Environmental Toxicology and Chemistry, Vol. 7, pp. 635–644, 1988
Printed in the USA. Pergamon Press plc

0730-7268/88 $3.00 + .00

EFFECTS OF FLUORENE ON MICROCOSMS DEVELOPED FROM FOUR NATURAL COMMUNITIES

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(*Received* 29 *June* 1987; *Accepted* 25 *January* 1988)

Abstract—Ecosystem-level responses were examined in microcosms developed from four different natural plankton communities and exposed to nominal concentrations of 0.12, 0.50, 2.0, 5.0 and 10.0 mg/L fluorene, a polynuclear aromatic hydrocarbon. The lowest observed ($p < 0.05$) effect level (LOEL) for dark respiration (R_{ni}) was 0.12 mg/L fluorene in three of the four communities during the first 14 d. The LOEL for R_{ni} in the fourth community was 5.0 mg/L. The LOEL of the net productivity/respiration ratio (P_n/R_{ni}) was 0.12 mg/L in all four communities. These results suggest that the responses of these microcosms were not completely independent of their source communities. The sensitivities or LOEL values from these microcosm experiments were as low as those reported for chronic bioassays of the three most sensitive of seven standard test organisms. Comparisons between the responses of these microcosms and those reported for experimental ponds exposed to the same concentrations of fluorene suggest that these microcosms accurately reflect the types of changes and concentrations that cause change in pond ecosystems. However, slight but significant changes in the ecosystem-level variables gave no indication of the almost complete elimination of some zooplankton populations at 5.0 and 10.0 mg/L in the ponds and at 2.0, 5.0 and 10.0 mg/L in the microcosms. This lack of correspondence between population- and ecosystem-level measurements suggests that test measurements made at one hierarchical level of organization may not always be appropriate for estimating effects at other levels.

Keywords—Aquatic ecosystems Microcosm Fluorene Toxicity Respiration Productivity/respiration ratio

INTRODUCTION

Toxicity tests using microcosms or laboratory-scale model ecosystems have been used to assess potential hazards to natural ecosystems because they have properties common to natural ecosystems that cannot be directly inferred from population studies of the organisms they contain [1–5]. The primary objective of microcosm toxicity studies in the recent past has been to describe the benefits of ecosystem-level tests in screening new chemicals for their potential hazard to the aquatic environment. Because of these recent efforts, information is now available on the responses of many different ecosystem tests to a wide variety of compounds [6–9]. The accuracy of hazard assessments made from most of these studies cannot be evaluated because comparable information is not available for natural systems. There is a need to describe the responses of several different types of model ecosystems and to compare these responses with those of natural systems.

One model ecosystem, the Leffler microcosm, uses natural populations equilibrated under laboratory conditions as inoculum [10]. The protocol is based on the assumption that the ecosystem-level properties of these naturally derived microcosms are independent of the species they contain and that microcosms derived from different water bodies will respond consistently to the same toxicant. These assumptions, and the fact that test results for naturally derived microcosms can be extrapolated to natural systems, are basic to the use of this test system to screen toxic chemicals.

The objectives of this study were to (a) describe

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the effects of fluorene on Leffler microcosms developed from four different water bodies, (b) determine whether the responses of these microcosms are independent of their source communities (i.e., to test the independence assumption mentioned above), (c) assess the sensitivity of these microcosm results by comparing microcosm sensitivity with that reported for single species bioassays and (d) test the assumption that these microcosm results can be extrapolated to natural systems by comparing the results of these experiments with results for experimental ponds exposed to the same concentrations of fluorene.

MATERIALS AND METHODS

Microcosm procedures

The Leffler microcosm was selected for these experiments because it is derived from a natural community, contains multispecies representatives of several trophic levels and is a likely candidate for use in screening toxic chemicals. Microcosms were prepared and operated according to the protocol developed by Leffler [10].

Briefly, each microcosm was prepared by adding 50 ml of a stabilized stock culture to 950 ml of Taub and Dollar (TD) nutrient solution [11] in a 1-liter Pyrex beaker. Replicate microcosms of each source community were cross-seeded twice a week during the first two weeks to reduce variation among replicates. Microcosms were allowed to stabilize for six weeks before fluorene was added (day 0); no other toxicant additions were made during the remaining 42 d of the experiment. Each system was reinoculated with 50 ml of stock culture every week during the experiment to provide the same species pool to all microcosms throughout the experiment. The incubation temperature was 20 ± 1°C and the light cycle was 12 h light (5,400 lux) and 12 h dark.

Stock cultures

All stock cultures were prepared using identical methods to assure that any difference in response would be the result of the unique characteristics of the natural source community. Stock cultures were prepared by mixing 2 liters of the source lake water with 36 liters of TD nutrient solution. This community was then allowed to adjust to incubation conditions for not less than three months before being used to start the microcosm community.

The four stock cultures used in the experiments were prepared from the plankton communities of Waverly Lake (WL), Freeway Lake (FL), Triangle Lake (TL) and Peavy Arboretum Pond (PA). Waverly Lake is a mesotrophic lake located in Albany, Oregon. Freeway Lake is a mesotrophic lake located about 2 km southeast of Albany in an agricultural drainage basin. Triangle Lake is a mesotrophic lake located about 45 km west of Eugene, Oregon, in the Coast Range mountains. Peavy Arboretum Pond, which is usually turbid, is located about 7 km northeast of Corvallis, Oregon, in an agricultural drainage basin. These lakes were selected to provide plankton communities with different histories and species compositions that could be used to test the assumption that ecosystem-level responses of naturally derived microcosms are independent of the source community.

Toxicant

Fluorene (2,2′-methylenebiphenyl), a by-product of the energy-related industries, was selected as the toxicant for these experiments. It is a U.S. Environmental Protection Agency priority pollutant [12], and more information is needed to assess its effect on aquatic ecosystems.

A stock solution of reagent-quality fluorene and acetone was prepared and analyzed before each experiment to assure that stock concentrations were accurate. Acetone carrier (0.5 ml) containing the appropriate amount of stock fluorene solution was added to each treatment microcosm. Five replicate microcosms were used for the control and carrier control and for each treatment level.

Exposure concentrations of 0.12, 0.50, 2.0, 5.0 and 10.0 mg/L fluorene were used in these experiments to match nominal concentrations reported for experiments conducted in experimental ponds [13,14]. This range of exposures represents concentrations above and below the solubility of fluorene in water (1.9 mg/L) [15]. The exposure regimen used simulates that of a spill or slug dose in the natural environment. Because we were interested in the response of the ecosystem to fluorene, exposure is the total amount of fluorene in all phases of the laboratory ecosystems. This differs from organism-level tests in which exposure to the individual is determined by the amount of toxicant in the aqueous phase. Nominal concentrations were used because we found a bias associated with sampling the heterogeneous mixture of water and slightly soluble fluorene. Fluorene micelles were more concentrated in the upper half of the microcosms where the samples were collected. Because the mixing intensity could not be increased without damaging the biota, the measured concentration always exceeded the nominal concentration.

Nominal values were considered to be more accurate because of this sampling bias, and also because care was taken to ensure the accuracy of the fluorene additions.

Fluorene analysis consisted of extraction of a 100-ml water sample with 20 ml of pesticide-quality ethyl acetate, followed by concentration of extract and analysis by GLC [16,17]. Extraction efficiency was determined with internal spikes, and precision (6%) was determined by duplicate analysis.

Fluorene loss rate

A separate experiment was conducted to determine fluorene loss rate and the potential contribution of biota to the loss rate. A mixture of fluorene and acetone was added at the concentrations used in these experiments to a series of 1-liter beakers containing reverse-osmosis-purified (RO) water only, and to another series containing biota and media from the WL source community. Fluorene concentrations were measured on days 0, 1, 3, 5, 7, 10 and 14, and loss rates were calculated [18]. Southworth's method [18] was used to calculate the half-life of fluorene resulting from volatilization alone in these beakers.

Response variables

The response variables monitored in these experiments included dissolved oxygen (DO), pH, redox potential (Eh), heterotrophic activity (HA), net primary productivity (P_n), dark respiration (R_{ni}) and P_n/R_{ni} ratio. These response variables were measured on days 1, 2, 3, 5, 7, 10, 14, 21, 28, 35 and 42. Before samples were collected or measurements made, microcosms were mixed well with a magnetic stir bar. All measurements and samples were taken 5 cm below the surface at the center of the microcosm. Measurements of DO, pH and Eh were made according to standard procedures [19,20]. The precision of these variables was determined for each sampling series by making five replicate measurements of 1 liter of nutrient medium without biota, near oxygen saturation, at 20°C. The DO meter was calibrated with the modified Winkler technique, and the average precision was 6% relative standard deviation. Eh was measured with a platinum electrode that had been calibrated with 192 and 258 mV poised solutions [21]. Average precision was 5%. Measurements of pH were made with a Ross electrode that had been calibrated using pH 7 and 10 buffers. Average precision was 0.23%.

An index of HA, the fraction of glucose decomposed to CO_2, was measured according to the methods of Kadota [22]. A 0.1-μCi ^{14}C-labeled glucose spike was added to a 10-ml microcosm sample; the sample was incubated for 4 h, then acidified with HCl and purged through a CO_2 trap. The activity of the collected $^{14}CO_2$ was determined by liquid scintillation counts.

P_n and R_{ni} were estimated by the amount of DO change during the 12-h light and dark periods, respectively. Corrections were made for O_2 exchange with the atmosphere [23]. The P_n/R_{ni} ratio was calculated using net productivity and dark respiration of consecutive light and dark periods.

Zooplankton counts were made weekly because others [14,24] had noted significant population reductions resulting from the same fluorene concentrations used in these experiments. Whole microcosm counts were made only of major groups at the order level. The precision resulting from this level of effort (less than 1 h/sample day) was sufficient to assess only major changes of about 70 to 80% mortality.

Statistical analysis

Data for each variable were analyzed to determine concentrations and times when treatments differed from the controls. Statistical analysis included a folded F test [25] to determine homogeneity of replicate variances. When replicate variances of a particular variable were not significantly ($p < 0.05$) different, a linear contrast (t test) with pooled variance was used to determine probability of difference between control and treatment groups. When replicate variances were significantly ($p < 0.05$) different, linear contrasts and degrees of freedom were calculated with a nonpooled variance [25].

RESULTS

Fluorene loss rates

Fluorene losses, with and without biota, were similar at all concentrations and by day 14 fluorene levels in all treatments, except in the 10 mg/L treatment had decreased to near the analytical limit of 0.025 mg/L (Fig. 1). Concentrations of fluorene in treatment microcosms of the four different communities were also near the analytical limit by day 14, suggesting similar loss rates in the microcosm experiments. Average loss rate coefficients for beakers with and without biota varied between 0.19 and 0.58 for all treatment concentrations. Half-lives calculated from these average coefficients were between 1.1 and 3.4 d. The half-life calculated for fluorene loss by volatilization alone was 2 d.

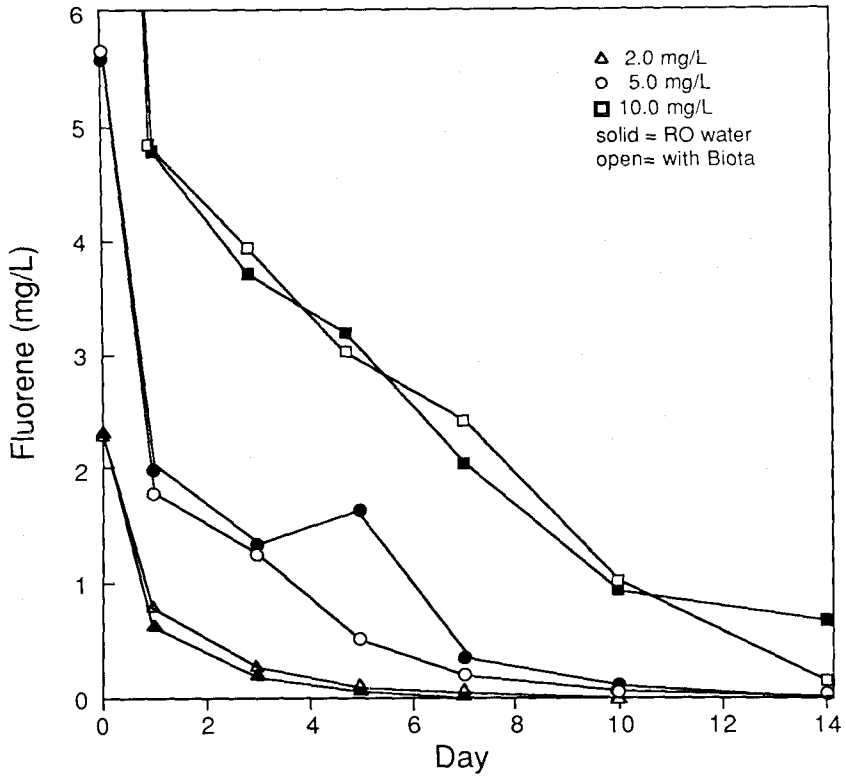

Fig. 1. Mean fluorene concentration (mg/L) in reverse-osmosis-processed water (solid symbols) and in Leffler microcosms with biota from the Waverly Lake source community (open symbols). Fluorene was added on day 0.

Ecosystem-level response variables

Some ecosystem-level response variables measured in these experiments were insensitive to the impact of fluorene. No dose-response relationship was evident for Eh or HA. Variation of Eh among collection dates was high and differences between treatment and controls were inconsistent. HA remained less than 25% in all experiments, and differences between treatment and control microcosms were significant ($p < 0.05$) in less than 20% of the comparisons.

Some ecosystem-level response variables were affected for only a short time or only at the highest fluorene concentration. During the first 7 d, about 60% of pH values in treatment microcosms exposed to 5.0 and 10.0 mg/L were significantly lower than those in their controls. Following day 7, differences were no longer significant and pH values in treatment microcosms were similar to or exceeded those in the controls. Although DO regimens in treatment microcosms were significantly different from those in the controls, the data are not presented here because they were controlled by primary production and respiration and added little additional information. P_n values in treatment microcosms of three of the four source groups were not significantly different from those in their controls. P_n in treatments developed from the FL community was significantly greater than that in the controls.

Conversely, fluorene stimulated R_{ni} in treatment microcosms from all four source communities (Fig. 2). Although the magnitude of change in TL was smaller than that in WL and FL, differences from control R_{ni} values were significant ($p < 0.05$) at similar times and concentrations as those found in WL and FL. During the first 14 d, these differences were significant in 85% of the measurements of treatment microcosms developed from WL, FL and TL. Differences between control and treatments developed from PA were as

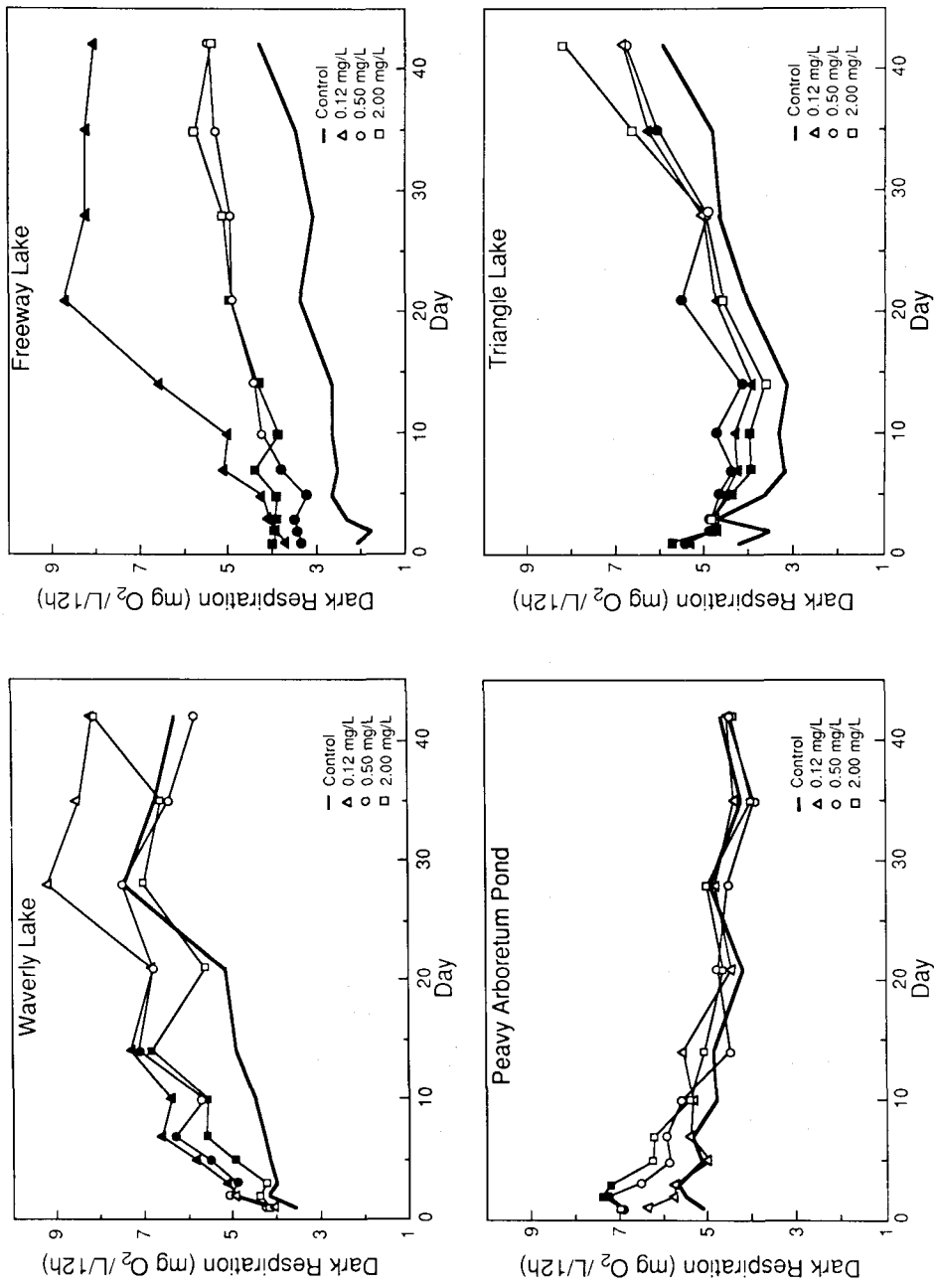

Fig. 2. Mean nighttime respiration (R_{ni}) in Leffler microcosms exposed to fluorene. The source communities used to develop these microcosms were from Waverly Lake, Freeway Lake, Peavy Arboretum Pond and Triangle Lake. Points of significant ($p < 0.05$) linear contrast from control are indicated by solid symbols.

small as those in microcosms developed from TL but generally were significant only for fluorene treatments of 5.0 and 10.0 mg/L.

Recovery times of R_{ni} in these systems were not the same for all communities. Respiration in treatments of WL and PA was consistently near that of the controls by day 14, whereas R_{ni} in FL and TL generally remained significantly different from their respective controls throughout the experiment.

The P_n/R_{ni} ratio was more consistent from one community to another than was R_{ni} (Fig. 3). During the first 14 d, 75% of the P_n/R_{ni} ratios in all treatment microcosms of these four groups were significantly less than those in their respective controls. After day 14, P_n/R_{ni} ratios in the treatment microcosms of all four communities recovered to those in the controls and differences were no longer significant.

R_{ni} and P_n/R_{ni} ratios in the carrier controls and acetone-free controls were compared to determine whether the 0.5-ml acetone carrier used in these experiments had an impact on the response variables. Differences were significant in only 15% of the comparisons. Acetone does not appear to be responsible for the changes in these variables.

Zooplankton

Cladocerans, ostracods and amphipods in all microcosms exposed to 2, 5 and 10 mg/L fluorene were almost completely eliminated immediately following the addition of fluorene. Copepods were almost completely eliminated at exposures of 2, 5 and 10 mg/L in treatment microcosms from TL and PA, and at exposures of 5 and 10 mg/L in WL and FL microcosms. The high variance associated with counting methods and among replicate microcosms precluded discrimination between controls and treatments causing lower mortalities. The copepods recolonized to control levels more quickly than did the cladocerans and ostracods. Copepods recovered in the 2.0, 5.0 and 10.0 mg/L treatments by days 7, 14 and 35, respectively; cladocerans recovered by days 21, 28 and 35, respectively. Recovery of the ostracods was similar to that of the cladocerans, but times were more variable among treatment groups. Recovery occurred between days 14 and 21 in the 2.0 mg/L treatments, between days 21 and 42 in the 5.0 mg/L treatments and between days 28 and 42 or later in the 10.0 mg/L treatments.

DISCUSSION

The response of an ecosystem exposed to a toxicant is in part dependent upon the persistence of the compound in the environment. Losses of fluorene from water both with and without biota were similar in this study, suggesting that biodegradation of fluorene was not significant. Henry's constant for fluorene is estimated to be 10^{-2} to 10^{-3} [15], which suggests that volatilization is a significant transfer mechanism [18]. The calculated half-life of 2.1 d for volatilization alone agrees well with the measured half-lives in this experiment, which suggests that volatilization was the primary mechanism of fluorene loss.

The ecosystem-level response variables used to describe the effects of a toxicant in the Leffler microcosms were not equally sensitive to fluorene. The chemical-physical state of these microecosystems as measured by Eh and pH was not significantly changed by fluorene. Autotrophic (P_n) and heterotrophic (HA) activities were also unaffected by fluorene. Fluorene, however, did increase ecosystem respiration (R_{ni}) and reduce the ratio between autotrophic activity and respiration (P_n/R_{ni}). Effects were noted at the lowest concentration tested and were significant in three of the four communities for R_{ni} and in all four for P_n/R_{ni} ratio during the first two weeks. R_{ni} recovered in two of the four communities by day 14, and P_n/R_{ni} ratios recovered in all four communities between days 14 and 21. Recovery of the P_n/R_{ni} ratio appeared independent of the recovery of R_{ni}.

The assumption that ecosystem-level responses of naturally derived microcosms are independent of their source communities was not completely supported by these experiments. The R_{ni} in the PA community was less sensitive to fluorene than was R_{ni} in the other three communities. However, the lowest observed effect level (LOEL) of P_n/R_{ni} ratios in all four microcosm communities was the same. Leffler [10] suggested defining the reproducibility of each community by its ability to rank the effects of a set of standard chemicals. The results of these studies suggest that a minimum acceptable sensitivity of a source community could be defined by a standard set of chemicals that are statistically distinguishable at a defined probability and exposure concentration.

Although changes in R_{ni} and P_n/R_{ni} ratio were significant at the lowest concentrations tested, the magnitude of the changes was slight and gave no indication of the almost complete elimination of zooplankton at fluorene exposures of 2.0, 5.0 and 10.0 mg/L. The lack of correspondence between ecosystem- and population-level responses supports the hypothesis that the effects measured at one hierarchical level cannot always be used to

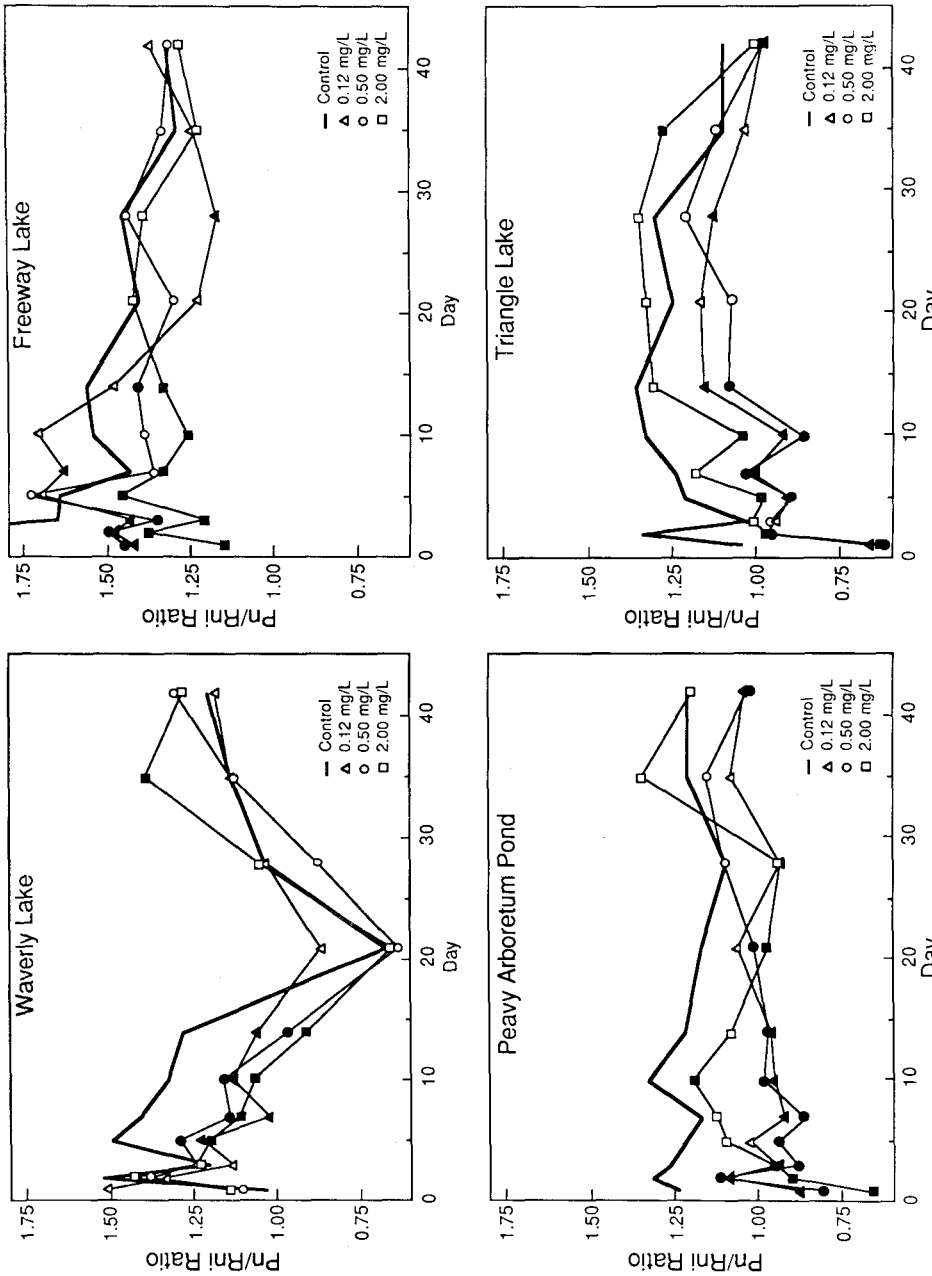

Fig. 3. Mean net primary productivity/respiration (P_n/R_{ni}) ratios in Leffler microcosms exposed to fluorene. The source communities used to develop these microcosms were from Waverly Lake, Freeway Lake, Peavy Arboretum Pond and Triangle Lake. Points of significant ($p < 0.05$) linear contrast from control are indicated by solid symbols.

infer the effects at another level of organization, if the levels are not appropriately related. O'Neil et al. [26] suggested that an ecosystem can be explained by dual hierarchies, population-community and process-functional, which are relatively independent of each other.

For any laboratory bioassay to be useful in assessing potential hazard to the natural environment, it should be precise, sensitive to a diverse array of toxicants and accurate. Accuracy, in this sense, is the measure of how well the information produced can be used to predict types of responses and toxicant concentrations causing damage or dysfunction in a natural environment.

Microcosm bioassays are an ecosystem-level analogue of the single-species chronic test. Chronic concentrations reported for three of the most sensitive of seven species [24] are presented in Table 1, along with LOEL values for response variables in the Leffler microcosms and experimental ponds. The Leffler microcosms were as sensitive to fluorene as were chronic tests of the most sensitive species.

The accuracy of the microcosm used in these experiments was evaluated by comparing microcosm responses to those reported for 0.08-ha experimental ponds [13,14] exposed to the same concentrations of fluorene as were used in these experiments.

Fluorene was added to the ponds at the start, and no other additions were made during the experiment. By day 14, fluorene was almost completely lost from all ponds. To compare pond volatilization losses with laboratory values, loss rates were calculated using the total amount of fluorene recovered in water, sediments and macrophytes on day 7 in the ponds [13]. Half-lives calculated from these data averaged 2.3 d, and the reported half-life calculated with Southworth's method was 4.2 d [12]. The similarity of volatilization loss rates in these two ecosystems may be fortuitous, since phase exchange coefficients are dependent upon variable environmental and hydrodynamic factors. Others, however, have noted less than one order of magnitude of difference between experimental and computed loss rates, lending credibility to these predictive techniques [18]. Nevertheless, the similar half-lives suggest that the fluorene exposures to the microcosm and pond ecosystems were similar and that responses should be equivalent if the microcosms accurately represent natural ecosystems.

Gross primary production ($P_{gross} = P_n + R_{24h}$) in the ponds exposed to fluorene was higher than in the controls, probably a result of increased community respiration [14]. The LOEL of gross primary productivity in the ponds, and of R_{ni} and P_n/R_{ni} ratio in the microcosms was 0.12 mg/L (Table 1). The responses in both the ponds and the microcosms were short-lived, and by day 14 both ecosystems had recovered to control levels.

As was noted in the microcosms, the magnitude of change in the ecosystem-level variable, P_{gross}, gave little insight into the major changes that occurred in the structure of the zooplankton community and the two fish populations measured in the ponds. Fluorene treatments of 5.0 and 10.0 mg/L resulted in greater than 90% zooplankton mortality [14] during the first 14 d. After this period, zooplankton recovered to levels near those in the control pond. These changes were accurately reflected by measurements of zooplankton in the Leffler microcosms.

Leffler [10] suggested that microcosm screening

Table 1. Comparison of chronic toxicity values for three sensitive species [24], and the responses and lowest observable effect levels (LOEL, mg/L fluorene) for experimental ponds [17], with responses and LOEL of Leffler microcosms

Chronic toxicity LOEL	Leffler microcosm LOEL	Experimental ponds LOEL
Daphnia magna		
21-d complete life cycle, 0.125 mg/L	Ecosystem processes (R_{ni}, P_n/R_{ni}, [H]) changed at 0.12 mg/L	Ecosystem respiration increased at <10 mg/L
Chironomus riparius		
30-d emergence, 0.6 mg/L	Zooplankton almost completely eliminated at 2.0, 5.0 and 10 mg/L	Zooplankton reduced 99% at 5.0 and 10.0 mg/L
Lepomis macrochirus		
30-d partial life cycle: survival, 0.5 mg/L; growth, 0.25 mg/L		Fish survival reduced 50% at 0.5 mg/L Fish growth reduced at 0.12 mg/L

systems can be developed that ignore individual population dynamics and focus on ecosystem-level parameters. The results from these experiments and the comparisons with single-species bioassays and with responses of the experimental ponds do not totally support this approach. The ecosystem-level responses in the Leffler microcosms accurately reflected the type of ecosystem-level changes in the ponds. The sensitivity levels of the ecosystem variables in the Leffler microcosm were comparable with those of pond systems and with chronic values for the most sensitive species as well. It was the additional measurements of the zooplankton population change within the microcosms, however, that most accurately reflected mortality and recovery times of structural components in the natural system. It appears that both ecosystem-level and population measurements from microcosm screening systems are of benefit in assessing the potential hazard of compounds to the environment.

At present, there does not appear to be a generally acceptable method for using microcosm or ecosystem-level response variables in the development of toxicity criteria for chemicals introduced into the environment. The experiments described here suggest that microcosm results are as sensitive as results of chronic tests of the most sensitive organisms of an array of standard test organisms. The cost differential between chronic bioassay and microcosm studies is not significant, but, more important, microcosms offer ecosystem-level information that cannot be inferred from conventional bioassays. The results of the studies presented here and those of others [23,27] that tested different compounds suggest that microcosms accurately reflect ecosystem-level changes in uncontrolled environments. The lack of correspondence between ecosystem-level measurements and changes in the zooplankton populations in the microcosm and the pond experiments also supports the hypothesis that levels of the population-community hierarchy cannot be used to infer changes in the process-functional hierarchy.

Because these microcosm experiments and other similar experiments accurately reflected concentrations that cause changes in natural systems, and because we agree that hazard assessments should provide information that allows the prediction of concentrations that will not cause harm at all levels of biological organization [28], we recommend that additional methods be developed to use ecosystem-level measurements along with the more conventional population- and organism-level measurements to develop toxicity criteria for chemicals introduced into the environment.

Acknowledgement—The authors would like to thank the following people for their contributions: Joseph Spitz and Donna Cox assisted in data processing and analysis; analytical chemical support was given by William Griffis and Claudia Wise; David Yount, Steven Hedtke and Kenneth Malueg provided technical reviews; and Evelyn Hunt provided editorial review of the manuscript.

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