Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems
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Evaluation of Tests to Predict Chemical Injury to Ecosystems: Microcosms

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ABSTRACT

Protocols for testing xenobiotic chemicals released into the environment are needed to assess the potential harmful effects they may have on non-human biota and ecosystems. Existing protocols are often too simplistic for extrapolation or too complex for analysis. Microcosms may constitute a useful protocol for testing chemicals because they strike a balance between these two extremes. A microcosm is an experimental preparation ranging in complexity along a continuum between bioassay scale and field plot scale. The point on the continuum where a microcosm preparation lies depends upon the hypothesis or question being addressed. The current status of microcosm methodology is in flux, but a number of ideas are emerging on which most workers are in agreement: the method is holistic; the method should not be employed alone, but rather in conjunction with appropriate mathematical models and validation experiments; there is an inverse relationship between realism (complexity) and precision (replicability) in microcosm performance. No consensus yet exists concerning the following aspects of microcosms: representativeness, i.e., the extent to which results can be extrapolated (and therefore the value of microcosms in screening protocols); standardization of design; and applicability of synthetic (gnotobiotic) microcosms.

1 INTRODUCTION

The need to develop protocols for the evaluation of chemicals released into the environment by mining, manufacturing and other industry, and by governmental activities becomes clear by merely considering their number: at least 1000 newly synthesized chemical compounds appear every year. It is obvious that the potential for ecological and human health impairment is large, and that the resources needed to adequately characterize all these substances are formidable. An efficient means for coping with this problem is widely sought, and the microcosm technique has been suggested as one possible tool.

Toxicology, the study of harmful effects of chemicals on life, is as old as alchemy, but environmental toxicology (called this for lack of a better name) is as recent as ecology; it is still in its infancy. The response of organisms to xenobiotic chemicals in classical laboratory preparations (for example, a fish in a tank) may perhaps always differ from the response of the same organism exposed to the same concentration of the chemical in its environment. To assess environmental effects, which subsume toxicological effects of xenobiotic chemicals, an experimental test system beyond laboratory scale is required.

It is now apparent that the results of many laboratory-scale experiments, including evaluation of toxicity, often diverge from the results of field-scale experiments designed to investigate the same phenomenon. Romeril's (1971) results suggested that the ^{65}Zn retention-time by laboratory oysters differed from that of oysters in their natural habitat. Till *et al.* (1979) presented evidence that ^{99}Tc did not behave in the natural environment as laboratory studies with potted plants suggested. Experiments by Hoffman *et al.* (1982) showed that plants exposed under field conditions indeed accumulated far less ^{95m}Tc than the laboratory-scale experiments had shown. Many other examples of such inconsistencies can be ferreted out of the environmental literature.

Should we suspect that laboratory-scale experiments are inappropriate for environmental toxicology and that truth lies mostly in field testing? It is the widespread impact of pollution, including the concomitant legislation developed to cope with it, that has produced a need for the testing of chemical effluents at field-scale (i.e., the scale of nature). Single-species laboratory testing (bioassay) simply cannot be quantitatively extrapolated to effects of the substance or substances in question in the ecosystem. In addition to the plethora of second-order and lower effects along the concatenations of the food web, an even more pragmatic matter may obscure the significance of the results: stocks of organisms adapted to the laboratory may respond to xenobiotics so differently from wild (natural) stocks that the results of tests with laboratory stocks may be meaningless.

Although the need for field-scale testing seems to be well accepted by both applied and theoretical ecologists, the difficulties of field-scale testing are formidable. Results are often complicated by so many unmeasured relationships that conclusions of only the grossest and simplest nature can be drawn. Perhaps the most pervasive impediment to the conduct of proper field studies, however, is cost.

This is the dilemma of the environmental toxicologist: the experimental paradigms available for the investigation of the behaviour and effects of xenobiotic chemicals are either too simplistic or too complex (and costly). The microcosm therefore appeared to be an ideal method for ecotoxicology.

There have been so many definitions of 'microcosm' that it could be suggested that the idea itself is too fuzzy to be of value in scientifically rigorous experimentation. This is understandable, inasmuch as many dictionaries,

ordinarily the final arbiter in such matters, offer a confusing mixture of meanings and nuances from one volume to another. Most workers, however, seem to seize the common thread of the concept of 'little world', a miniature, and in this sense, a model, not containing all the elements of the whole, and to realize that the terms 'microcosm' and 'macrocosm' are not the antipodes of an epistemological sphere. Again, not all dictionaries will help much here, except to provide an idea that 'macrocosm' refers to a universe (in this case, an ecosystem) and is thus not a large microcosm. Clarification of terminology is required if the concepts are to be clear. For instance, the neologism 'mesocosm' has recently entered the vernacular, presumably as a synonym for 'medium microcosm'. However, 'microcosm' is quite sufficient; all that is required to be a microcosm is to be a model; that is, (*reductio ad absurdum*) minus at least one element of the whole (universe). The point will be made below that the design of a microcosm experiment should follow from the hypothesis to be tested, or at least with specific objectives in mind; the size of experimental preparation ('meso-cosm') should not be preselected and the experiment designed accordingly. The conceptual problem is resolved when one considers what should be, but often is not, obvious: there is a continuum of experimental paradigms from the simplest possible bench scale to the most complex field scale (Goldstein, personal communication). The choice of the paradigm—the point on the continuum—is a function of the question (hypothesis) being asked.

A microcosm, in the present sense, is an experimental preparation ranging in complexity between laboratory bench scale and field scale. The specific design of a microcosm, that is, the subset of the ecosystem that is to be modelled and tested, will be determined by the framing of the hypothesis to be tested.

It follows that the choice of scale at which one designs a microcosm is a trade-off between simplicity, therefore replicability; and complexity, therefore realism. Most of the literature seems to agree that the pivotal issue in the design and use of the microcosm technique is the question of the scale of the subset of an ecosystem over which one can maintain experimental precision and still derive useful, realistic information.

2 CURRENT STATUS OF MICROCOSM RESEARCH

To review the field of microcosm research is to review the work in ecosystem theory, ecology, limnology, microbiology, soil science, physical and biochemistry, toxicology; it becomes pointless to enumerate further. Clearly, the microcosm technique has appealed to a large segment of environmental science community. It is the purpose of this discussion to explore the commonalities of the technique as applied by the various disciplines, rather than to present a discussion of individual results or an exhaustive literature review.

There have been several recent documents stemming from gatherings of researchers, who use microcosm techniques, at which the state-of-the-art was

assessed. The various workshops and symposia have built upon one another, and the attendees and authors overlap considerably. There was perhaps an inevitable redundancy, but a clear progress was evident as succeeding results were presented. It is not the purpose here to discuss and critique the microcosm literature, but to attempt to distil from the most recent work a synthesis of a working theory and methodology of microcosm experimentation that can be useful for ecotoxicology.

Gillett and Witt (1979) presented the results of a workshop convened as a part of a symposium in 1977 (Witt *et al.*, 1979) to evaluate the use of terrestrial microcosms, present and future, for testing chemicals in the environment. A second symposium was held in 1978 to consider the broad topic of microcosms in ecological research (Giesy, 1980). Several workshops were held on the topic 'Methods for Ecological Toxicology' and the results compiled in a report (Hammons, 1981). A similar compilation (without workshops), 'Testing for Effects of Chemicals on Ecosystems', was edited by Cairns (1981). Finally, a workshop, 'New Perspectives in Ecotoxicology', considered toxicology in an ecosystems context (Levin, in preparation). Although a few contributors were common to all these efforts, the total number of contributors was several score. The objectives of each document are somewhat different, but there are several points of agreement common to all seven, and no major disagreement about the use of microcosms in ecotoxicology.

2.1 Microcosms: Definitions and Principles

The symposium 'Microcosms in Ecological Research', held in 1978, is the second of the two principal compilations of microcosm research pertinent to the present effort. Although there is no synthesis paper, there are 52 papers discussing microcosm theory, aquatic microcosms of several types, and terrestrial microcosms.

In the editorial preface, Giesy (1980) assesses the field from his own perspective. According to Giesy, the first principle of microcosm design is to be aware that the technique is holistic. That is, one should design 'from the top down', working out the hierarchial structure and maintaining the integrity of closely related processes. The opposite method, to be avoided, would be to include in a single contained experiment several components not closely related in a natural ecosystem. This idea does seem to be reflected in most of the symposium papers and therefore represents a consensus opinion about how to conduct ecological research in microcosms.

Microcosms are models, 'conceptual and operational bridges' as Giesy (1980) puts it, between the simplicity of the laboratory test and the complexity of the natural ecosystem. As models, microcosms should not be employed alone; that is, appropriate validation/verification experiments in laboratory or field, and an appropriate mathematical model are, in some combination, required before the

results of a microcosm study can be accurately assessed in terms of experimental precision and realism.

Leffler (1980), following Caswell (1976), distinguishes between models (in this case microcosms) that predict and models that explain. The latter, heuristic models, are themselves of two classes: exploratory, or inductive, and hypothesis-testing, or deductive. The first class, inductive, can provide data needed to design specific or deductive experiments (the second class) which will provide conclusions of a quantitative nature, directly applicable to the natural ecosystem.

The fact that microcosms are physical models renders classification schemes unwieldy, and the difficulty of representing the technique in such a manner follows from the differences in the ecosystem processes that the microcosm are designed to test. For example, it can be useful to contrast microcosms as large or small, aquatic (lotic, lentic, or marine) or terrestrial, open or closed, artificial or natural, inductive or deductive, and on and on.

Classification of microcosms, then, should be thought of as a convenience and not a descriptor of the paradigm. It is apparent that several of the classifiers could apply to one paradigm, e.g., large closed natural lotic deductive microcosm. Structural relationships (biomass distributions, air mass trajectories, for example) are not amenable to microcosm preparations. Functional relationships (rates, fates, dynamics, for example) are amenable, and it is thus at the process level that models of subsets of ecosystems can be built for the testing of ecosystem-level hypotheses.

Inasmuch as microcosms are models of specific ecosystems, it is difficult to apply the results of a given microcosm experiment to other ecosystems. This suggests that the use of microcosms for screening purposes is quite limited. One approach to circumvent this problem is the construction of artificial or synthetic microcosms. The synthetic (for lack of a better word) microcosm technique was pioneered by Metcalf and his associates (1971a,b), and by Taub as reported in Taub and Crow (1980) and Taub *et al.* (1980). The use of synthetic components to put together a microcosm (also called a gnotobiotic microcosm) has the obvious advantage of providing better statistical replicability. Immediately apparent is the question of realism and the violation of the principle of 'top down' ecosystem subset modeling.

Adequate replicability of microcosm preparations with natural rather than synthetic components has been demonstrated, even though the variability of microcosm experiments is a function of the complexity of the design. Studies testing the experimental variability of microcosms have included the establishment of duplicate experiments (Huckabee and Blaylock, 1974) and the ability of aquatic microcosms to 'track' or follow the process under consideration in the natural environment (Pilson *et al.*, 1979).

Three experiments with duplicate microcosms were conducted to test the ability of excised stream-bank microcosms to indicate the distribution of cadmium, mercury, and selenium in ecosystem compartments over a 6-month

period. The variability of each experiment was determined by destructive mass-balance expressed as the percentage of radioisotope of the element in question retained in each ecosystem compartment. The percentage distribution of both Cd and Hg in the major compartments (soil, sediments, water and tank liner) was found to vary by less than 12% in the two duplicate preparations but the maximum variability in the two Se experiments was only 3%. These results show that rates and routes of Cd, Hg and Se transport (and by implication of other metals as well) in duplicate microcosm preparations are constant enough to provide a valuable assessment tool.

Pilson *et al.* (1979) showed that marine microcosms could be made to very closely simulate certain biological and chemical conditions in an adjacent bay. They found that chlorophyll-*a* concentration in the water of nine microcosm preparations over the course of the test were as similar to one another as they were to the source water from Narragansett Bay. Although complex multivariate statistical analysis was essential in interpreting the results, Pilson *et al.* (1979) concluded that the microcosms were of low enough variability to provide adequate tools for ecological and biochemical experiments. However, there is no *a priori* reason why a hypothesis could not be posed that would be of ecosystem relevance and that could be tested in a synthetic system. The distinction between a bioassay and a simple microcosm is here seen to blur.

Are microcosms, synthetic or natural, suitable models for the routine screening of potentially toxic chemicals? Probably not, at least not yet; the level of complexity of the simplest microcosm is more than needed for a 'yes' or 'no' answer to the question of potential for harm and thus of the need for more specific evaluation. An appropriate generic mathematical process model, with both biogeochemical and toxicological modules, would help identify the best paradigm. Such a generic model would serve as an organizer for the appropriate research, much of which could be ideally accomplished with site-specific microcosm preparations. The modelling of biogeochemistry is sufficiently advanced that such modules are already at hand. However, adequate toxicological models are not yet available. Nevertheless, appropriate biogeochemical variables (for example, water quantity, soil properties, uptake rates, residence times, depuration rates, etc.) for toxicological testing can be obtained currently.

An issue closely related to screening in microcosm design is standardization. Harte *et al.* (1980) claim the standardization of certain design and operational procedures would provide systems that replicate well and behave more like natural systems. On the other hand, Giddings (1980) argues against standardization because 'each scientist creates his own experimental tools in a process involving careful reasoning, intuition, and occasional leaps of faith'.

The lack of agreement on these issues is apparent in contradictions between several authors in Giesy (1980) who claim alternately that microcosms are useful or not useful for toxicity tests, useful or not useful for assessing fate of chemicals, should or should not be used to predict ecosystem response to chemicals. I prefer

not to list specific references here so as to avoid unintended invidious comparisons.

2.2 Microcosms in Practice

Gillett and Witt (1980) discuss six primary terrestrial microcosm types:

(1) physical model ecosystem (here used more restrictively than the more inclusive definition of 'physical model'),
(2) terrestrial microcosm chamber,
(3) plant–soil–water microcosm,
(4) soil core microcosm, and
(5) soil ecosystem respirometer.

These types have received most attention because results obtained with them, or close variants of them, have been published as reports in the technical literature (for references see Gillett and Witt, 1980).

Gillett and Witt (1980) offer a concise enumeration of the advantages and disadvantages of microcosm techniques over bioassays and field tests.

2.2.1 Advantages

'1. Microcosms can demonstrate interactions without risk to larger systems, such as biomes or populations.
2. Microcosms provide indexes of temporal and spatial distribution that suggest where and what to look for regarding fate and effects of chemicals in the natural environment.
3. Microcosm systems are practical and cost effective for examining hundreds or thousands of chemicals.
4. Microcosms provide an intermediate step in verifying the predictive process from simple laboratory tests to what might be expected in the complexities of the field.
5. If a comparative approach is used among chemicals, conditions and types of systems, microcosms may permit evaluation of effects in complex systems at an early stage of investigation, again increasing predictability of the screening process.
6. Microcosm technology leads to a fundamental analysis of processes and problems, giving a broader perspective to interactions and potential effects of classes of chemicals on systems and providing a better understanding of system relationships.
7. Microcosms provide information that is more useful, or more directly applicable, to guide "safe" manufacturing, use and disposal practices than do laboratory tests alone.'

2.2.2 Disadvantages

'1. Microcosms are not self-sustaining and are too short-lived to demonstrate a number of ecologically significant processes, such as natural succession or other multigeneration–multispecies phenomena.
2. Simple properties are most readily measured in less complex systems; however, simple systems provide good prediction only to the extent that we recognize how these properties interact in ecosystems.
3. Not all significant processes of a given environment or ecosystem can be included in a particular microcosm.
4. Biological effects and chemical disposition can be indicated only in a relative sense in microcosms; we do not know how accurately these are reflected in scaling from the field or biome to the microcosm.
5. A microcosm system is only as useful as the hypothesis that the system tests: it is limited by the validity of assumptions and the sum of the precision of techniques used to test its operating hypothesis.' (Reproduced by permission of the US Department of Energy)

Gillett and Witt (1980) single out point five above for emphasis: the importance of designing microcosm experiments based upon a specific hypothesis about toxicant chemodynamics and ecosystem response.

Several key processes of ecosystem chemodynamics were discussed by Gillett and Witt (1980) as factors to be considered in the framing of hypotheses amenable to the microcosm technique. They caution that transport of chemicals in microcosm preparations suffers from difficulties related to scale (soil depth, air flow, temperature cycles).

Transformation reactions of chemicals in microcosms are considered by Gillett and Witt (1980) to be among the processes more amenable to study in microcosm preparations. The one drawback is the apparent lack of realism in photolytic reactions in microcosms.

Bioaccumulation is one of the stronger indicators of potential for ecological harm by a chemical, and Gillett and Witt (1980) claim that in spite of the reported insensitivity of terrestrial microcosms to bioaccumulation processes, certain soil parameters can provide an accurate estimate of bioconcentration.

Whether or not a chemical causes detrimental biological effects is the main reason for concern about chemicals in the environment, and it follows that microcosms, if they are to be of value in the assessment of chemical injury to the environment, must be useful for studies of effects. However, this assumption carries a heavy load of oversimplification. Microcosm preparations inevitably diverge in time from the ecosystem they represent. Therefore, the information to be gained, if environmentally relevant, must be gathered in days or weeks, perhaps a few months, at best. Microcosms are perforce useful for short-term effects, not for chronic processes operating over months or years. System processes that have been studied with microcosm preparations include energy

relationships, nutrient cycling, and chemical transformations. Population interactions that have been studied with microcosm preparations include plant–microbe interactions, arthropod population interactions, and predator–prey interactions.

Gillett and Witt (1980) speak of the usefulness of microcosms to evaluate gestalt effects (routes, chemical species or transformation products, interactions with dietary and other components of the natural environment, etc.) in subchronic tests. They report that in spite of the fact that many of the mechanisms are unknown, the soil core microcosm showed the greatest potential promise for determining terrestrial ecosystem vulnerability to chemical damage.

Several research recommendations are presented by Gillett and Witt (1980). The conferees' suggestions fell in two divergent groups: standardization and improvement of technology. In the first category, the accent was upon routine screening of regulated chemicals, and in the second with trade-offs between system replicability and realism. The incorporation of mathematical modelling was regarded as essential before microcosm preparations could be fully exploited.

The overlap in the objectives of the workshop reported by Gillett and Witt (1980) with the current effort are broad enough to merit quoting the specific recommendations:

2.2.3 Recommendations for Terrestrial Microcosm Research

'1. Use reference compounds in comparative tests of new chemicals.
2. Test the chemical at the level anticipated in the environment, then at multiples and fractions of that level.
3. Obtain kinetic data (time–space–concentration profiles).
4. For bioaccumulation and biotransformation studies, use trophic levels equivalent to the environment modelled.
5. Use standard organisms in constructed microcosms.
6. Use at least two soils with properties within different ranges. (The following require substantial research to establish their thrust in standardization and to resolve inconsistencies, *inter alia*.)
7. Use unformulated, technical-grade (commercial) products; appropriate formulations should also be tested.
8. Use radioactive isotopes (major or nominal ingredient only).
9. Perform mass balance on chemical and transformation products.
10. Provide determination of transformation products to the extent of analytical feasibility.
11. Determine the biological activity of transformation products.'

'The use of microcosms for screening chemicals that requires the fate and effects of chemicals in the system be reasonably similar to (and thereby predict) those in the environments modelled. For validation, verification, mathematical modelling,

and design criteria, the following are suggested:

1. Incorporate photochemical action.
2. Achieve realistic airflows.
3. Simulate temperatures more accurately throughout the system.
4. Define the errors caused by the structure of the system (e.g., wall effects and scaling problems).
5. Develop nondestructive plant tests and tests of plant–soil (rhizosphere) interaction. (For example, include a plant species for mutagenicity testing, such as *Tradescantia* or *Arabidoposis spp.*).
6. Establish criteria for fauna appropriate to the scale of the system and test objectives.
7. Identity parameters of reproducibility which can serve as operational criteria.
8. Develop both predictive and analytical mathematical models.
9. Perform field trials specifically to verify accuracy of microcosms'.

In addition to the conference proceedings discussed above, there is a large literature on microcosms in the open scientific journals and various internal reports. Pease *et al.* (1982) constructed for EPRI a very useful tabulation (which includes some previously mentioned contributions in Giesy (1980)) for aquatic microcosms (Tables 1 and 2). They include physical (72 studies) and biological (70 studies) characteristics involving methodology for the testing of various chemicals. This tabulation provides a concise (but incomplete) description of the field and will serve as an entrance to the literature.

2.3 Summary

2.3.1 Definitions

Microcosms are experimental preparations of subsets of ecosystems that range in complexity along a continuum from laboratory bench scale to field (natural) scale; there is an overlap at each end of the continuum of complexity with laboratory bench scale and field plot scale.

2.3.2 Advantages of Microcosm Technique Versus Laboratory-scale and Field-scale Testing of Xenobiotic Chemicals

Microcosms are more realistic than laboratory tests and more manageable (replicable) than field tests.

2.3.3 Disadvantages of Microcosm Technique Compared with Laboratory-scale and Field-scale Testing of Xenobiotic Chemicals

There is usually an inverse relationship between realism (complexity) and precision (replicability) in microcosm performance. In some special cases this

Table 1 Physical characteristics of small microcosms.

Citation	Construction design	S/V ratio	Substrate	Light	Temperature (°C)	Flow and turnover rate	Water description	Treatment	Mixing
Abbott (1967)	5-gal glass jar	—	Bay sediment	Daylight type fluorescent; LD 12:12	Ambient (not controlled)	Remained standing after introduction of treatment	Bay water	Reagent grade sodium nitrate and sodium dihydrogen phosphate	Constant, simulates ambient
Adler *et al.* (1980)	150-l glass 0.7 m	—	Narragansett Bay sediment in 169-cm^2 benthic box	Cool white fluorescent, benthic box covered	—	1/35 days	Narragansett Bay	Trace metals added, sediment behaviour in small *vs* large microcosm	Revolving plastic honeycomb
Admiraal (1977)	30 × 40 × 9 cm PVC silicon tubing	—	Natural-sand or mud flat; kept in 3 separate layers	8 hours fluorescent light	7, 14	2.5 min turnover circulation	Sea water	Aged and glass fibre filtration; 240 μ mole nitrate; 9.4 μmole orthophosphate	Circulating pump
	Small container	—	Sediment near sewage outlet	—		Closed loop	—	—	Stirring
Allen and Brock (1968)	40-ml medium in 35-mm diameter glass test tubes	—	—	—	2, 5, 10, 18, 25, 30, 35, 40, 45, 50, 60, 65, 70, 75, 80, 85	1–2 days fresh medium added	Distilled water with chemicals added	Temperature	Constant aeration
Barsdate *et al.* (1974)	1-l glass Erlenmeyer flask	—	Ground *Carex* leaves	—	25	None	Pond	^{32}P tracer	Not recorded
Beyers (1962)	29.3 × 12.2 × 18.3 cm, stainless steel and glass	119.15 cm^3/l	Sediment	150 watt reflector spot bulb LD 12:12	23 + 2	Replace evaporated water, only distilled water	San Marcos River, hot spring	Light, temp. and grazing	Constant aeration with airstone
Bourquin *et al.* (1974)	6.5-l battery jars	—	Salt marsh mud	LD 12:12	28 constant	—	—	Malathion	Slow aeration

Table 1 (*Contd.*)

Citation	Construction design	S/V ratio	Substrate	Light	Temperature (°C)	Flow and turnover rate	Water description	Treatment	Mixing
Byrfogle and McDiffett (1979)	3-l Erlenmeyer flask plugged with cotton	—	—	9 fluorescent bulbs; 4 Sylvania incandescent bulbs LD 12:12	21 ± 1	—	Autoclaved pond water with Algagro concentrate added	Diluted simazine	Aerated except for a 36-hour period, pH and DO monitoring
Child (1972)	Airtight translucent bags	—	Spanish moss	8000 foot-candles	25	Closed system	Terrestrial	^{14}C	None
Confer (1972)	200-l aquaria	—	Attached and planktonic organisms	—	24 summer 20 winter	Continuous 8 l/day	Dechlorinated tap water	Phosphorus tracer	Constant flow of water
Cooke (1967)	400-ml glass Pyrex beakers	—	—	150–180 ft candles; LD 12:12	21	Evaporated water replaced with distilled water	Farm pond stock	Nitrate, phosphorus and $CaCO_3$	—
Cooper (1970)	5 9-gal polyethylene canisters interconnected	—	Sediment from Trinity Bay	496–806 ft candles	Light: 13, 24, 35 Dark: 4. 5, 16.5, 27	Variable according to treatment	Saltwater and freshwater input; simulates estuary	Freshwater flow and retention dye studies, hydrological simulation, and effluent loading	Constant stirring
Cooper (1973)	15-l glass aquaria	—	Autoclaved sediment	1850 + 25 lux	21	None	Filtered pond water #20 mesh	Predation. Added various Nos. of spotfin shiners to graze	None
Crouthamel (1977)	28 × 18 × 13 cm transparent vessels; volume @ 4 litres	—	—	400 ft candles; LD 14:10	22 ± 2	Batch microcosms maintained at 4 litres for duration	Aged tap water	Fish (*Gambusia*) predation @ 2 30-min periods per week	Once/week for 6 wk initially

Davis *et al.* (1977)	37.5-l	—	Present, but no description	Natural, full intensity	—	40 l/hr	Filtered estuarine water through 1.6 mm fibreglass screen, S = 21–29‰		
DePinto *et al.* (1980)	25 × 35 cm, 15-l opaque, polyethylene cylinders	—	Adirondack sediments 3 cm deep	No light present	—	Continuous flow *vs* batch mode system	3 Adirondack lakes	$Ca(OH)_2$, $CaCO_3$ agricultural limestone, fly ash	Once every 3 days
Dolen and Wagner (1978)	—	—	—	—	—	None	—	Oil and oil surfactant	
Eichenberger (1972)	Channel 25 cm wide 10–20 cm depth; flow 41 per second	—	—	Outdoor	—	4 l/sec	Groundwater	Sewage 0, 1, 5, and 12%	—
Elmgren *et al.* (1980)	1.8 m diameter 5.5 m height cylindrical tank	—	Narragansett Bay; sediment 0.3 m deep	—	—	0.3 l/min, turnover 30 days	Narragansett Bay	No. 2 fuel oil	4 2-hour periods per day
Evans and Henderson (1977)	600 l fibreglass tanks, gel coated (plastic piping)	—	Sediments	12 l/2% of ambient	—	13 l/m to 0.6 l/m	Seawater, Kaneohe Bay	Sewage	—
Everest (1978)	75-l, 51 × 51 × 97 cm plastic laundry tubs	—	Cordgrass and saltmarsh soil	—	—	'Tidal Machine'	Seawater, 1 part natural' 9 parts synthetic	^{32}P fluorometron	Simulates tidal flux
Ferens and Beyers (1972)	250-ml flasks	—	—	—	—	None	—	Gamma radiation	—
Fraleigh (1971)	500-ml Erlenmeyer flask	—	—	750–850 footcandles, LD 12:12	20 + 1	—	—	Nutrients-P concentration, 1/2 strength Taub 36 medium	—
Fraleigh (1978)	250 ml Erlenmeyer flasks	—	—	—	—	—	—	Different P concentrations along with Taub 36 medium, vitamins, nitrate	—

Table 1 (*Contd.*)

Citation	Construction design	S/V ratio	Substrate	Light	Temperature (°C)	Flow and turnover rate	Water description	Treatment	Mixing
Fraleigh and Wiegert (1975)	1 × 2 m 1.2 × 24 m wooden troughs	—	—	Variable	35–54	30 l/min 15 l/min stream microcosm	Hot springs	Temp; light; nutrients	—
Giddings and Eddlemon (1977)	Slate bottom, glass aquaria 25 × 16 × 19 cm and 60 × 30 × 40 cm; 3.5 cm of substrate added to each aquaria	—	Sand and lake mud	Cool white fluorescent lights; 16 000 lux, LD 12:12	18	Spring water replaced water removed for samples and distilled water replaced water that evaporated	Spring water filled aquaria; water allowed to stand for 1 week, permitting it to clear. 15 mg wet algae/sq cm of surface area added to each aquaria	Sodium arsenate injected after 7 weeks of sampling water quality	—
Giesy (1978)	96.4 m long 0.6 m wide 0.3 m deep concrete channel. Pools 1.5 × 3 × 91 m	—	Black PVC plastic, silicon, sand, high organic silt	—	—	94.6 l/min 1.3 cm/s	Synthetic to simulate natural surface run-off	Cadmium © 3 conc on decomposition of leaf litter	—
Gile and Gillett (1979)	1 × 0.75 × 0.61 m glass box	—	Gravel, sand, organic matter, sea sand, clay	2500 foot-candles LD 12:12	30 day 18 night	—	Terrestrial	^{14}C-Dieldrin	—
Gillett and Gile (1976)	1 × 0.75 × 0.61 m glass box; Plexiglas lid	—	Sand, gravel and potting soil	LD 16:8	21–24	Added as needed from a common water source	Terrestrial and terrestrial/aquatic	DDT, methoxychlor, dyfonate, aldrin, dieldrin	—
Goodyear *et al.* (1972)	244 cm diameter × 45 cm deep; vinyl plastic bottom; corrugated steel side	—	—	Outdoor ambient	—	—	—	Fertilizers	—

Gorden *et al.* (1969)	250-ml Erlenmeyer flasks plugged with cotton	—	—	4 cool white fluorescent, 2 incandescent, and frosted plastic thermal barrier	21 ± 2	—	—	Bacterial activity-effects on primary producers	—
Guthrie *et al.* (1974)	0.33 × 0.50 m, 50-l cylindrical polyethylene tanks	—	—	—	5 regimens	Replace evaporated water daily with autoclaved reservoir water	Reservoir water	Thermal loading	—
Guthrie *et al.* (1979)	Oyster colony	—	Oyster shells	Seasonal	—	—	Jones Bay-Galveston	Heavy metals	—
Harrison and Davies (1977)	2-l borosilicate flat bottom boiling flasks	—	—	50% incident solar (outdoor)	Ambient	10–50% dilution/day	—	Nutrient ratios (Si, N)	Mixing bar, 60 rpm
Hollibaugh (1978)	21 Erlenmeyer flasks; 100 μm mesh	—	—	Dark	—	—	Seawater	Phosphate; silicate trace metals, vitamins	Hand swirling 2 times/day
Isensee *et al.* (1973)	25.4 × 5.2 × 17.8 cm glass aquaria filled to 4-l	—	—	—	22 ± 1	Evaporated water replaced with distilled water	Standard reference water + NH_4NO_3 + K_2HPO_4	^{14}C-labelled; cacodylic acid and dimethylarsine	Constant aeration
Jassby *et al.* (1977a)	700-l cylinder 60.9 cm radius 75.8 cm height fibreglass with non-toxic seal	—	River sand 4 cm deep; particles 0.3–3 mm washed in HCl	High output fluorescent LD 12:12	19 ± 10	Replace evaporated water (1 cm) every week	Demineralized water	Nutrients and organisms	Constant aeration
Jassby *et al.* (1977b)	700-l cylinder 60.9 cm radius 75.8 cm height fibreglass with non-toxic seal	—	River sand 4 cm deep particles 0.3–3 mm washed in HCl	High output fluorescent LD 12:12	19 ± 10	Replace evaporated water (1 cm) every week	Demineralized water	Nutrients; trophic structure	Aerated 1.2 l/min
Johnson and Burke (1978)	14 m^3, fibreglass	—	—	—	—	Flowthrough; 30 day turnover	—	Arsenate reduction	Stirred

Table 1 (*Contd.*)

Citation	Construction design	S/V ratio	Substrate	Light	Temperature (°C)	Flow and turnover rate	Water description	Treatment	Mixing
Kawabata and Kurihara (1978)	300-ml flasks	—	—	3500 lux	24	—	Taub start solution	Nutrients and aquatic oligochaete	—
Kehde and Wilhm (1972)	2.4 × 0.15 × 0.07 m marine plywood coated with white nontoxic enamel	—	—	250 footcandles LD 12:12	30–34	Current, 8 cm/s	Natural stream water	Nitrate/phosphate	—
Kersting (1975)	3 compartment Plexiglas A = 7 l B = 175 ml C = 175 ml	—	Sand with organic material removed	A = LD 14:10 fluorescent lamps B = shielded C = completely dark	18	600–700 ml/day	Filtered water from eutrophic lake	Diuron (herbicide)	Compartment A is stirred
Kevern and Ball (1965)	Stream riffle pool (2 × 20 cm) 246-l water recycled	—	—	Incandescent	Controlled to +1	Flowthrough system but details not recorded	Distilled water and inorganic nutrients	Temp, light, flow, photoperiod, and chelator	
Knaus and Curry (1979)	8 m × 1 m × 12 cm wood coated with fibreglass	—	Water saturated sod	—	—	50 l/min	Distilled water and Mn	Radioactive tracer ^{56}Mn	—
Leffler (1977)	500-ml Erlenmeyer flasks stopped with cotton and cheesecloth	—	—	Cool white fluorescent light with frosted plastic plates. 1900 ft candles	24 + 1	2	250-ml of Taub multivitamin solution in each flask	Bacterial, algal, protozoan and metazoan taxa injected into 10 systems. Systems were exposed to a variety of stresses to evaluate their response	—

Leffler (1978)	—	—	—	—	—	—	—	Radiolabelled nutrients ^{59}Fe, ^{32}P	—
Lichtenstein *et al.* (1978)	49 × 39 × 26 cm stainless steel, compartmentalized	—	Lake mud and soil	—	—	125 ml/min terrestrial + aquatic	Simulated rain	^{14}C-phosphate	—
Metcalf *et al.* (1971a)	10 × 12 × 20 in glass aquaria	180 in²/71	Washed quartz sand	5000 foot-candles	80°F ± 1	—	Standard reference water	Radiolabelled DDT, DDE, DDD Methoxyclor	Aeration
Metcalf *et al.* (1973) and Lu *et al.* (1975)	Glass aquaria	—	Sand	LD 12:12	86°F ± 1	—	Aquatic/ terrestrial	DDT, methoxychlor, and DDT analogues; lead, cadmium, and sewage sludge	—
Neill (1972)	Crystallizing bowls (18.5 × 10 cm) 500 ml of mixed algae cultures added. Clear window glass plates placed on top of bowls	—	—	Bank of 10 fluorescent lamps cool white, 40 watt; 300 (± 2.5) foot candles LD 12:12	27 + 1.5	—	Unchlorinated well water	Fish introduced into microcosm to study predation	Every 4 days with large paddle in plastic can and randomly redistributed after mixing. This procedure was followed for 17 weeks. For the next 18 weeks, cultures were mixed every 7 days using the same method. No mixing after treatment began
Neill (1974)	2-l crystallizing bowls with 1500 ml of water	—	—	—	—	—	—	Interspecific competition	4–7 days homogenization of stock cultures

Table 1 (*Contd.*)

Citation	Construction design	S/V ratio	Substrate	Light	Temperature (°C)	Flow and turnover rate	Water description	Treatment	Mixing
Neill (1975)	2-l crystallizing bowls	—	—	White fluorescent lamps	27 + 1.5 −20	—	Unchlorinated well water	Interspecific competition	4–7 days initial mixing. No mixing after start of experiment
Nixon (1969)	Series I: 2 750-ml Roux flasks with cork stoppers, fitted with spinal needles for inoculation	—	—	Bank of fluorescent lights, 2150 lux LD 12:12	25	—	10% salt and Guillard's Medium (f)	Successional changes of gnotobiotic microcosms observed over time in aerated microcosms with seawater medium (10% salinity) and brine water (15% salinity)	Slow bubbling with air for 2–3 weeks
	Series II: 5 750-ml with 25 ml of seawater with sea salts and Guillard's Medium (f). Rubber stoppers with small tubes with removable cap, plugged with cotton and cheesecloth over ends of tubing to prevent predation by shrimp	—	—	As in Series I	As in series I	—	Seawater with sea salt and Guillard's Medium	Successional changes in non-aerated gnotobiotic microcosms over time in enriched seawater	No mixing at any time
Odum and Hoskin (1957)	400-ml recirculating stream apparatus	—	Plastic screen windows	150 watt photo flood lights	—	0.1–0.08 m/s; 10 min circulation	—	Light, temp., flow on aufwuchs	Aeration

Ollason (1977)	100 × 600 mm glass cylinder	—	—	20 watt fluorescent bulbs; adjusted to 3 light regimens	20 + 1	20 h sampling; 450 ml/day with addition of nutrient	Distilled water	Light	Aeration
Patten and Witkamp (1967)	600-ml, 4-cm dia. glass	—	Silt-loams	—	25	2–3 days addition of sterile water	—	12% ethylene oxide	Aeration
Perez *et al.* (1977)	166 l, 19.5 × 9 × 21 cm plastic polyvinyl chloride	Simulates ambient	Narragansett Bay sediment	Cool white fluorescent lamps	Ambient	1.4 cm/s	Narragansett Bay water	Variation in water turnover, turbulence incident radiation and ratio of pelagic volume to benthic volume	Electric motor
Porcella *et al.* (1975)	75 × 15 cm lucite	—	Lake sediment	50 fluorescent lamps	25 ± 2	Daily removal of 10% of the water; replace with nutrients	Deionized water	Light, nutrients, mercury, nitrogen	Water mixed with magnetic stir bar
Ramm and Bella (1974)	3-1 and 500-ml glass Erlenmeyer flasks	—	Sediment	Dark	20	None	Seawater	NaCl, NaOH, Na_2S, Na_2SO_4, $BaCl_2$	Prior to sampling
Richardson (1930)	60 1-2 gal, 1 10-gal, and 1 20-gal glass aquaria	—	—	Full sunlight for part of the day was most successful	—	—	—	—	Non-circulating
Ringelberg (1977)	100 l autotrophic: 7.5 l heterotrophic; 56 l decomposer chamber	—	—	—	17–19	0.3 and 0.9 $l\,h^{-1}$	—	Addition of toxic substances	Magnetic stirrer
Samsel *et al.* (1972)	15 500-ml flasks	—	—	LD 12:12	—	None	Sterilized pond water	^{60}Co irridation 5 ppm, 10 ppm, and 15 ppm oxygen stresses	30 days homogenization prior to experiment
Scura and Theilacker (1977)	20-l glass carboys, 10-l container for fish	—	—	500–700 foot candles	23–25	—	Enriched seawater	Chlorinated hydrocarbons	Aeration

Table 1 (*Contd.*)

Citation	Construction design	S/V ratio	Substrate	Light	Temperature (°C)	Flow and turnover rate	Water description	Treatment	Mixing
Sebetich (1975)	11.5-cm specimen dishes, glass	—	Glass cover slips and microscopic slides	129 lux fluorescent; LD 16:8	20	0.00029 ^{32}P in microcosms containing water and a snail; 0.1035 ^{32}P in the 4 compartment microcosms	Lake Nelson NJ; distilled water	Radio phosphorus treatment	Diatoms aerated prior to experiment
Taub (1976)	Glass bottles	—	—	LD 12:12	25	—	Filtered autoclaved arboretum pond water	Interspecific competition	—
Taub and Crow (1980)	Gallon jars, glass	—	Synthetic sediment	—	—	None	Synthetic lake water	Cropping, competition	
Thomann *et al.* (1973)	—	—	Sediment	—	25	—	Local saline and brackish water	Mixture of pesticides added weekly	—
Trabalka and Eyman (1976)	Photographic tray with surface area 0.2 m^2	—	Field collected sediment	Banks of 3 fluorescent lighting: 1 operated on LD 12:12; 2 on continuous cycle	19 ± 1	Maintained with the addition of spring water diluted with distilled water	Mixture of spring and distilled water	Plutonium 237 nitrate	—

Walter-Echols and Lichtenstein (1977)	18 × 6 cm glass cylinder	—	Agricultural soil, lake mud	14 days dark	20 ± 2	—	Tap water	Phorate sulphoxide, phorate sulphone, phoratoxon, phoratoxon sulphoxide, and phoratoxon sulphone	—
Ward and Matsumura (1978)	20-ml glass culture tubes with untightened screw caps	—	5 g sediment	—	24	—	Lake water approx. 18 ml/tube	Filtered through Whatman No. 1 filter paper; some with nutrients added, TCDD	—
Whittaker (1961)	228-l indoor aquaria; 9.15 × 92 cm outdoor aquaria glass, porcelainized steel, concrete	—	Sediment and rocks	Fluorescent light	22–26	—	Uncontaminated river water	^{32}P	—

— Not discussed in text.

Table 2 Biological characteristics of a small microcosm. Copyright © 1982, Electric Power Research Institute, EPRI EA-2283, *Feasibility of Large-Scale Aquatic Microcosms*. Reprinted with permission

Citation	Inoculation	Duration/maintenance	Biological categories	No. species present
Abbott (1967)	Bay water and sediments	Nutrients were added on day 1 and 147 days later	Phytoplankton, molluscs, snail	At least 3 present
Adler *et al.* 1980	Bay water and sediments	220 days	Phytoplankton and benthic organisms	
Admiraal (1977)		Water replaced 1/wk; 1 container with silicate enriched water pumped in 5–10 days	Bivalves, phytoplankton, diatoms	4 benthic diatoms
Allen and Brock (1968)	Inoculum of mixed flora from various sources		Bacteria, protozoa	Unknown
Barsdate *et al.* (1974)	3 flora/fauna assemblages and ground *Carex* leaves		Bacteria, *Tetrahymena*	Mixed natural flora and fauna
Beyers (1962)	1-l sediment and 3-l water and 30-g *Valisneria*	1st month—cross seeding; water level maintained with distilled water	Plants, algae, protozoa, oligochaetes, snails	4–7 species per cycle
Bourquin *et al.* (1974)	Salt-marsh mud and water	After 7 days malathion treatment. Samples on 10, 20 and 30 days	Plants, decomposers	Unknown
Bryfogle and McDiffett (1979)	Algae, autoclaved pond water, and 60-ml mixed stock inocula diluted to 2:1 by adding distilled water	85 days	Algae, phytoplankton	3 *Chlorella, Coelastrum, Ankistrodesmus*
Child (1972)	Spanish moss festoons with associated flora and fauna	200 days	Spanish moss (producer), arthropods (consumer), diptera (consumer)	75 species present
Confer (1972)	Unfiltered pond water and fertilization	3 series: I—5 months, II—8 months, III—13 months	Filamentous algae, planktonic algae, snails, ostracods	12 after 3 months' operation

Cooke (1967)	Taub F & G solutions, fish tank water, 100 mg $CaCO_3$, 0.07 mg of laboratory stock microcosm	100 days—no maintenance except adding of distilled water	Autotrophs (algae, diatoms), heterotrophs (ostracods, copepods, daphnids)	25 genera
Cooper (1970)	—	8 months	Zooplankton	18 species
Cooper (1973)	Filtered pond water and 250-g autoclaved sediments	Initial 2 days aeration; cross-culture daily for 2 weeks; after P/R > 1 herbivore added	Algae, zooplankton, fish	7
Crouthamel (1977)	Aged tapwater, 1-l inoculum of water with mixture of planktonic algae	12 vessels were mixed together and redistributed 1/week for the 1st 6 weeks after start. After a 6-week homogenization period, contents were maintained and isolated.	Microcrustaceans, fish	25 individuals of 9 different species
Davis *et al.* (1977)	Unfiltered seawater from tidal estuary	Harvest periods at 30, 60, and 120 days	Phytoplankton, macrophytes, benthic invertebrates, fish (one instance)	—
DePinto *et al.* (1980)	Adirondack acid water and sediment and slurry of various neutralizing materials.	Brief mixing before water chemistry samples every 3 days for 48 days	—	—
Dolen and Wagner (1978)	—	60 days	Algae, fungi, protozoa, bacteria	—
Eichenberger (1972)	0, 1, 5, 12% sewage added to outdoor groundwater stream simulator	20 days/test	Autotrophs, heterotrophs	—
Elmgren *et al.* (1980)	Narragansett Bay water and sediment; continuous flow and intermittent mixing	> 1 year	Phytoplankton, zooplankton, tunicates, macrobenthos, meiobenthos, microbenthos	15

Table 2 (*Contd.*)

Citation	Inoculation	Duration/maintenance	Biological categories	No. species present
Evans and Henderson (1977)	Sediment scoops from two sites in Kaneohoe Bay transferred to 2 flowthrough microcosms	135 days and 92 days—conditions simulate ambient	Polychaetes, crustacea, oligochaetes, insects, nematodes, sponges, diatoms	34
Everest (1978)	18 × 18 × 25 cm sections of salt marsh with associated organisms compose terrestrial part; aquatic reservoir is 1 part seawater; 9 parts synthetic seawater allowed to stabilize two weeks	Treatment ^{32}P non-destructive radioassay at 1, 2 and 4 tidal cycles after intro of ^{32}P and at 4, 8, 16, and 32 days. Destructive radioassay at 2, 4, and 8 weeks	Algae, diatoms, rooted macrophytes, molluscs, arthropod, annelid, insects	3 identified others present
Ferens and Beyers (1972)	Half flasks received irradiated inocula; 1/2 control inocula	40 days; microcosms sacrificed every 3 days	Algae	2
Fraleigh (1971)	5.0-ml of 'climax' microcosm into 250 ml fresh medium. Phosphorus added in the initial medium of 1/2-strength Taub's-36. Nitrate, thiamin, and vitamins added to stock solution	After inoculation, constant temp maintained (20 ± °C). Microcosms were kept in shallow trays of water to maintain high humidity. Light regimen used LD 12:12. Sodium concentration altered to provide a constant sodium to potassium ratio	Algae, rotifers, ostrocod	8
Fraleigh (1978)	Taub medium (with nitrate and vitamins) inoculated with 5 ml of climax microcosm and varying P concentrations	Batch microcosms sampled at intervals for standing crop, biomass, and chlorophyll at 80-day intervals.	Algae, rotifers, ostracod, bacteria	8
Fraleigh and Wiegert (1975)	Outdoor microcosm from a thermal spring algal community	—	Algae, grazers, brineflies	—

Giddings and Eddlemon (1977)	Sand substrate, spring water, algal inoculum, and adult snails	Distilled water replaced evaporated water. 7 weeks equilibration; then contaminant added—conclusion 140 days	Filamentous algae, benthos, snails, insects, zooplankton	—
Giesy (1978)	Stream microcosm (EPA-ERDA), leaf litter envelopes incubated with cadmium contaminated stream water	28 weeks	Aquatic macrophytes, periphyton, micro and macroinvertebrates, fish, terrestrial organic detritus	6 mentioned
Gile and Gillett (1979)	Planted rye grass on 1/2 surface and alfalfa seed planted on other half. Seeds covered with soil mixture and washed sea sand. Dieldrin fate tested. (Terrestrial microcosm)	Organisms added throughout experiment	Gastropods, insects, crustaceans, earthworms, nematodes, vole, plants	—
Gillett and Gile (1976)	Terrestrial microcosm added soil mixture; start light/temp cycle; 3 days	Day 0 add pesticide, plant seeds, per schedule; 56 day termination	Grasses, annelids, nematodes, molluscs, insects, mammals, birds, reptiles	12 species present
Goodyear *et al.* (1972)	5 levels of fertilization tested in flowthrough system with 15 pools; all pools receive initial plankton inoculum collected near Savannah River plant	May 26 initial inoculum and fert.; 2 Jun add mosquito fish; 13 Aug 2nd fert; 10 Sept terminate	Plankton, invertebrate fish	*Gambusia affinis* and other (actual number unknown)
Gorden *et al.* (1969)	2.5-ml well water mixed with mature microcosm	—	Phytoplankton, bacteria	—
Guthrie *et al.* (1979)	Inocula from 2 sources (Lakes Keowee and Hartwell) 1 tank heated, 1 ambient	Evaporated water replaced daily	Bacteria	Lake Keowee: 10, Lake Hartwell: 17, Bacterial genera

Table 2 (*Contd.*)

Citation	Inoculation	Duration/maintenance	Biological categories	No. species present
Guthrie *et al.* (1974)	Oyster colonies from 2 bays were collected as examples of naturally occurring microcosms	Oyster colony samples iced and returned to lab in 6 hours; flesh of all organisms removed, emulsified, and analysed for heavy metals	Molluscs, arthropods, polychaetes	5 species noted
Harrison and Davies (1977)	Natural phytoplankton assemblage from an existing controlled experimental ecosystem	2–3 weeks per experiment	Phytoplankton	6
Hollibaugh (1978)	Phosphate, silicate, vitamins, and trace metals added to raw seawater samples from Halifax Harbour	After pretreatment, water was separated into flasks. Substrate added to flasks. Stock solutions were prepared for each experiment	—	—
Isensee *et al.* (1973)	Batch aquatic microcosms inoculated with *Daphnia*, *Physa*, and *Gambusia* plus aged aquarium water; 5 days later herbicides added	1 ml water sample; 2 days for radioactive analysis; exposure 32 days; microcosm terminated	Protozoa, diatoms, rotifers, snails, fish, cladocerans	4 identified, others present
Jassby *et al.* (1977 a,b)	Batch microcosms enriched with nutrients and inoculated with 3.5 l of lake water plus 70.0 l of demineralized water	100-day duration	Diatoms, protozoa, rotifers, cladocerans, ostracods, copepods, periphyton, insects, molluscs, fish	22 species present
Johnson and Burke (1978)	30-m sediment and/or a 4.5-m water column. Water samples collected weekly from MERL microcosm experiment. (Benthic/pelagic microcosm)	Flowthrough system with bay water being replaced approx. 30 days	Phytoplankton	2

Kawabata and Kurihara (1978)	Batch microcosms	40-day duration	Bacteria, algae, protozoa	—
Kehde and Wilhm (1972)	Natural stream water plus nutrients; closed system with flow pumps	Complete mixing for 9-day colonization period; snails added for grazing; 92-day duration	Periphyton, blue-green algae, molluscs, green algae	6 algal genera; 1 snail—*Physa gyrina*
Kersting (1975)	Flowthrough microcosm compartmentalized A, B, and C are inoculated (lake water) with algae; B with *Daphnia*; C with bacteria substrate (sand)	Comp. A is lighted, B-shield, C-dark after 1 wk, toxicant added; duration 10 wks.	Algae, zooplankton, bacteria	2 (without bacteria)
Kevern and Ball (1965)	Recirculating stream microcosm; distilled water and inorganic nutrients seeded by introducing periphyton-covered stones from warm water streams	Periodic addition of nutrients; colonization period of 10 months, after which physical/chemical perturbations were tested.	Algae, bacteria	3
Knaus and Curry (1979)	Recirculating stream microcosm inoculated with water-saturated sod and black willow shoots	One single pulse of ^{56}Mn added to water samples daily for 20 days	—	1
Leffler (1977)	Batch microcosms inoculated with laboratory aquaria water, farm pond, cemetery urns, and established microcosms	Cross-seeding for 2 weeks and subsequent 2 months for development; 4 types chosen plus reference microcosm maintained for about 2 1/2 years	Green algae, blue-green algae, diatoms, protozoa, bacteria	Type A—10 species Type B—17 Type C—21 Type D—25
Lichtenstein *et al.* (1978)	Compartmentalized terrestrial aquatic; soil added to terrestrial chamber; lake mud to aquatic chamber; corn planted after 28 days; fish and aquatic plants added after day 44	Aged pesticide residue and fresh tested simulated rainfall on days 42 and 49; terrestrial chamber terminated day 50; aquatic terminated on day 70	Plants and fish	3

Table 2 (*Contd.*)

Citation	Inoculation	Duration/maintenance	Biological categories	No. species present
Metcalf *et al.* (1971a)	Terrestrial/aquatic; day 1—sand and water organisms added at intervals	Day 2—*Daphnia*, snails, algae, sorghum and plankton culture; day 26—mosquito larvae; day 30—mosquitofish added; terminated on day 33	Algae, protozoa, snail, diatoms, insects, fish	19
Metcalf *et al.* (1973)	Terrestrial/aquatic; day 1—sand and water organisms added at intervals	Day 2—*Daphnia*, snails, algae, sorghum and plankton culture; day 26—mosquito larvae; day 30—mosquitofish added; terminated on day 33	Algae, protozoa, snail, diatoms, insects, fish	—
Neill (1972)	Batch microcosms inoculated with stock culture obtained from Texas ponds and lakes	Cross-seeding every 4–7 days for more than 8 months until no statistical difference detected between populations; defined as equilibrium	Algae, crustacea, bacteria	32 (without bacteria)
Neill (1974)	Batch microcosms inoculated with stock culture obtained from Texas ponds and lakes	Cross-seeding every 4–7 days for more than 8 months until no statistical difference detected between populations; defined as equilibrium	Algae, crustacea, bacteria	35 (without bacteria)
Neill (1975)	Fish introduced and allowed to feed for 45 min every 3 days	Stock cultures uncontrolled by light and temp., and nutrient conditions to provide variation. Weekly additions of new individuals	Algae, fish, crustaceans	20 green and blue-green algae

Nixon (1969)	Batch microcosms; inoculated with pure cultures of algae, bacteria, and brine shrimp (gnotobiotic system)	Series I—aerated; Series II—unaerated, Crombie tube added in Series II to prevent overgrazing; 200 days	Algae, bacteria, crustaceans	8
Odum and Hoskin (1957)	Recirculating stream microcosm inoculated with a blade of eel grass and water from Silver Spring, Fla., plus inorganic fertilizer	—	—	—
Ollason (1977)	Flowthrough microcosm inoculated with batch cultures from horse trough	Fresh nutrient (400 ml) added daily; photoperiod 4 days; samples taken every 12 h; 60-day duration	Algae, protozoa, rotifers, bacteria	—
Patten and Witkamp (1967)	4 batch microcosm inocula types: (a) sterile leaves; (b) leaves with microflora; (c) leaves, microflora and millipedes; (d) same as c placed on 1-cm soil	8-ml sterile water added every 2–3 days with 10-cm Hg vacuum; terminated after 18 days	Microflora and millipedes	—
Perez *et al.* (1977)	Batch microcosms; inoculated with filtered (1-mm mesh) seawater and benthic box filled with Narragansett Bay sediment, including organisms	Maintenance to simulate ambient conditions closely (30–35 day duration)	Diatoms, algae, protozoa, benthic epifauna and infauna, ctenophores, zooplankton	Species not identified
Porcella *et al.* (1975)	Sediment water microcosms filled with 15-cm of sediment and 9-l water.	10% volume water replaced with fresh media which was 5°C cooler than microcosm; gas pressure readjusted; 189 day duration	Insects, green algae, blue-green algae, and ostracods	Species not identified

Table 2 (*Contd.*)

Citation	Inoculation	Duration/maintenance	Biological categories	No. species present
Ramm and Bella (1974)	Extract of algae with soluble carbon; O_2 removed and pH adjusted to 7.5–8.0; 2-ml inocula containing anaerobes added; flask shaken to disperse inocula	Flasks sealed air tight, incubated in dark at 20 °C, samples withdrawn with a syringe	Anaerobes	Not identified
Richardson (1930)	Water collected from river with abundance and variety of insect larvae, snails, worms, crustacea, small invertebrates. Fish introduced	5 years	Insect larvae, worms, crustacea, small invertebrates, fish	—
Ringelberg (1977)	Autotrophic chamber inoculated with *Chlorella vulgaris* and *Scenedesmus quadricauda*—herbivore chamber inoculated with *Daphnia* sp.	3 years +	Algae, herbivore, decomposers	7 species noted on 180 day of experiment
Ringelberg and Kersting (1978)	3-compartment microcosm: filtered (0.45 μm) lake water and 2 pure algal cultures: *Daphnia* in herbivore chamber (large scale *vs* small scale compared)	Recirculating pump exchanges water and nutrients; lake water replaces samples distilled water to replace evaporation: autotrophic chamber stirred constantly; duration 720 days for large, 400 days for small	Algae, diatoms, bacteria	10 identified species, other present

Samsel *et al.* (1972)	Sterilized pond water inoculated with equal numbers of copepod, cladoceran, ostracod, plant, and pure culture of green algae and *Euglena*	Before treatment—equilibration time 1 week; treatment (O_2 and gamma rays); microcosms maintained at constant temp and photoperiod for 6 weeks	Copepod, cladoceran, ostracod, green algae, aquatic plants, protozoa	4 identified species, 2 genera
Scura and Theilacker (1977)	Algae and rotifers cultured in a medium to which chlorinated hydrocarbons were added. Rotifers fed to anchovy larvae	Algae kept in constant light: day 10 rotifers added; day 20 anchovy larvae hatch; day 25 rotifers fed to anchovy; and day 45 termination.	Phytoplankton, rotifers, dinoflagellates	3 discussed
Sebetich (1975)	2, 3, and 4 compartment microcosms; 7 combinations of sand, water, snail and diatoms tested	Equilibration time 1 day for those containing snails and diatoms; for those containing both sand and water and snail/diatom equilibrate separately for 1 day	Snail, diatom	Species not identified, 2 genera
Taub (1976)	Batch microcosms with nutrients added inoculated with algae and mixture of grazers	After inoculation, microcosms perturbed and monitored for 5 weeks	Algae, protozoa, bacteria, rotifers, ostracods	7 species identified, others present
Taub and Crow (1980)	Synthetic lake water and sediments. 10 species of algae inoculated on day 0. Grazing began on day 4 with introduction of animals. Protozoa and rotifers added and ostracods and amphipods individually added	—	Algae, protozoa, rotifers, ostracod, amphipod	18 species, plus bacteria and other microorganisms

Table 2 (*Contd.*)

Citation	Inoculation	Duration/maintenance	Biological categories	No. species present
Thomann *et al.* (1973)	Saline and brackish water and sediment with associated microflora and fauna	Pesticides added weekly at 50 μg/ml. Mineral salt with yeast extract used to isolate bacteria. Pesticide levels monitored monthly	Bacteria	—
Trabalka and Eyman (1976)	Water in 1-year old microcosm inoculated with plutonium-237 nitrate	Maintained with the addition of spring water, diluted with distilled water to prevent mineral build-up. 90-day duration	Aquatic macrophytes, algae, goldfish, snails amphipod	12 genera observed
Walter-Echols and Lichtenstein (1977)	Phorate sulphoxide treated loam soil, slowly added to tap water	2 week incubation period	—	—
Ward and Matsumura (1978)	^{14}C-TCDD added to two batch microcosms	Incubation periods from 1 hour to 589 days	—	—
Whittaker (1961)	^{32}P used as a tracer to monitor phosphate ions in water with H_3PO_4	—	Insects, fish	6

—Not discussed.

situation is of little or no concern, for example, measuring gross distribution of a xenobiotic chemical in system compartments.

2.4 Implementation of Microcosm Testing

The experimental design of a microcosm test depends on the objectives of the experiment which may be: (1) the testing of hypotheses (deductive) or (2) exploratory (inductive) testing. Experimental design is a pragmatic, case-specific problem. Standardization of microcosm design is of limited value.

Microcosms are subsets (models) of ecosystems, not miniature ecosystems. Realism and replicability of microcosm experiments have to be defined and interpreted in this context.

Results of microcosm experiments are incomplete without appropriate verification (for example, in field tests).

There are 15 points that summarize the current status of microcosm applications in ecotoxicology.

(1) It has yet to be determined which ecosystem variables are most suitable for specific microcosm application.
(2) Microcosms are limited by scale relationships (size dependencies of organisms and area : volume ratios) and are unstable in time.
(3) Microcosm design requires an ecosystem perspective.
(4) As complexity of microcosm design increases, realism and cost tends to increase and precision tends to decrease.
(5) There are few *a priori* rules for the inclusion of common factors in microcosm design; generic-design microcosms are of very limited use.
(6) Microcosms have a place in a tiered scheme of environmental hazard assessment of chemicals.
(7) Microcosm experiments are of limited use until they are validated by field experiments.
(8) Mathematical models must play a central role in all environmental hazard assessment because of the inherent limitations of empirical data.
(9) Microcosms provide information at the process level; they are subsets of ecosystems, not miniature ecosystems. Processes include energy relationships, nutrient cycling, chemical transformations, and organism responses at the population or community level.
(10) Both 'synthetic' (gnotobiotic) and 'natural' (excised or reconstructed) microcosms are of potential use in hazard assessment.
(11) Population or community level metabolic processes are more appropriate than organism-level processes for environmental hazard assessment with microcosms.
(12) Criteria for employing results from microcosm tests in the regulatory process have not been developed.

(13) The usefulness of microcosms for routine screening of potentially hazardous substances is not clear.
(14) No laboratory system or microcosm is ready for use as a test protocol or for prediction of the environmental hazard of a toxic chemical.
(15) Some specific microcosms tests can now be recommended for development of hazard assessment:
  (a) Plant–microbe interactions
  (b) Arthropod population interactions
  (c) Soil core preparation
  (d) Predator–prey interactions

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