct TIEs.

Most water samples were collected with a rosette-mounted 30-L General Oceanics Teflon®-lined Go-Flo bottle (General Oceanics, Miami, FL, USA). A 10-L Teflon-lined Niskin bottle (General Oceanics) was used for stations in Jamaica Bay and upper Newark Bay (New York State and New Jersey, USA) and for the TIE samples. To minimize cross-contamination between stations, samplers were rinsed with 10% HCl and then with deionized water. Samples were withdrawn into 1-gallon, labeled cubitainers previously rinsed twice with sample water and were stored in the dark at 4°C on-board the vessel. Samples were transferred to the testing laboratory (Science Applications International Corporation Environmental Testing Center, Narragansett, RI, USA) on ice in coolers.

Measuring toxicity

Toxicity tests began within 48 h after the samples were collected. The salinity of the water samples was adjusted, if necessary, to 28 to 30 ppt with hypersaline brine. Tests for *A. punctulata* and *C. parvula* followed U.S. Environmental Protection Agency procedures for complex effluents [1] except that development to first cleavage endpoint was used for the sea urchins in all but the TIE samples. (Development to first cleavage is easier to identify than fertilization.) Test procedures for the TIE samples used the standard fertilization endpoint for the sea urchin. The endpoint was fertilization instead of first cleavage in order to minimize the delay between determining initial toxicity and selecting which samples to subject to the Phase I TIE.

The sperm-cell test on the sea urchins began by exposing their sperm for 1 h to 5 ml of sample or control water in each of three replicate 20-ml glass scintillation vials. Eggs were then added and the exposure continued for an additional 20 min for the standard sperm-cell test or an additional 70 to 80 min for first cleavage. At the end of the exposure, adding 1 ml of 10% buffered formalin in seawater ended the test. One hundred individuals were examined and scored as not fertilized, fertilized, or developed to first cleavage (when included).

Tests with *C. parvula* began by adding five female branch tips (7–10 mm long) and one male branch (1.5–2.0 cm long) to each exposure flask. Tests were conducted in 125-ml borosilicate Erlenmeyer flasks containing 100 ml of sample or control water. Males and females were exposed together for 2 d, followed by a recovery period for the females of up to 9 d for cystocarp development (evidence of sexual reproduction). Exposure chambers were hand-swirled twice a day. At the end of the recovery period, the numbers of cystocarps per female were counted. The results from each of the four replicates are reported as the mean number of cystocarps per plant.

Toxicity identification evaluations

The toxicity identification evaluation (TIE) methods used procedures described by the U.S. Environmental Protection Agency [2]. Only Phase I TIEs, which look for differences in toxicity before and after physical and chemical manipulations of the sample, were used for characterizing the causes of toxicity. If significant toxicity was observed in the initial tests on a series of samples, subsets of the samples were subjected to a complete Phase I TIE, which used only *A. punctulata.* In the complete TIE, subsamples were aerated to minimize or reduce the effects of oxidizable or spargeable components. Other subsamples were passed through a C18 solid-phase extraction column to remove nonpolar organic compounds, surfactants, and some metals. Ethylenediaminetetraacetic acid was added to separate samples by making cationic metals less bioavailable. Some subsamples were filtered to 0.45 μm to remove or minimize the toxic effects of suspended solids or components bound to them. The pH was adjusted to 7, 8, and 9 in additional subsamples to see whether the toxicity depended on pH. Sodium thiosulfate was added to a final subset of samples to eliminate oxidative species such as chlorine, bromine, iodine, ozone, chlorine dioxide, copper, and manganese.

Data analysis

Because the untransformed data met the test for normality, they were analyzed without being transformed. The data for *C. parvula* were expressed as absolute counts. The data for *A. punctulata* were expressed as percent fertilization or percent development to first cleavage. When data for natural seawater and brine controls were both used, they were pooled. From the data for *A. punctulata* and *C. parvula* at the four stations during the June 1991 sampling event, we estimated the components of variance attributable to station, time of sampling, and depth of sampling.

Plots of the residuals for *A. punctulata* and *C. parvula* were used to compare the variabilities in the estuary, in an area, and at a station. Residuals are a convenient way to normalize variability to differences in means. Residuals for the entire estuary were calculated by subtracting its overall mean from the mean value for each of the 51 stations. Calculating separate sets of residuals for each of the two large-scale sampling events removed seasonal effects. Station residuals were estimated by calculating residuals for each of the four stations from the June 1991 preliminary sampling event by using the overall mean for all 36 samples.

Table 1. Summary of toxicity tests for the June 1991 preliminary assessment of within-station variability for the Hudson/Raritan Estuary (New York State and New Jersey, USA). Samples were collected 1 m below the surface (surface), just below the pycnocline (middle), and 1 m above the bottom (bottom). Samples were taken sequentially during a slack high tide, a slack low tide, and the next slack high tide

Station	Tidal stage	*Arbacia punctulata*			*Champia parvula*		
		Surface	Middle	Bottom	Surface	Middle	Bottom
HR-6	High 1	97	94	85	91	85	82
	Low	94	88	77	91	74	63
	High 2	91	84	90	54	58	46
HR-11	High 1	45	69	63	101	88	83
	Low	91	78	91	87	86	81
	High 2	89	59	83	81	79	57
NB-2	High 1	99	99	101	68	83	102
	Low	98	100	100	78	86	88
	High 2	100	101	99	80	101	89
RB-6	High 1	64	82	58	48	59	76
	Low	69	78	84	83	72	76
	High 2	81	74	65	61	58	63

RESULTS

Toxicity to both species in the June 1991 small-scale sampling (Table 1) varied considerably but was not linked in any obvious way to time of sampling or depth of water. There was also no consistent relationship between the toxicity of a sample to *C. parvula* and to *A. punctulata* (i.e., the fact that a sample exhibited some toxicity to one species was a poor predictor of toxicity to the other). Table 2 shows the components of variance estimated for the factors of the June samples for *A. punctulata* and *C. parvula.* Small-scale variability for *A. punctulata* was not as important as among-station variability. *Champia parvula* was the opposite, i.e., variabilities with tide and depth were at least as large as variability among stations.

Table 2. Estimated components of variance from the June 1991 sampling event for the Hudson/Raritan Estuary (New York State and New Jersey, USA)

Cause of variance	*Arbacia punctulata*	*Champia parvula*
Stations	147	33
Tides	29	99
Depths	0	38
Error (unexplained)	81	61

The toxicities to the two species were just as unrelated during the two broad-scale sampling events as during the preliminary June samplings (Figs. 2 and 3). The data for *A. punctulata* show that water quality improved significantly from July 1991 to January 1992 in four of the seven areas, those areas being the Hudson River, East River, Kill van Kull, and Raritan Bay. But the water generally became more toxic to *C. parvula* in January 1992, although the toxicity in some parts of the Hudson River and Raritan Bay had decreased significantly relative to July 1991.

The total variability (expressed as residuals) during June was similar for both *A. punctulata* and *C. parvula* (Figs. 4 and 5, respectively). As stated earlier, most of this variability for *A. punctulata* was among station, whereas tides and depth contributed substantially for *C. parvula.* Variability in toxicity among all stations was greater in July than January for *A. punctula* (Fig. 4) but was more-or-less reversed for *C. parvula* (Fig. 5).

Additional samples were taken in 1993 and 1994 and tested on *A. punctulata.* (Only a few stations were sampled from each area in August 1993, and not all areas were sampled in 1994.) None of the samples from 1993 were toxic to *A. punctulata* (Table 3). At the two 1994 stations (ER5 and NB1), all 10 replicate samples from March were toxic to *A. punctulata.* In July, however, almost none of the 10 replicates were toxic (Table 3).

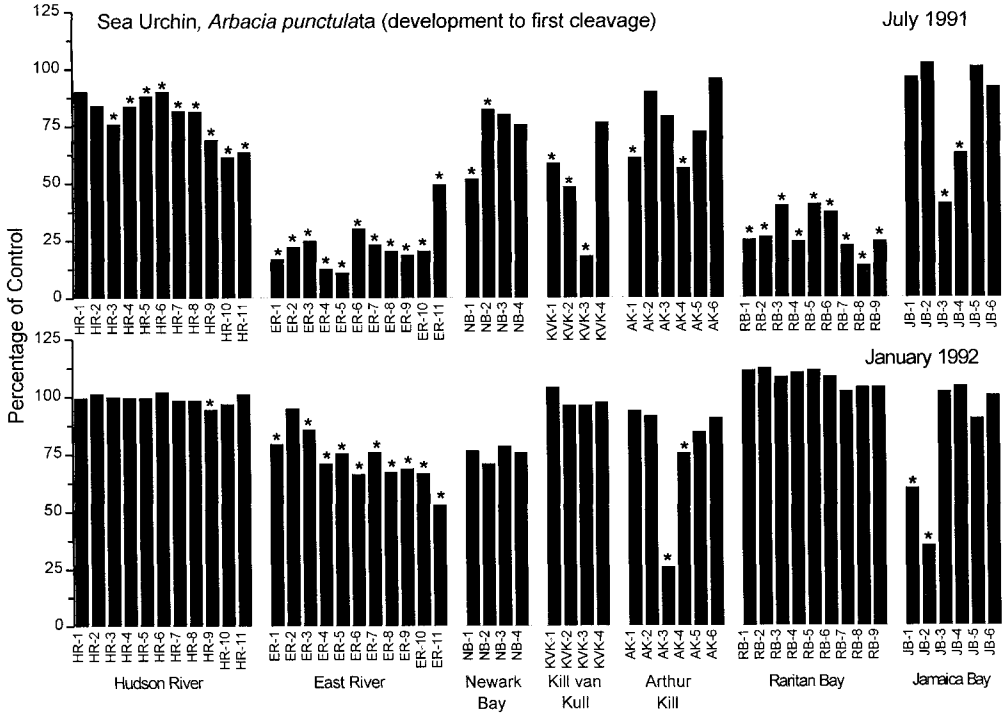

Fig. 2. Results of toxicity tests on the sea urchin *Arbacia punctulata* from the broad-scale sampling events in July 1991 and January 1992. Asterisks indicate samples statistically below their respective performance controls. All samples were taken at the surface during slack high tide.

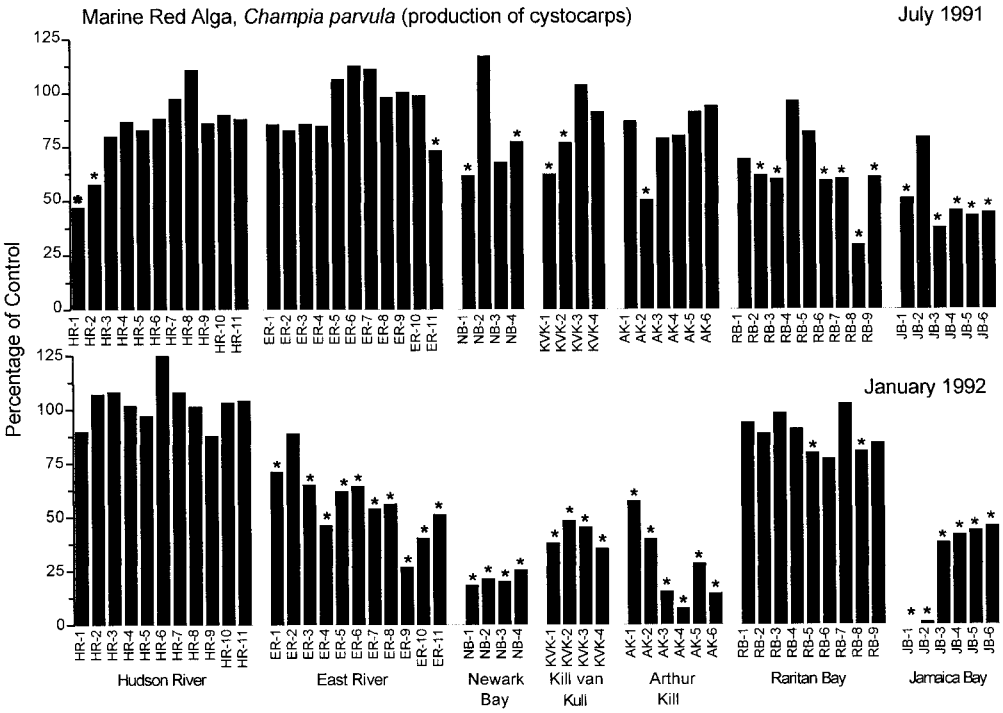

Fig. 3. Results of toxicity tests on the marine red alga *Champia parvula* from the broad-scale sampling events in July 1991 and January 1992. Asterisks indicate samples statistically below their respective performance controls. All samples were taken at the surface during slack high tide.

Six of the more toxic samples from March 1994 were tested on *A. punctulata* with the full TIE procedure (three samples each from stations ER5 and NB1; Table 4). The baseline data are for subsamples that were not manipulated. Aerating and filtering the samples barely changed their toxicity. Treating them with C18, adding ethylenediaminetetraacetic or sodium thiosulfate, and manipulating the pH significantly reduced the toxicity of all six samples to various degrees, however.

DISCUSSION

Large-scale sampling studies such as those reported here are rare. Field sampling for measuring the toxicity of ambient water is often limited to collecting single grab samples from various stations within the area of concern, generally near point sources of pollution. However, point sources were deliberately avoided in this study. This avoidance allowed us to better characterize the extent and pattern, if any, of the toxicity of the ambient water column in the Hudson/Raritan Estuary.

The objectives of the June 1991 sampling were to address the magnitude of small-scale variations in toxicity and to determine the best depth and the best time within a tidal cycle to sample during the broad-scale sampling events. Although the range of variability (i.e., the residuals) of the June samples for both species were similar, the variability for *A. punctulata* was mostly due to station-to-station variability whereas that for *C. parvula* was just as likely from tide and depth. This variability obscured any other consistent relationship between toxicity and time or depth of sampling. Therefore, all subsequent samples were taken at the more convenient surface and high tide.

Many of the samples from the two broad-scale sampling events were toxic to the two species. In fact, samples from

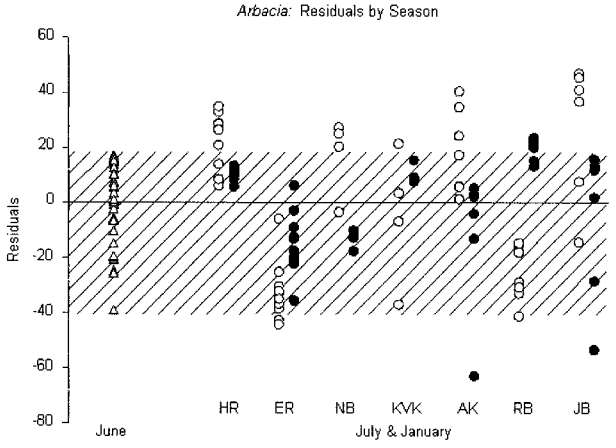

Fig. 4. Residuals from the toxicity tests using the sea urchin *Arbacia punctulata* from the small-scale sampling event (June, triangles and shaded area) and the broad-scale sampling events (July and January, open and closed circles, respectively). See text for explanation of how the residuals were calculated.

Table 3. Summary of mean responses from sea urchin sperm-cell tests for samples collected as part of the toxicity identification evaluation (New York State and New Jersey, USA). Values in parentheses for August and September 1993 represent the total number of stations sampled. Values in brackets for March and July 1994 represent the total number of samples collected during about 1 h at a single station (ER-5 and NB-1) for both months. Areas not sampled are indicated by NS

	August 93, area mean	September 93, area mean	March 94		July 94	
			Station mean	Range	Station mean	Range
Hudson River	99 (4)	97 (3)	NS		NS	
East River	99 (3)	NS	38 [10]	13–65	92 [10]	83–96
Newark Bay	98 (2)	99 (2)	27 [10]	9–55	81 [10]	76–93
Kill van Kull	100 (1)	102 (1)	NS		NS	
Arthur Kill	98 (2)	90 (3)	NS		NS	
Raritan Bay	98 (2)	NS	NS		NS	
Jamaica Bay	101 (1)	NS	NS		NS	

Table 4. Summary of toxicity identification evaluation results for 6 of the 20 samples collected from station NB1 and ER5 in March 1994. Values are percentage of fertilized eggs from sperm-cell tests using *Arbacia punctulata*

Sample	Baseline	Aeration	C18	EDTA	Filtration	pH 7	pH 8	pH 9	Sodium thiosulfate
NSW control	98	100	90	97	96	88	97	90	97
Brine control	96	97	95	93	96	86	70	92	99
NB1-1	18	24	93	84	32	12	46	78	69
NB1-3	25	37	91	86	37	14	41	79	53
NB1-9	30	38	94	88	43	30	65	80	59
ER5-1	28	38	94	87	48	5	59	74	71
ER5-3	32	23	95	89	42	12	53	89	61
ER5-9	46	29	97	85	37	25	80	71	73

every station were toxic to either of the species during at least one of the sampling events. Neither species was consistently more sensitive than the other, however; depending on the event or the station, either species could be more sensitive. These results are consistent with earlier findings from the U.S. Environmental Protection Agency's Complex Effluent Toxicity Testing Program [3]. They underscore the importance of testing toxicity with more than one species and collecting samples during different times. Equally important is the fact that many of the samples were not toxic to either of the test species, even though they are generally considered to be among the more sensitive for this type of testing.

Although these tests characterized the toxicity in surface waters of the estuary, they did not identify its sources. The later small-scale sampling events in this study were conducted as part of attempts to perform TIEs to identify the likely major contributors to the toxicity. The results from the Phase I characterizations were similar for all six of the samples—none of the manipulations affected the toxicity of the negative control (clean natural seawater) or the brine control. The baseline toxicity tests demonstrated that the unaltered samples had not lost their toxicity at the time of the TIE manipulations. While none of the manipulations were designed to detect single toxicants and were therefore unable to yield definitive results, the pattern of reduced toxicities among the various manipulations was consistent with toxicity coming from cationic metals [2,4].

One of the most important results of this study was the large variations in small-scale toxicity—the response by both *A. punctulata* and *C. parvula* varied greatly within parts of the estuary and from one collection event to the next. In some cases, the results from a single station varied more than they did over multiple stations. Without knowing about these small-scale variations, one might be tempted to draw unwarranted conclusions about differences between stations or sampling events based on location or season alone. Knowing about small-scale variability when characterizing toxicity is critical for interpreting differences among data sets from different areas or different sampling events. The most obvious explanation is that water columns are not static. Large-scale currents (especially over tidal cycles) bring much of the water of an area past a single station. Statistically significant differences between samples from a given area or between two sampling events may not be attributed to station or season unless they exceed differences at a single station.

The data from this study suggest that increasing the number of stations may not automatically be the best way to evaluate the variations of toxicity in ambient waters. When resources are limited, taking more samples at fewer stations will be nearly as good. With some knowledge of currents, flushing times, and estimated variabilities of toxicity, an efficient sampling scheme can be designed that will accurately characterize both the extent and the variability of water-column toxicity within a region.

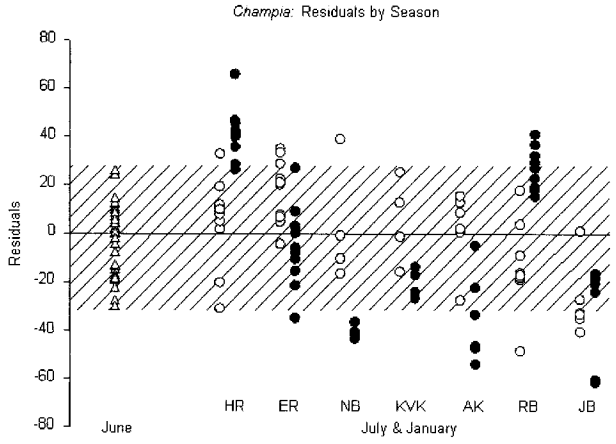

Fig. 5. Residuals from the toxicity tests using the marine red alga *Champia parvula* from the small-scale sampling event (June, triangles and shaded area) and the broad-scale sampling events (July and January, open and closed circles, respectively). See text for explanation of how the residuals were calculated.

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