PNL-3640
UC-11

The Incipient Toxicity of Lithium to Freshwater Organisms Representing a Salmonid Habitat

R. Emery
D. C. Klopfer
J. R. Skalski

July 1981

Prepared for the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Operated for the U.S. Department of Energy
by Battelle Memorial Institute

Battelle

PNL-3640

PACIFIC NORTHWEST LABORATORY
operated by
BATTELLE
for the
UNITED STATES DEPARTMENT OF ENERGY
Under Contract DE-AC06-76RLO 1830

Printed in the United States of America
Available from
National Technical Information Service
United States Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22151

Price: Printed Copy $_____*; Microfiche $3.00

*Pages	NTIS Selling Price
001-025	$4.00
026-050	$4.50
051-075	$5.25
076-100	$6.00
101-125	$6.50
126-150	$7.25
151-175	$8.00
176-200	$9.00
201-225	$9.25
226-250	$9.50
251-275	$10.75
276-300	$11.00

PNL-3640
UC-11

THE INCIPIENT TOXICITY OF LITHIUM TO FRESHWATER ORGANISMS REPRESENTING A SALMONID HABITAT

R. Emery
D. C. Klopfer
J. R. Skalski

July 1981

Prepared for the
U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Richland, Washington 99352

ABSTRACT

Eventual development of fusion power reactors could increase the mining, use and disposal of lithium five-fold by the year 2000. This study has investigated potential effects from unusual amounts of lithium in aquatic environments. Freshwater organisms representing a Pacific Northwest salmonid habitat were exposed to elevated concentrations of lithium. Nine parameters were used to determine the incipient toxicity of lithium to rainbow trout (Salmo gairdneri), insect larvae (Chironomus sp.) and Columbia River periphyton. All three groups of biota were incipiently sensitive to lithium at concentrations ranging between 0.1 and 1 mg/L. These results correspond with the incipient toxicity of beryllium, a chemically similar component of fusion reactor cores. A maximum lithium concentration of 0.01 mg/L occurs naturally in most freshwater environments (beryllium is rarer). Therefore, a concentration range of 0.01 to 0.1 mg/L may be regarded as "approaching toxic concentrations" when assessing the hazards of lithium in freshwaters.

CONTENTS

FIGURES

TABLES

THE INCIPIENT TOXICITY OF LITHIUM TO FRESHWATER ORGANISMS REPRESENTING A SALMONID HABITAT

INTRODUCTION

During the 1960's, scientists became increasingly aware that the element lithium was unusually toxic to plant life. In 1968, the U.S. Federal Water Pollution Control Administration recommended that lithium ion (Li^+) concentrations in irrigation water be limited to 5 ppm (U.S. FWPCA 1968). However, no standards have been established for Li^+ in natural aquatic environments by the Environmental Protection Agency (U.S. EPA 1976).

Natural concentrations of Li^+ in freshwater environments vary with different salinity regimes. Data for major rivers in North America show a median Li^+ concentration of 0.0011 mg/L (Durum and Haffty 1961). Maximum concentrations among these data approached 0.01 ppm. Minimum Li^+ concentrations were below detectability. These observations are consistent with those of water supply specialists who investigated over 100 freshwater systems in the U.S. (Durfor and Becker 1964).

Other investigators reported somewhat lower concentrations of Li^+ in freshwater environments that they investigated. The highest concentrations of Li^+ they reported in some North American ponds and streams approached 0.003 mg/L (Cowgill 1976, Vine 1976). However, in aquatic systems with higher salinity, often identified as brackish waters with total salinity exceeding 250 mg/L, Li^+ concentrations approached 0.1 mg/L (Vine 1976). Of all inland waters considered collectively, highest Li^+ concentrations are in salt lakes and hot springs. In these environments where total salinity exceeded 500 ppm, maximum Li^+ concentrations approach 10 mg/L.

Based on all of this information, it is reasonable to conclude that the "normal spectrum of freshwater environments" (for which this assessment seeks to protect) can tolerate Li^+ concentrations up to 0.01 mg/L.

Effects of Li^+ on aquatic life now becomes a more-important issue because the development and use of fusion reactors will increase the demand for lithium

by several-fold over the next few decades. Fusion rectors will use lithium to generate deuterium and, possibly, as a liquid-metal coolant. The present total demand for lithium by the U.S. is 2.7×10^6 kg/yr. This will likely increase to at least 1.2×10^7 kg/yr by the turn of the century (Vine 1976).

There is 6.9×10^8 kg of lithium presently available in rock ores, and 4.0×10^9 kg in fines associated with salt lakes. However, by the year 2030, these resources may be sufficiently depleted to necessitate extracting lithium from seawater where there is 2.5×10^{14} kg available. For at least the next 50 years, the major source of lithium for fusion technology will be brines associated with inland aquatic environments (Vine 1976).

Compounds of lithium that would most likely enter freshwater environments from mining, refining, fabrication, and use are the carbonates, fluorides, oxides, hydroxides, and sulfates. Fluorides and carbonates of lithium appear to be the most probable environmental contaminants.

Literature on the subject of Li^+ toxicity to aquatic life is sparse. What has been reported is often difficult to interpret. Difficulties lie in answering the fundamental assessment question that pertains to the incipient toxicity of Li^+. Of greatest importance to the initial stages of an environmental hazards assessment is to ascertain a "threshold" or incipient level of toxicity. Above such a concentration biological effects are known to occur, and below it no effects have been recorded. This information is essential for assessing risks and hazards of any chemical substance.

The purpose of this investigation is to determine what biological damage might occur if Li^+ concentrations (as in fluoride and carbonate compounds) exceeded tolerable levels in freshwater environments. The scope of this investigation is limited to habitats of salmonid fishes. Our objective is to identify the lowest concentrations of Li^+ that produces measurable effects in our experimental organisms. Biological parameters for which effects were measured fall into two categories:

1. Rainbow trout (Salmo gairdneri) life cycle:

    a. Egg integrity--The integrity of the eggs' protective membranes in elevated Li^+ concentrations.

b. Fertilization success--The ability of eggs having integrity to become fertilized in elevated Li^+ concentrations.

c. Completion of embryogenesis--The ability of the fertilized eggs to complete embryogenesis in elevated Li^+ concentrations.

d. Hatchability--The ability of surviving trout embryos to hatch in elevated Li^+ concentrations.

e. Fry survival--The ability of sac fry to complete their "buttoning-up" process after having undergone embryogenesis in elevated Li^+ concentrations.

f. Juvenile survival--The ability of juvenile trout to survive elevated Li^+ concentrations.

2. Colonization and habitation of Columbia River biota:

a. Biomass--The ability of periphyton biomass to become established in elevated Li^+ concentrations.

b. Photosynthesis rate--The ability of colonized periphyton to perform photosynthesis in elevated Li^+ concentrations.

c. Insect habitation--The extent to which insect larvae will remain in sediments exposed to elevated Li^+ concentrations.

EXPERIMENTAL APPROACH

Floride and carbonate compounds of Li^+ were used in our toxicity bioassays. The six rainbow trout parameters were examined in an experimental sequence of three test runs. Measurements of the river-biota parameters involved four test runs. Experimental details of these tests are described in Appendix A.

In all tests, the objective was to identify the lowest concentration of Li^+ that produced measurable effects in each parameter. These Li^+ concentrations are defined statistically as LRCT's (Lowest Rejected Concentration Tested, Skalski 1981) using chi-square tests for equal proportions in independent

samples (Tables A1-A3, A5-A7). Analyses of the effects of Li^+ on trout embryos (Tables A1-A3) took into account the successful completion of all previous stages of embryonic development.

RESULTS

Our primary mission was to determine LRCTs of Li^+ using rainbow trout, Columbia River periphyton and chironomid larvae as test organisms. These concentrations defined incipient levels of Li^+ toxicity and provide fundamental reference points for hazards assessment and regulation. Here are the results of our search for LRCTs in nine biological parameters.

Trout Egg Integrity:

LRCTs were relatively high in the first test (76 and 108 mg Li^+/L, Fig. 1). The second and third tests produced LRCTs ranging from 4.3 to 8.8 mg Li^+/L. For this parameter the incipient level of toxicity for Li^+ seems to approach 1 mg/L. Above this concentration the integuments of trout eggs began to deteriorate.

Trout Egg Fertilization:

The process of fertilization was surprisingly tolerant to elevated Li^+ concentrations (Fig. 2). Our test results suggest that Li^+ concentrations of <70 mg/L may actually enhance fertilization--perhaps as a membrane conditioner or as a biocidal agent against hostile microbes. These results appear too consistently to dismiss as pure anomaly. The only LRCTs that could be identified were in tests 1 and 3 in Li_2CO_3 solutions. Respectively, these are 70 and 108 mg Li^+/L, and too high to be of real concern for most assessment situations.

Trout Embryogensis:

Embryogensis displayed a broad range of tolerance to Li^+ (4.6 to 108 mg/L, Fig. 3). Tests 2 and 3 demonstrated that LRCTs could occur below 10 mg Li^+/L. Therefore, Li^+ may be regarded as incipiently toxic to this parameter at concentrations in excess of 1 mg/L.

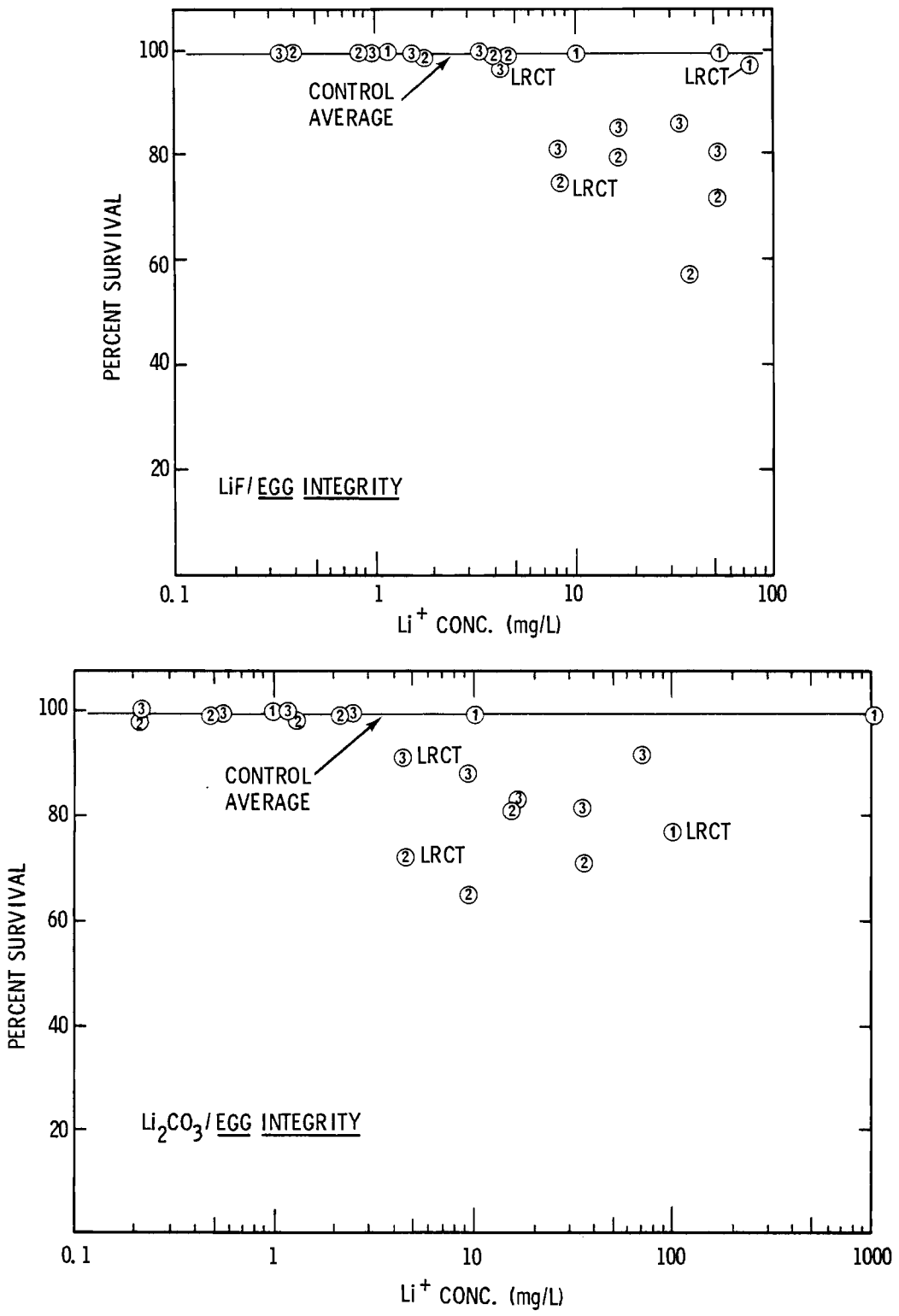

FIGURE 1. Effects of elevated Li^+ concentrations on trout egg integrity. Results (circles) display test numbers respectively.

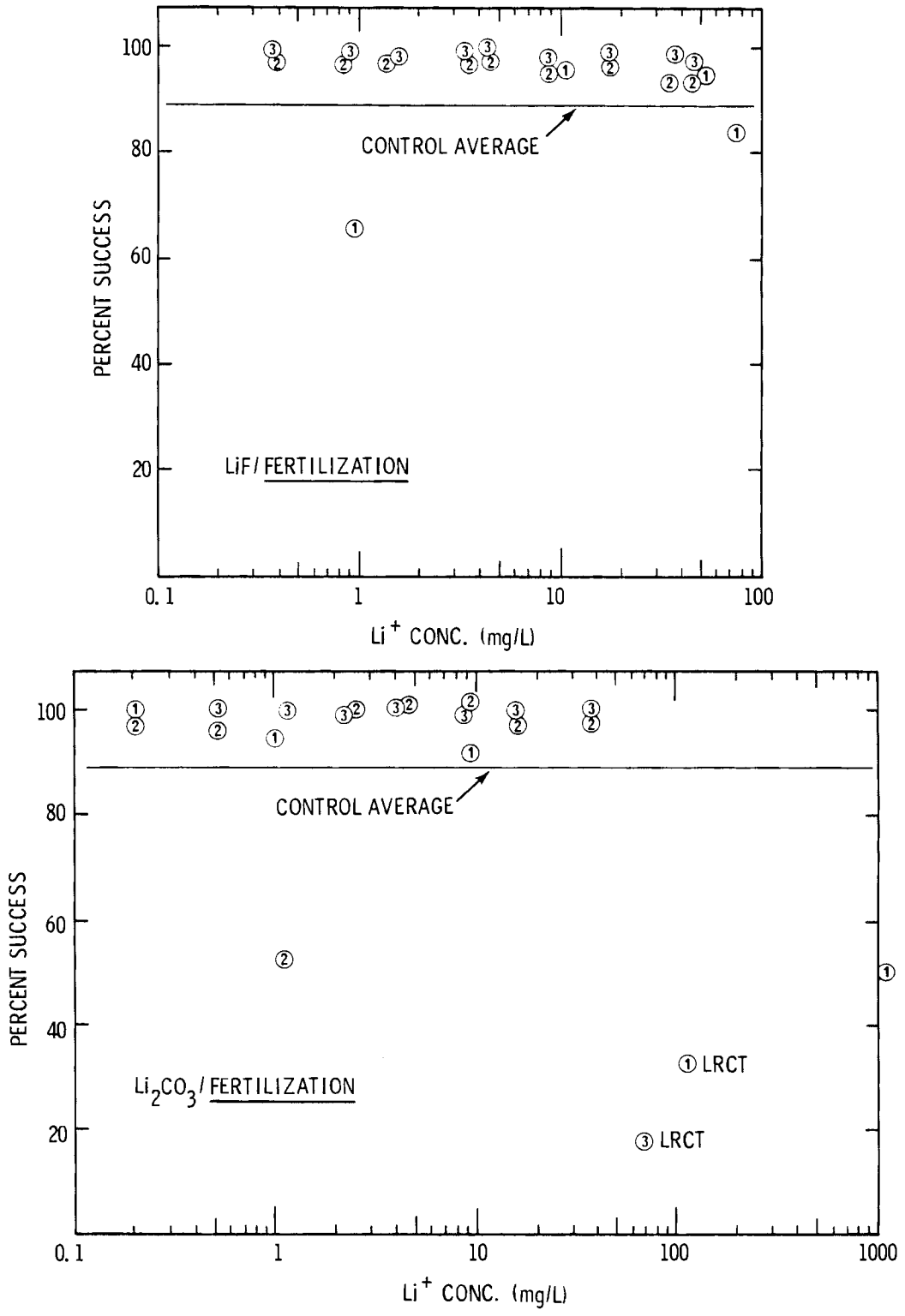

FIGURE 2. Effects of elevated Li^+ concentrations on trout egg fertilization. Circles display test numbers.

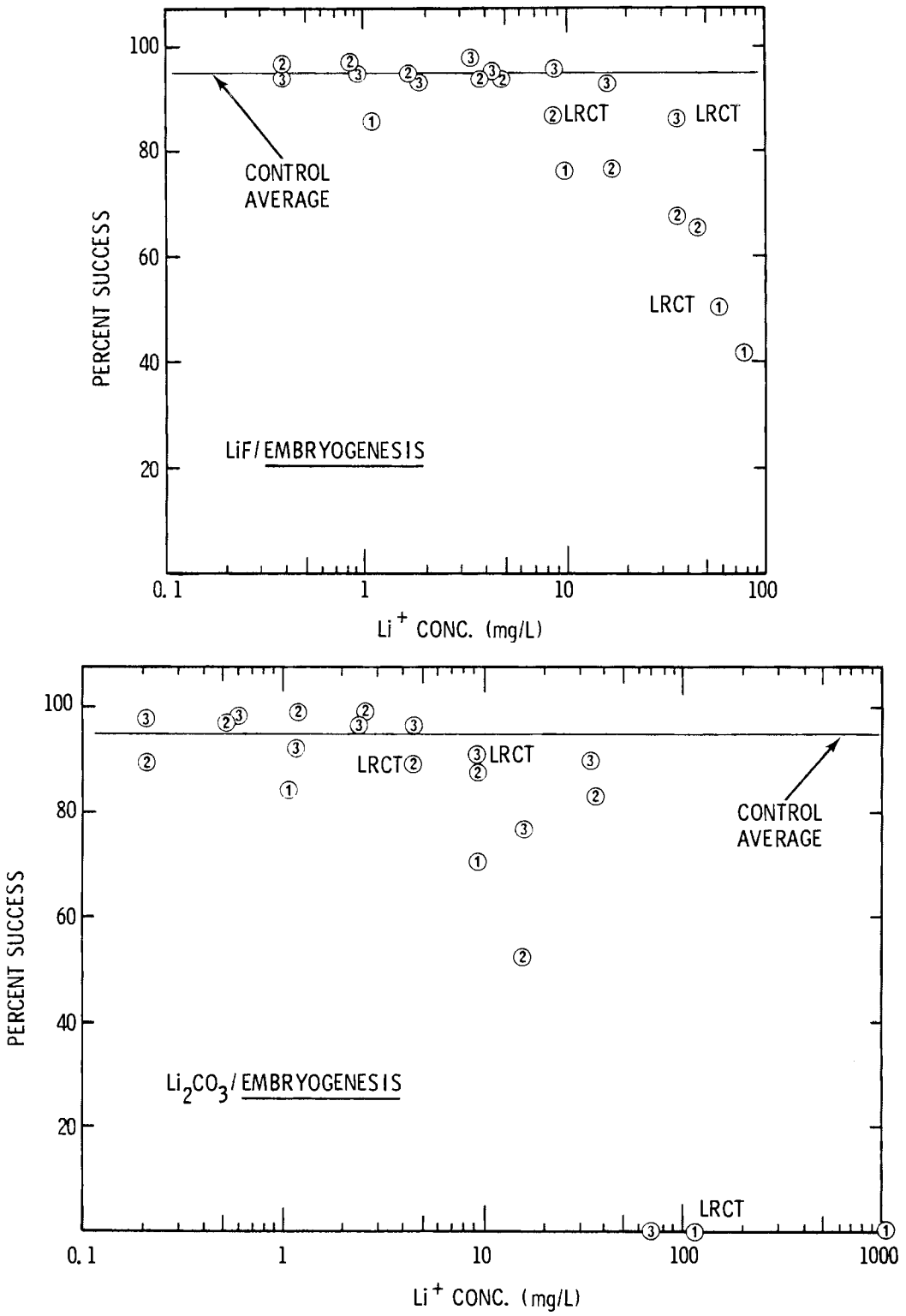

FIGURE 3. Effects of elevated Li^+ concentrations on the success of trout egg embryogensis. Circles display test numbers.

Trout Egg Hatchability:

LRCTs for this parameter fell in a narrow range of 3.6 to 10.1 mg Li^+/L (Fig. 4). The consistency of these observations tends to confirm the others--Li^+ is incipiently toxic to trout eggs about 1 mg/L.

Trout Sac-fry Survival:

LRCTs for this parameters are nearly identical to those of hatchability. They appear in a narrow range from 2.4 to 10.1 mg Li^+/L (Fig. 5). Once again our observations seem confirmed.

Juvenile Trout Survival:

The range of LRCTs for this parameter is narrow (0.6 to 1.3 mg Li^+/L, Fig. 6). Our results show almost complete survival in 0.5 mg Li^+/L, but almost no survival above 1.0 mg/L. This parameter provides evidence that Li^+ may be incipiently toxic below 1 mg/L.

Periphyton Biomass:

Three out of the four LRCTs for this parameter are below 1 mg/L (Fig. 7). All are grouped in a narrow range extending from 0.3 to 1.4 mg/L. These LRCTs are nearly identical to those obtained form juvenile trout bioassays. It also appears that the colonization and growth of periphytic algae is reduced in Li^+ concentrations approaching 0.1 mg/L.

Periphyton Photosynthesis:

This parameter seemed to show inconsistaent response to elevated concentrations of Li^+ (Fig. 8). It's LRCTs range from 3.5 to 27.8 mg Li^+/L. Nevertheless, it appears that Li^+ is incipiently toxic to the photosynthetic process at concentrations approaching 1 mg/L.

Insect Habitation:

The habitation of chironomid larvae was affected consistently by elevated Li^+ concentrations (Fig. 9). LRCTs range from 0.4 to 1.4 mg Li^+/L. These experiments have demonstrated in replication that chironomids are sensitive to Li^+ at concentrations approaching 0.1 mg/L.

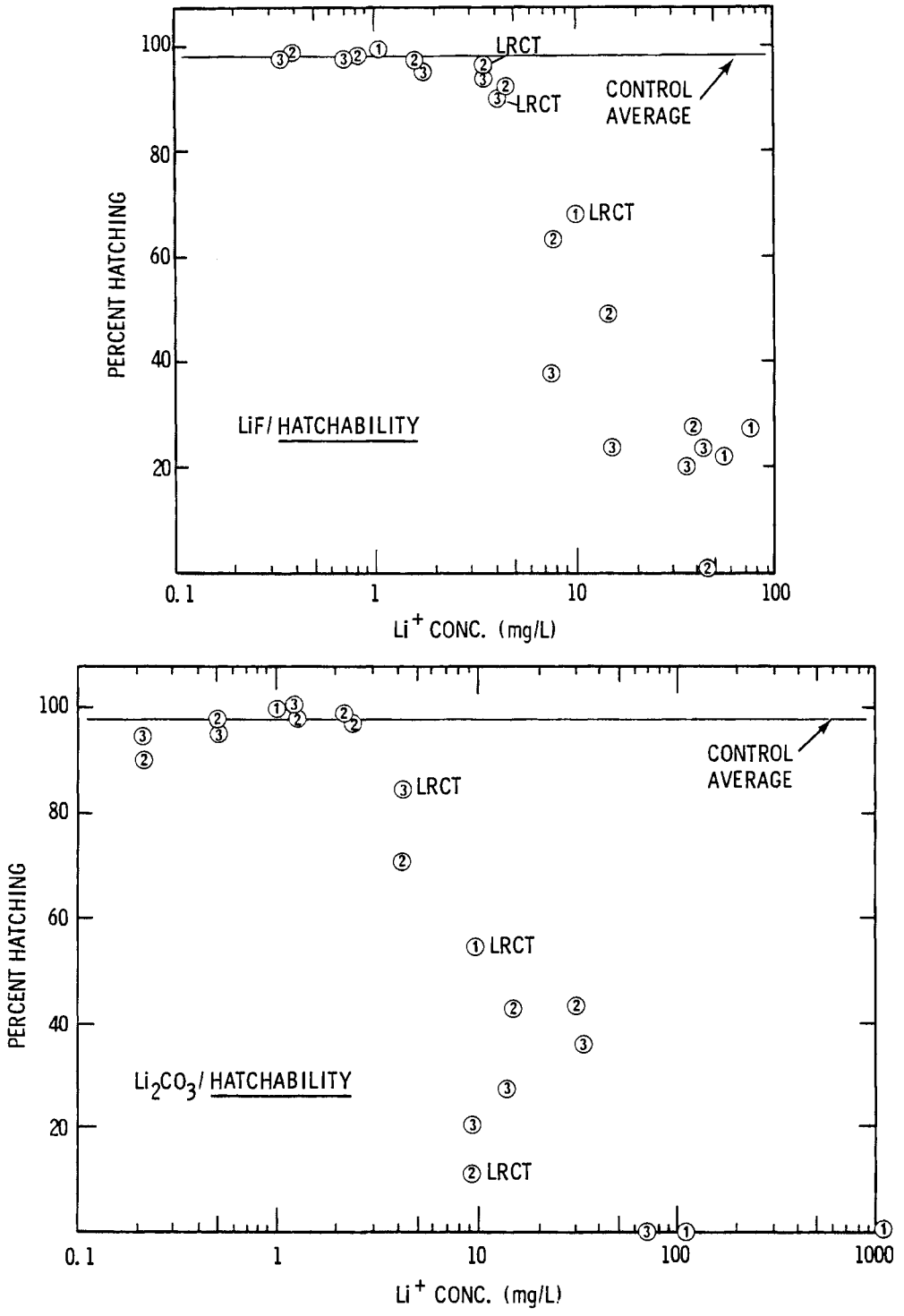

FIGURE 4. Effects of elevated Li^+ concentrations on hatching success of trout eggs. Circles display test numbers.

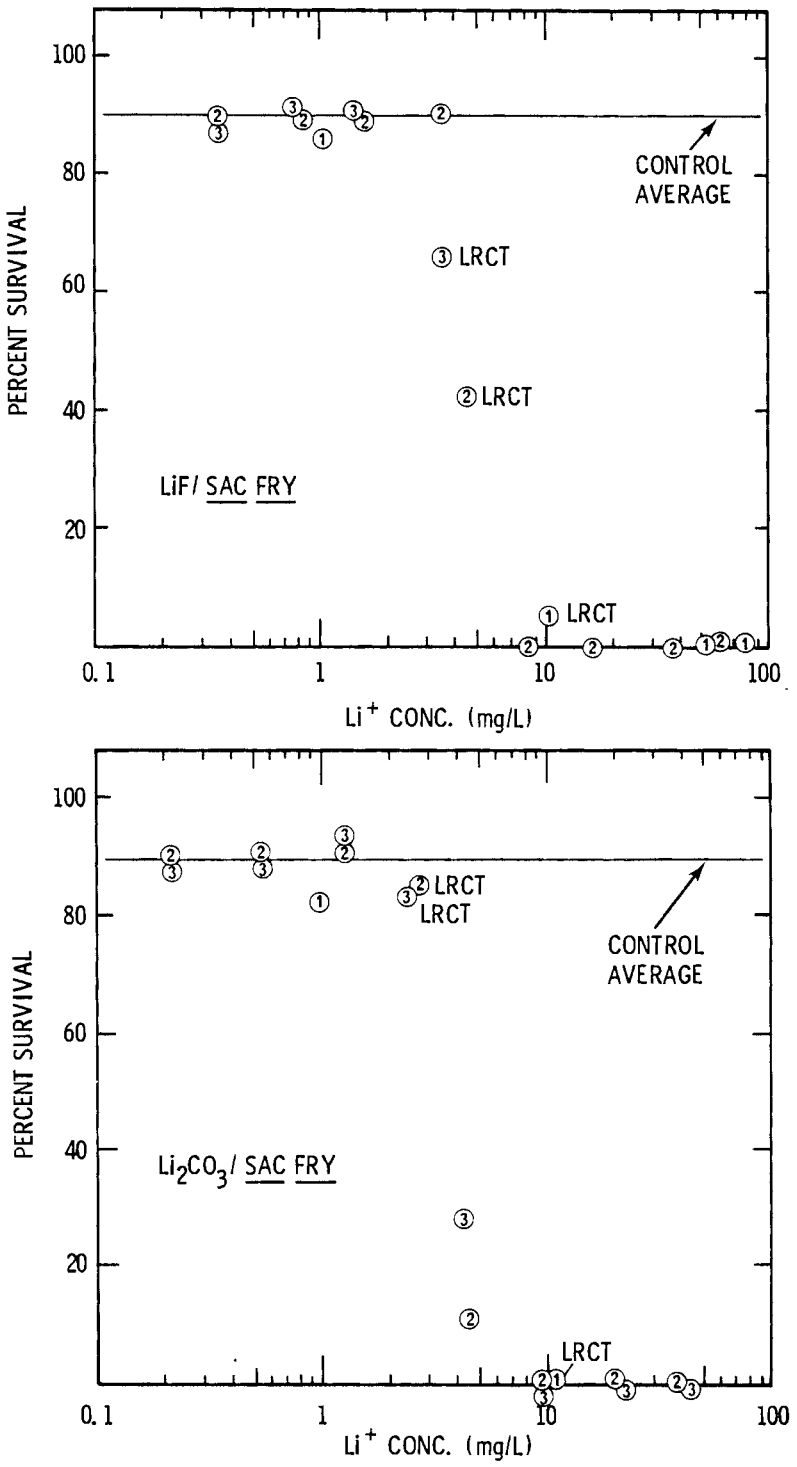

FIGURE 5. Survival of trout sac fry undergoing embryogenesis in elevated Li^+ concentrations. Circles display test numbers.

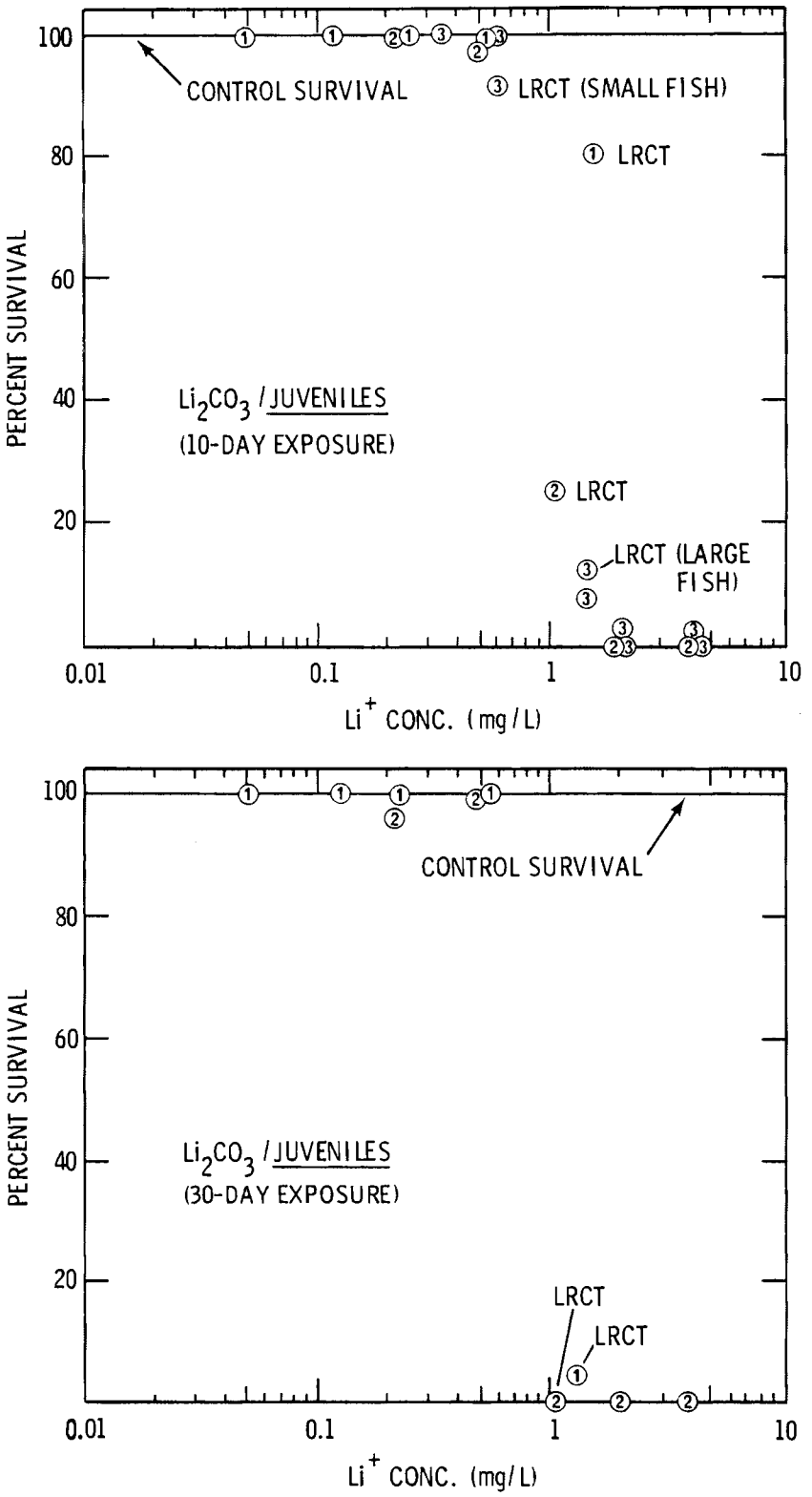

FIGURE 6. Survival of juvenile trout in elevated Li^+ concentrations. Circles display test numbers.

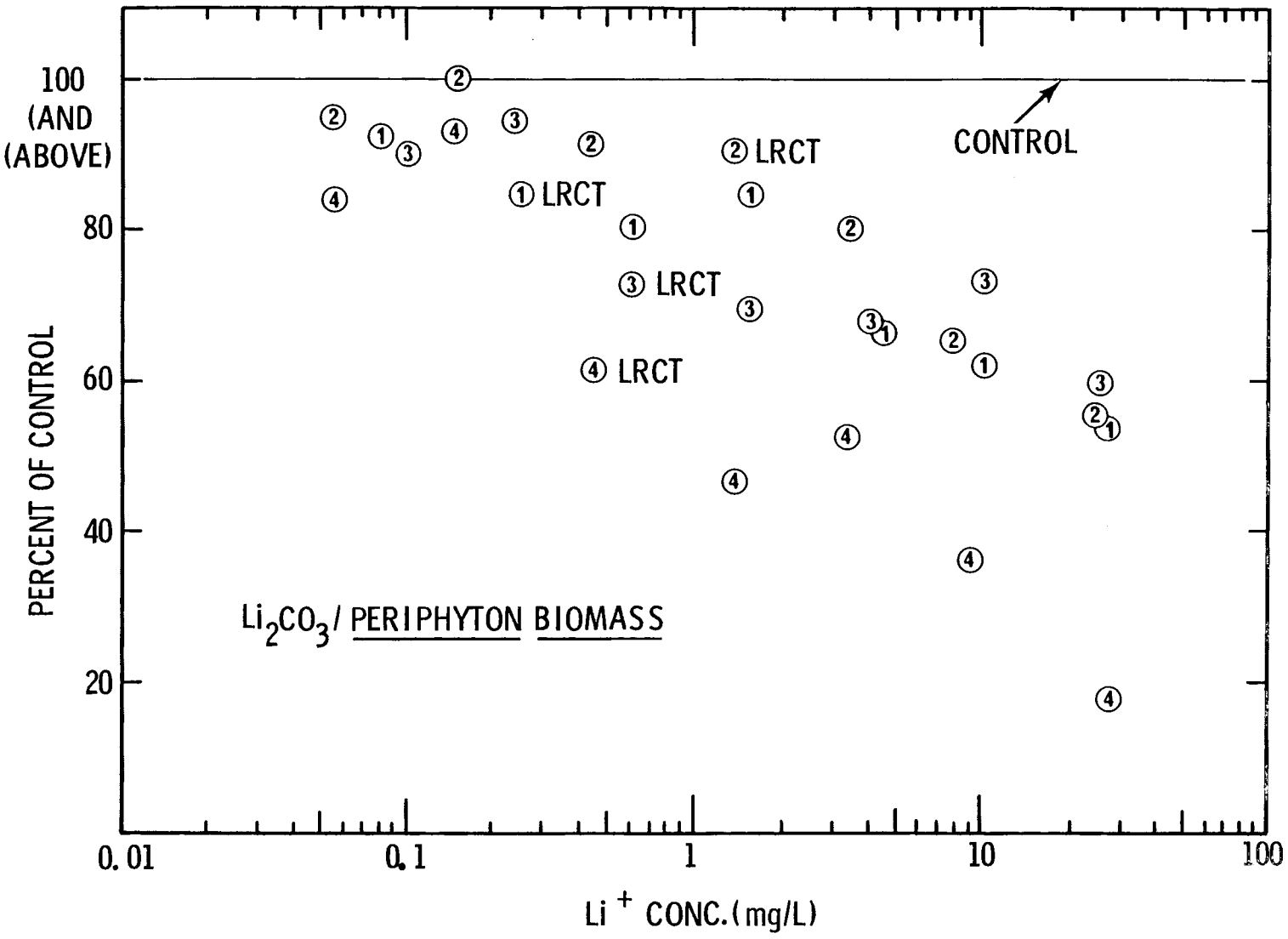

FIGURE 7. Effects of elevated Li^+ concentrations on relative accumulations of periphyton biomass. Circles display test numbers.

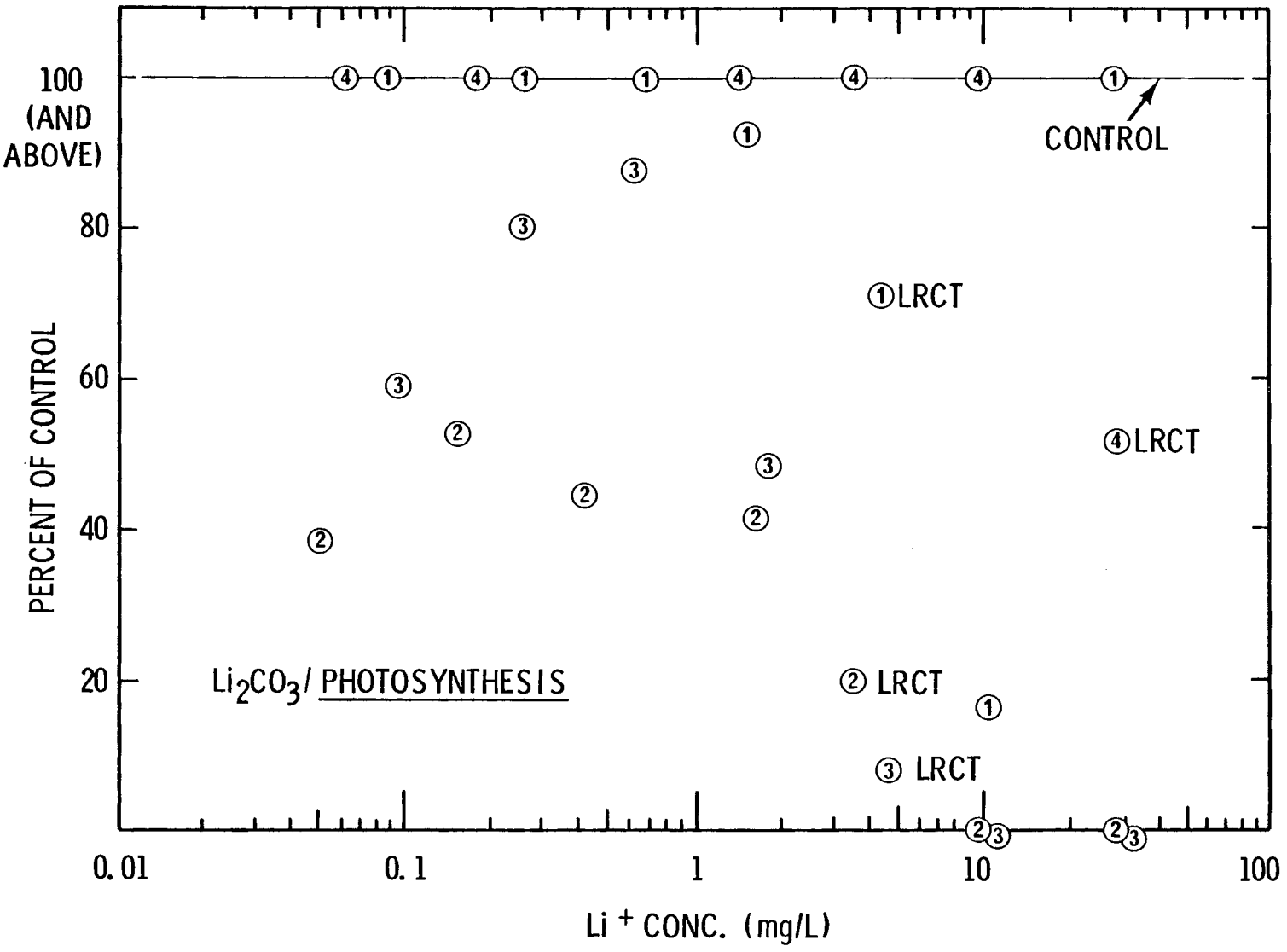

FIGURE 8. Effect of elevated Li^+ concentrations on relative photosynthetic rates of periphyton. Circles display test numbers.

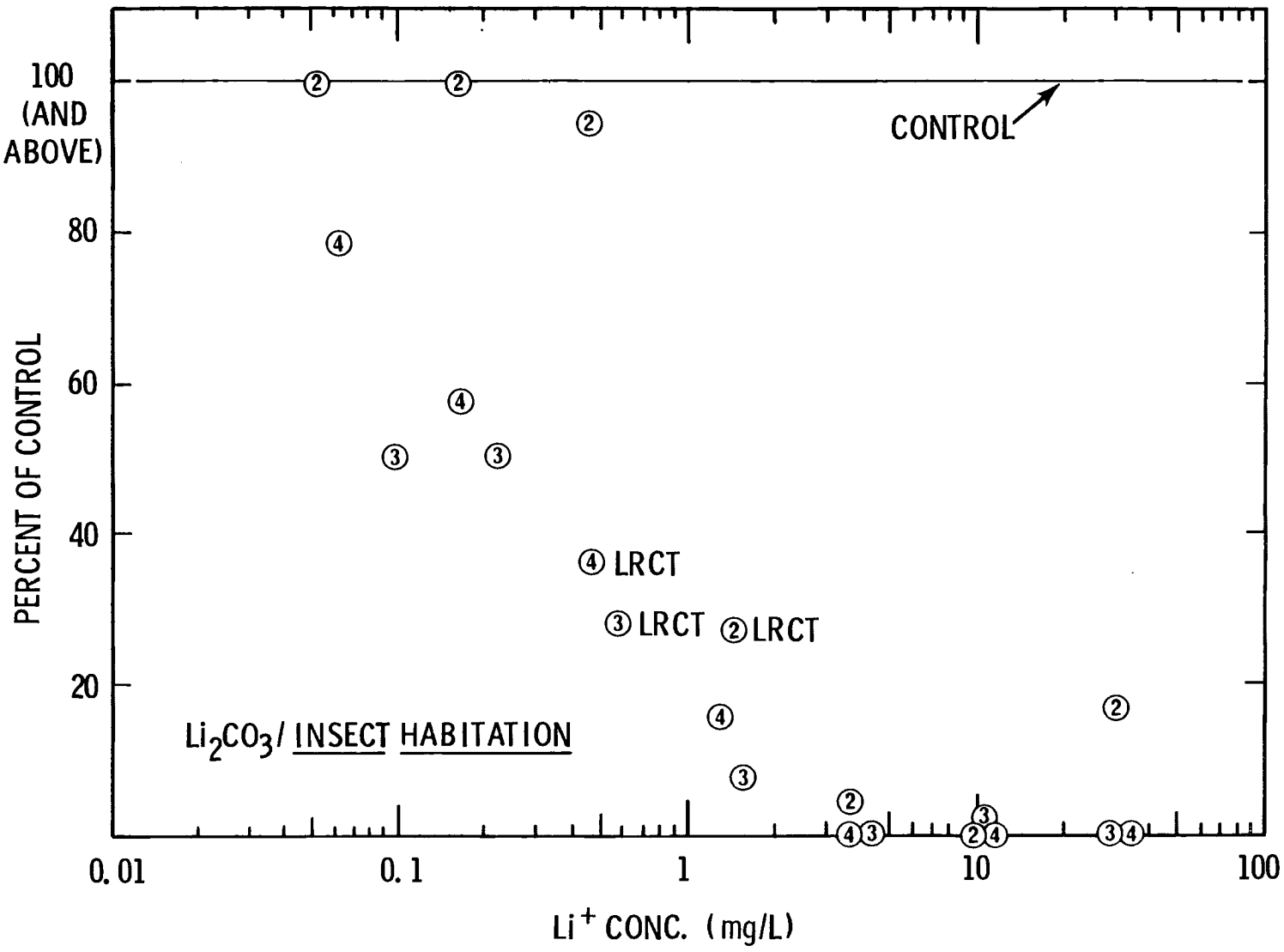

FIGURE 9. Effects of elevated Li^+ concentrations on the relative habitation of chironomid larvae. Circles display test numbers.

DISCUSSION

Our LRCT concentrations appear to be the lowest Li^+ toxicity data reported for aquatic organisms. Only one other investigator reported data similar to ours. Angelovic (1961) reported LiF was toxic (LD_{50}) to rainbow trout at a molecular concentrations ranging from 5.4 to 7.5 mg/L. Expressed as concentrations of Li^+, 1.9 to 2.7 mg/L, these results approximate our own.

There seems to be no earlier data available that defines the toxicity of Li_2CO_3 on aquatic life. King (1953), however, reported that Li_2CO_3 was toxic to the fruit fly (Drosophila) at molecular concentrations of 295-516 mg/L. Expressed as concentrations of Li^+, these range from 55 to 97 mg/L.

There are several reports of the toxicity of LiCl to fish and invertebrates. Toxic concentrations of LiCl to fish were >100 mg/L (Doudoroff and Katz 1953, Ellis 1937, Meinck, Stoof and Hohlshutter 1956). However, Anderson (1948) and Bringmann and Kuhn (1959) report that LiCl is toxic to Daphnia magna in a molecular concentration range of 7 to 16 mg/L (1.1 to 2.6 mg Li^+/L).

The few remaining reports defining Li^+ toxicity to aquatic life are practically uninterpretable. Their expressions of Li^+ toxicity all exceed 1000 mg/L.

Considering all of the rainbow trout parameters, the lowest LRCT was 0.6 mg Li^+/L (juvenile survival, Table 1). Columbia River periphyton communities were affected similarily. Algae biomass was reduced at Li^+ concentrations of 0.3 mg/L, and photosynthesis was suppressed at 3.5 mg/L. These minimum LRCT observations were confirmed additionally by the habitation of chironomid larvae. They were sensitive to 0.4 mg Li^+/L.

From this it is clear that incipient toxicity to important biota in this kind of salmomid habitat falls in the range of 0.1 to 1.0 mg Li^+/L (Fig. 10). The complete array of LRCT data, including LRCT for large and small trout, extend from 0.3 to 108 mg Li^+/L. About three-quarters of the LRCT occur below 10 mg Li^+/L and nearly one-fifth were at or below 1 mg Li^+/L.

TABLE 1. The LRCT expressions defining incipiently toxic concentrations of Li^+ for all tests and experimental parameters.

PARAMETER	STATISTICALLY DEFINED LRCT CONC. ($mg Li^+/L$, $\alpha = 0.05$)								LOWEST LRCT
	TEST 1		TEST 2		TEST 3		TEST 4		
	LiF	Li_2CO_3	LiF	Li_2CO_3	LiF	Li_2CO_3	LiF	Li_2CO_3	
RAINBOW TROUT:									
• EGG INTEGRITY	76.0	108.0	8.8	4.6	4.3	4.6	(b)		4.3
• FERTILIZATION	---[a]	108.0	---	---	---	70.3			70
• EMBRYOGENESIS	54.8	108.0	8.8	4.6	37.4	9.4			4.6
• HATCHABILITY	10.1	10.0	3.6	9.2	4.3	4.6			3.6
• SAC-FRY SURVIVAL	10.1	10.0	4.4	2.4	3.3	2.3			2.3
• JUVENILE SURVIVAL		1.3		1.0		0.6			0.6
COLUMBIA RIVER ORGANISMS:									
• PERIPHYTON BIOMASS		0.3		1.4		0.6		0.4	0.3
• PHOTOSYNTHETIC RATE		4.2		3.5		4.2		27.8	3.5
• INSECT HABITATION				1.4		0.6		0.4	0.4

(a) LRCT NOT DETECTED
(b) BLANK INDICATES NO TEST PERFORMED

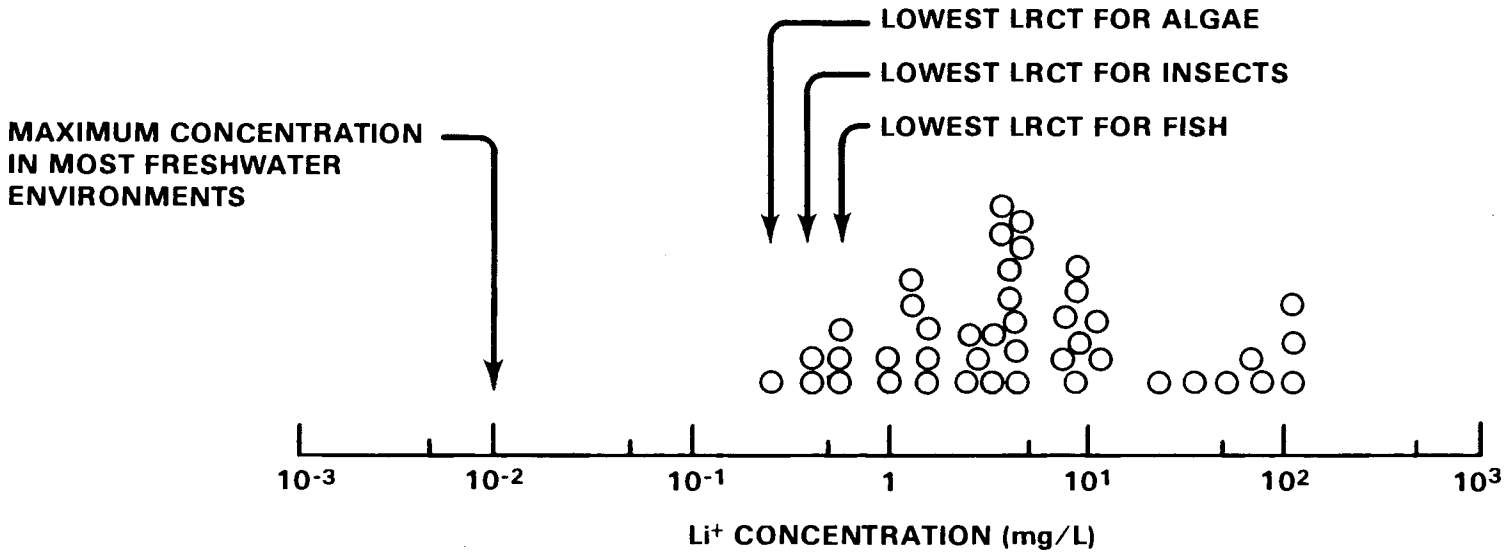

FIGURE 10. An array of all LRCT data from Figures 1 through 9 and Table 1.

These results suggest that regulators of the mining, transportation, use and disposal of Li^+ have two key concentrations to consider for protecting most aquatic environments. These concentrations form a zone-for-regulation that is one order-of-magnitude wide. Regulators could consider Li^+ concentrations of <0.01 mg/L as acceptable for nearly all aquatic systems. But concentrations >0.1 mg Li^+/L would create potential problems. Algae populations would begin to decline at 0.4 mg/L, and trout populations at 0.6 mg/L. Hence, concentrations of Li^+ ranging from 0.01 to 0.1 mg/L define a zone-for-regulation.

Concentrations of Li^+ >1 mg/L are certain to cause significant effects on some aquatic biota, and concentrations >10 mg Li^+/L could severely damage certain aquatic populations. The duration of exposure, and structural/functional characteristics of the environment exposed, would influence greatly the actual impact of Li^+ on biota.

A useful perspective is obtained by comparing the natural occurrence and toxicity of Li^+ with that of the beryllium ion (Be^{++}). The toxicity of Be^{++} to aquatic life has been investigated sufficiently to allow for a comparative interpretation. Both elements have similar chemical properties and close atomic identities. Third position in the period table of elements is occupied by Li^+, and Be^{++} occupies the fourth.

Perhaps the most relevant factor in this comparison between Li^+ and Be^{++} is their common use in fusion technology. Certain core components of fusion reactors will require both elements, with Be^{++} serving as a neutron multiplier. Hence, the mining, use and disposal of these elements will increase proportionately as fusion technology advances over the next several decades.

Today's pre-fusion demands for Be^{++} by the U.S. are approximately 2×10^4 kg/yr (Powell 1975)--two orders of magnitude lower than that of Li^+. Contingent upon specific reactor designs, our demands for Be^{++} could increase to 8×10^4 kg/yr by 2000. As construction of fusion reactors commences, demands could increase to 1×10^6 kg/yr in 2010. Demands for Be^{++} will probably level off by 2030 at a rate of 1.5×10^6 kg/yr--about one-tenth of

projected demands for Li^+. Powell (1975) points out that the types of fusion reactors actually used, and the likely changes in reactor design, may alter these demand projections considerably.

The most assessable Be^{++} deposit is in Utah. This is associated with the mineral bertrandite at Topaz Mountain. Powell (1975) concludes that this "is now the most important commercial beryllium ore deposit in the world." Utah will also supply fusion technology with Li^+ from its salt lakes.

In aquatic environments Be^{++} is rarer than Li^+. Beryllium occurs naturally in freshwater environments only at very low concentrations (<0.001 mg/L). A USGS study of numerous water supplies in the U.S. reported only one sample with detectable Be^{++} (0.00075 mg/L, Durfor and Becker 1964). In freshwaters of California, Be^{++} concentrations were consistently below detectability (<0.0003 mg/L, Silvey 1967). Hem (1970) suggests that natural concentrations of Be^{++} in freshwaters are extremely low because it occurs in particulate rather than in dissolved form. However, there is a consensus of opinion that if Be^{++} enters freshwater environments as a result of fusion technology, it would appear as a sulfate ($BeSO_4$). This form is more soluble than naturally-occurring Be^{++} ores.

The toxicity of Be^{++} to freshwater organisms has been investigated most thoroughly by researchers at the Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, near Dayton, Ohio. They have exposed both fish and amphibians to elevated Be^{++} concentrations. Slonim and Slonim (1973) exposed the common guppy (Lebistes reticulatus), and Slonim and Ray (1975) the salamander larvae (Ambystoma spp.), to $BeSO_4$ solutions in soft and hard water.

Using 96-hour exposure tests, they found that Be^{++} in soft water (hardness = 22 mg/L as $CaCO_3$) was 100 times more toxic than hard water solutions (hardness = 400 mg/L). Median tolerance limits in soft-water Be^{++} solutions were 0.1 to 0.3 mg/L, while TL_m's in hard water were 20 to 22 mg Be^{++}/L. Our lowest LRCT data defining Li^+ toxicity correspond with the lowest of these TL_m concentrations. [Experiments with Li^+ performed in soft water (hardness = 70 mg/L) showed incipient toxicity occurring between 0.1 and 1 mg/L.] In all Be^{++} tests where hardness exceeded 100 mg/L, TL_m concentrations were >1 mg/L.

Results of these studies were consistent with the findings of Tarzwell and Henderson (1960) who tested common guppies, minnows (Pimephales promelas) and sunfish (Lepomis macrochirus) in softand hard-water solutions of $BeSO_4$. All other investigators of Be^{++} toxicity have reported higher toxic thresholds. The incipient toxicity of Be^{++} appears to be comparable to that of Li^{+} in soft water. But maximum concentrations of Be^{++} occurring naturally in freshwaters are one order of magnitude lower than those of Li^{+}. These observations suggest a range of 0.001 to 0.1 mg Be^{++}/L as "approaching toxic concentrations."

Additionally important are the observations of reduced toxicity in hard-water solutions of Be^{++}. Although there is no experimental evidence, the toxicity of Li^{+} may be reduced similarily in hard water. Hardness concentrations might be increased artificially in some freshwater environments as a treatment for accidental spills of Li^{+} or Be^{++}.

SUMMARY AND CONCLUSION

This study has investigated the toxicity of Li^{+} to important components of a salmonid community typical of the Columbia River basin. Our expressions of toxic Li^{+} concentrations are lower than any reported in the literature. We have identified concentrations of Li^{+} that are incipiently toxic (LRCTs) to critical stages in the development of rainbow trout. We have also identified LRCTs for the accumulation of biomass and photosynthetic activity of Columbia River periphyton. And in addition, we identified LRCTs for the habitation of insect (chironomid) larvae.

Concentrations of Li^{+} greater than 0.1 mg/L affected all of our test parameters (Fig. 10). We must conclude, therefore, that some freshwater populations could begin to decline as Li^{+} concentrations approached 1 mg/L. Beyond 1 mg Li^{+}/L, certain and significant biological effects could be expected to occur in both plant and animal populations.

A comparison of these results with data defining the toxicity of Be^{++}, a chemically similar component of fusion reactor cores, indicates that incipiently toxic concentrations of both elements correspond. However, Li^{+} occurs more abundantly than Be^{++} in aquatic environments.

Since most freshwater environments can tolerate Li^+ concentrations of $\leq$0.01 mg/L, our results suggest that regulators of the Li^+ economy consider a range of 0.01 to 0.1 mg/L as "approaching toxic concentrations".

ACKNOWLEDGEMENT

For their participation in this research, we wish to thank Steven Barraclough, Robert Genoway, and Ted Poston of the Ecological Sciences Department, and Kathi Hanson and Roger Schirmer of the Biology Department, here at Pacific Northwest Laboratory.

REFERENCES

American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1971. Standard Methods for the Examination of Water and Waste Water. Amer. Pub. Health Assoc., Washington, DC.

Anderson, B. G. 1948. The apparent thresholds of toxicity of Daphnia magna for chlorides of various metals when added to Lake Erie water. Trans. Amer. Fish. Soc., 78:96.

Angelovic, J. W. et al. 1961. Temperature and fluorosis in rainbow trout. J. Water Pollut. Control Fed., 33:371.

Bringmann, G. and R. Kuhn. 1959. The toxic effects of waste water on aquatic bacteria, algae, and small crustaceans. Gesundheits Ing., 80:115.

Cowgill, V. M. 1976. "The effect of the thermal regime on the annual distribution of selected elements in Linsley Pond (North Bradford, Connecticut)." In: Environmental Biochemistry, Proc. of the Isoken. Symp. 2:731-741.

Doudoroff, P. and M. Katz. 1953. Critical review of literature on the toxicity of industrial wastes and the compentents to fish, II. The metals and salts. Sewage and Industrial Wastes, 25:802.

Durfor, C. N., and E. Becker. 1964. Public water supplies of the 100 largest cities in the United States, 1962. U.S. Geol. Survey Water Supply Paper 1812, Washington, D.C.

Durum, W. H., and J. Haffty. 1961. Occurrence of minor elements in water. U.S. Geol. Survey Circ. 445, Washington, D.C.

Ellis, M. M. 1937. Detection and measurement of stream pollution (related principally to fish life). U.S. Dept. of Commerce, Bur. of Fisheries Bull. 22, Washington, D.C.

Emery, R. 1981. The relevance of ecological theory to environmental hazard assessments. Battelle, Pacific Northwest Laboratory, PNL-SA-9274, Richland, Washington.

Hem, J. D. 1970. Study and interpretation of the chemical characteristics of natural waters. U.S. Geol. Survey Water Supply Paper 1473, 2nd ed., Washington, D.C.

King, R. C. 1953. Effects of alkali metal ions on development of Drosophila, with special reference to lithium induced abnormalities. Proc. National Acad. Sci., 39:403.

Leitritz, E. and R. C. Lewis. 1976. Trout and salmon culture (hatchery methods). State of California, Dept. Fish and Game, Fish Bull. 164, Sacramento, California.

Meinck, F., H. Stoof and H. Hohlshutler. 1956. Industrie Agwasser (Industrial Waste Waters). 2nd Ed., Gustar Fisher Verlag, Stuttgart 536, 44 D.M.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Research 1:21-29.

Powell, J. R. 1975. Beryllium and lithium resource requirements for solid blanket designs for fusion reactors. Brookhaven National Lab., BNL-20299, Long Island, New York.

Silvey, W. D. 1967. Occurrence of selected minor elements in the waters of California. U.S. Geol. Survey Water Supply Paper 598, Washington, D.C.

Skalski, J. R. 1981. Statistical inconsistencies in the use of no observed-effect levels in toxicity tests. In: Aquatic Toxicology and Hazard Assessment, Fourth Conference, ASTM STP 737, D. R. Branson and K. L. Dickson, Eds. American Society for Testing and Materials, pp. 377-387.

Slonim, C. B. and A. R. Slonim. 1973. Acute toxicity of beryllium sulfate to the common guppy. Journal Water Pollut. Contr. Fed. 45:2110-2122.

Slonim, C. B. and E. E. Ray. 1975. Acute toxicity of beryllium sulfate to salamander larvae (Ambystoma spp.). Envir. Contam. and Tox. 13:307-312.

Tarzwell, C. M. and C. Henderson. 1960. Toxicity of less common metals to fishes. Industrial Wastes 5:12.

U.S. Environmental Protection Agency. 1976. Quality Criteria For Water. Washington, D.C.

U.S. Federal Water Pollution Control Administration. 1968. 1968 Report of the Committee on Water Quality Criteria. Washington, D.C.

Varian Techtron. 1971. Analytic Methods for Flame Spectroscopy. Varian Techtron, Ltd., Melbourne, Australia.

Verduin, J. 1964. Principles of primary productivity: photosynthesis under completely natural conditions. In: Algae and Man, E. F. Jackson, ed., Proc. NATO Advanced Study Inst., 1962, Louisville, KY, Plenum Press, NY, pp. 221-238.

Vine, J. D. (ed.). 1976. Lithium Resources and Requirements by the Year 2000. Proc. of Golden, Colorado, Conf., U.S. Geol. Survey Paper 1005, Golden, Colorado.

APPENDIX A

EXPERIMENTAL METHODS

APPENDIX A

EXPERIMENTAL METHODS

1.0 RAINBOW TROUT EXPERIMENTS

1.1 EARLY DEVELOPMENTAL STAGES

To observe effects of elevated concentrations of Li^+ on trout egg integrity, fertilization, embryogenesis, and hatchability, we used the recirculating exposure chambers shown in Figure A1. Each system contained 9 L of water and recirculated that volume once every 6 hr. This size and amount of circulation permitted excellent development of up to 1000 eggs in control chambers. In all trout egg experiments, we used four replicated bioassay chambers at each test concentration of Li^+. The total number of eggs at each test concentration was distributed equally among the replicates.

1.1.1 Chemical Parameters

Test solutions of Li^+ were prepared by diluting batch concentrations to the intended concentrations identified in Tables A1 through A3. Concentrations of Li^+ in the water of these test chambers were monitored evey 48 hours throughout these 30- to 40-day test periods. These Li^+ concentrations were measured on a Varian Model AA6 flame atomic absorption spectrometer. These analyses were performed by PNL's Analytic Chemistry Group, Biology Department, using techniques described by Varian Techtron (1971).

Temperatures of these experimental systems were maintained by a 10°C water bath. Each test unit (Figure A1) rested three-fourths in one of two large water baths. Temperatures were monitored continuously using a Yellow Springs Instruments (YSI) Model 81A temperature recorder.*

Additional data were also collected from test and control systems weekly. Measurement of dissolved oxygen were made using a YSI DO probe and Model 51A meter. An Orion Research Model 901 ionalyzer meter was used to measure pH.

* Trade name specification does not imply endorsement by PNL or DOE.

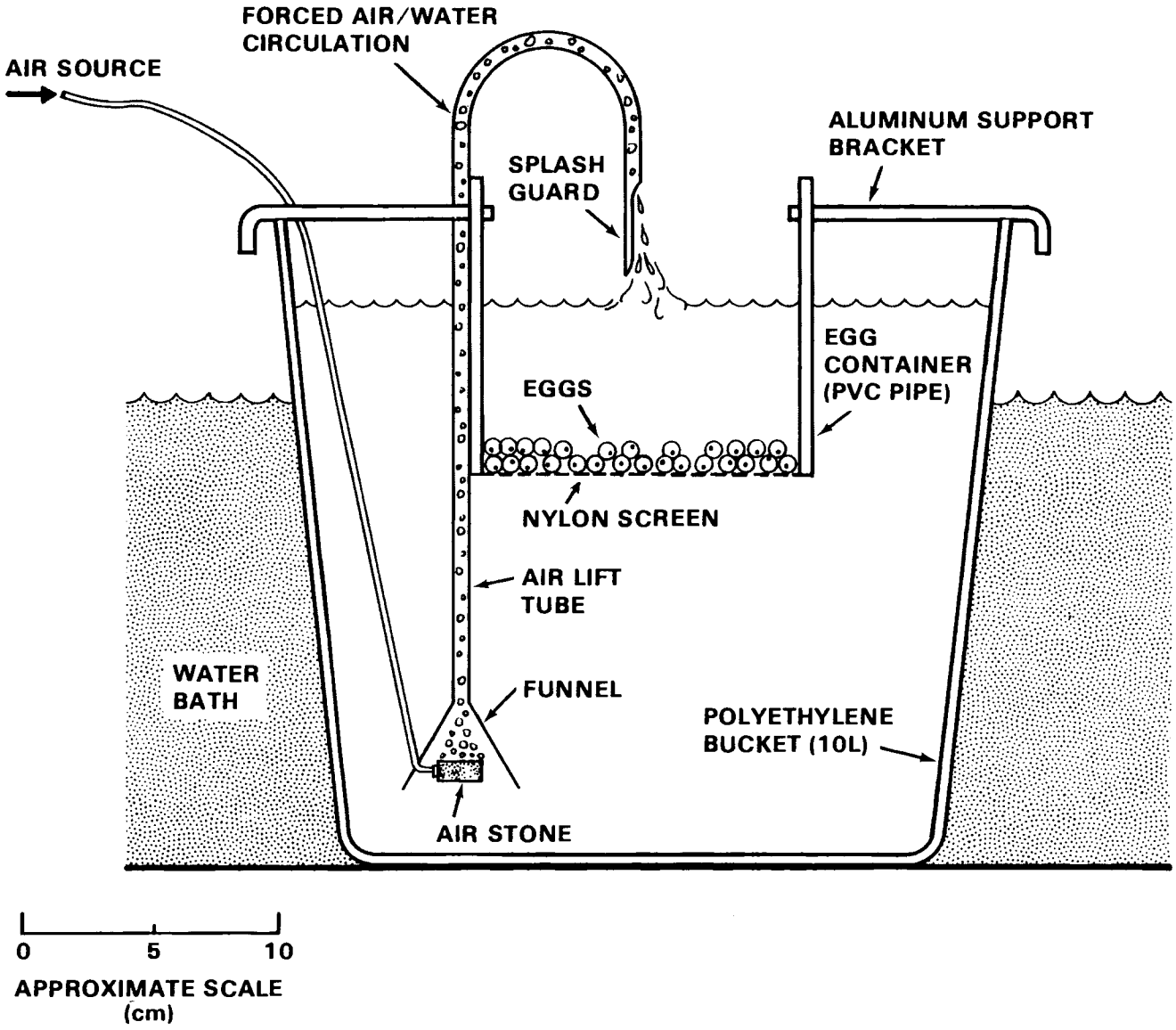

FIGURE A1. A recirculating exposure chamber for testing trout egg development in elevated Li^+ concentrations.

TABLE A1. Experimental conditions and results of the first test where early stages of trout development were exposed to elevated concentrations of Li^+. Results producing LRCTs are enclosed in boxes.

TEST 1:

PARAMETERS	CONTROL	LiF SOLUTIONS				Li_2CO_3 SOLUTIONS			
EXPERIMENTAL CONDITIONS									
INTENDED Li^+ EXPOSURE CONC. (mg/L)	0.0	1.0	10.0	70.0	SATURATION	1.0	10.0	100.0	1000.0
MEASURED Li^+ CONC. (mg/L) • MEAN	<0.01	1.03	10.00	54.81	76.0	1.01	9.97	108.0	1071.0
• ± c.i.[a]	---	±0.10	±0.60	±4.70	±6.67	±0.09	±0.73	±6.30	±40.5
• (n)[b]	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(6)	(3)
TEST DURATION (DAYS/DATES)	31 TO HATCHING -- 73 TO BUTTON-UP/MARCH 28 TO JUNE 7, 1978								
TEMPERATURE (RANGE, °C)	9.9 - 10.9 TO HATCHING -- 10.3 - 12.5 TO BUTTON-UP								
DISSOLVED OXYGEN (RANGE, mg/L)	9.3-10.3	9.3-10.2	9.1-10.2	9.4-10.2	8.7-10.5	9.6-10.4	7.7-10.3	10.1-10.5	10.0-10.1
pH (RANGE)	7.6-8.2	7.8-8.1	7.8-8.2	7.7-8.3	7.7-8.2	7.8-8.5	7.7-9.0	9.7-10.6	10.9-11.0
TOTAL ALKALINITY (RANGE, mg/L AS $CaCO_3$)	56 - 63					63-70	123-140	~650	~650
TOTAL HARDNESS (RANGE, mg/L AS $CaCO_3$)	64 - 70								
EXPERIMENTAL RESULTS									
EGG INTEGRITY (VIABLE EGGS / TOTAL EGGS)	2995/3051	1004/1007	1063/1069	1188/1201	**1180/1208**	1060/1066	1074/1080	**775/1003**	1075/1122
FERTILIZATION (FERTILIZED EGGS / VIABLE EGGS)	2557/2995	942/1004	1024/1063	1136/1188	995/1180	1004/1060	994/1074	**268/775**	552/1075
EMBRYOGENESIS (EGGS COMPLETING EMBRYOGENESIS / FERTILIZED EGGS[c])	1310[d]/2122	628/725	612/787	**448/882**	289/679	660/781	574/808	**0/250**	0/552
HATCHABILITY (EGGS HATCHING / EGGS COMPLETING EMBRYOGENESIS[c])	1295/1310	626/628	**417/612**	101/448	77/289	657/660	**316/574**	-0-	-0-
SAC-FRY SURVIVAL (FRY SURVIVAL TO BUTTON-UP / EGGS HATCHING[c])	993/1099	478/554	**28/394**	0/101	1/77	508/615	**5/316**	-0-	-0-

[a] STATISTICAL CONFIDENCE INTERVAL ABOUT THE MEAN AT $\alpha = 0.05$
[b] NUMBER OF OBSERVATIONS
[c] SOME EGGS WERE SUBSAMPLED AND PRESERVED OR LOST DURING ANALYTIC PROCEDURES
[d] ACCIDENTAL LOSSES OF SOME CONTROL EGGS

TABLE A2. Experimental conditions and results of the second test where early stages of trout development were exposed to elevated Li^+ concentrations. Results producing LRCTs are enclosed in boxes.

TEST 2:

EXPERIMENTAL CONDITIONS

PARAMETERS	CONTROL	LiF SOLUTIONS								Li_2CO_3 SOLUTIONS							
INTENDED Li^+ EXPOSURE CONC. (mg/L)	0.0	0.25	0.50	1.1	2.2	4.4	8.7	17.0	35.0	70.0	0.25	0.50	1.1	2.3	4.4	8.7	17.0	35.0	70.0
MEASURED Li^+ CONC. (ppm) • MEAN	< 0.02	0.38	0.82	1.67	3.59	4.36	8.79	17.51	35.73	45.91	0.21	0.51	1.18	2.36	4.64	9.19	17.75	35.90	70.80
• ± c.i. [a]	---	±0.02	±0.04	±0.06	±0.07	±0.11	±0.23	±0.56	±0.99	±1.05	±0.01	±0.06	±0.03	±0.05	±0.17	±0.23	±0.59	±1.16	±0.86
• (n) [b]	(14)	(13)	(7)	(7)	(7)	(13)	(7)	(7)	(7)	(13)	(13)	(7)	(7)	(13)	(7)	(7)	(13)	(7)	(16)
TEST DURATION (DAYS/DATES)	32 TO HATCHING -- 66 TO BUTTON-UP/MARCH 28 TO JUNE 7, 1978																		
TEMPERATURE (RANGE, °C)	10.3 - 10.8 TO HATCHING -- 10.3 - 12.5 TO BUTTON-UP																		
DISSOLVED OXYGEN (RANGE, mg/L)	8.3-10.8	10.0-10.2	9.3-10.8	6.8-10.8	9.2-10.7	7.3-10.7	9.0-10.8	9.0-10.8	9.2-10.9	10.0-10.8	7.7-10.8	9.6-10.8	9.4-10.9	10.1-11.0	10.0-10.9	9.5-10.9	10.1-11.0	9.2-10.7	10.2-10.8
pH (RANGE)	6.8-7.9	7.2-7.8	7.2-7.8	6.8-7.8	6.8-7.8	7.0-7.8	7.2-7.8	7.2-7.9	7.4-8.0	7.2-7.9	6.9-7.6	7.4-7.8	7.4-7.7	6.8-7.9	7.2-7.9	7.4-8.6	7.8-9.1	7.7-9.7	8.8-10.2
TOTAL ALKALINITY (RANGE, mg/L AS $CaCO_3$)	56 - 63										57-65	59-67	64-72	73-80	88-98	121-131	180-195	306-330	560-579
TOTAL HARDNESS (RANGE, mg/L AS $CaCO_3$)	64 - 70																		

EXPERIMENTAL RESULTS

EGG INTEGRITY (VIABLE EGGS / TOTAL EGGS)	904/918	476/482	454/459	412/424	429/438	458/458	**236/318**	343/430	159/276	278/379	495/512	438/445	432/443	435/440	**454/622**	223/342	340/418	254/358	185/378
FERTILIZATION (FERTILIZED EGGS / VIABLE EGGS)	866/904	462/472	440/454	398/412	419/429	449/458	225/236	330/343	149/159	260/278	481/495	419/438	420/432	418/435	440/454	217/223	322/340	242/254	156/185
EMBRYOGENESIS (EGGS COMPLETING EMBRYOGENESIS / FERTILIZED EGGS[c])	825/864	446/462	421/438	381/398	394/419	425/449	**197/222**	256/330	96/140	172/260	429/477	407/419	412/420	409/416	**393/440**	191/216	180/322	200/242	0/156
HATCHABILITY (EGGS HATCHING / EGGS COMPLETING EMBRYOGENESIS[c])	808/825	435/445	415/421	372/381	**379/394**	394/425	125/197	126/256	27/96	10/172	387/429	401/407	404/412	396/409	282/393	**18/196**	77/180	89/200	-0-
SAC-FRY SURVIVAL (FRY SURVIVAL TO BUTTON-UP / EGGS HATCHING[c])	714/808	391/435	372/415	334/372	342/379	**164/394**	0/125	0/126	0/27	0/10	350/387	363/401	366/404	**335/396**	29/282	0/18	0/77	0/87	-0-

[a] STATISTICAL CONFIDENCE INTERVAL ABOUT THE MEAN AT $\alpha = 0.05$
[b] NUMBER OF OBSERVATIONS
[c] SOME EGGS WERE SUBSAMPLED AND PRESERVED OR LOST DURING ANALYTIC PROCEDURES

TABLE A3. Experimental conditions and results of the third test where early stages of trout development were exposed to elevated Li^+ concentrations. Results producing LRCTs are enclosed in boxes.

TEST 3:

EXPERIMENTAL CONDITIONS

PARAMETERS	CONTROL	LiF SOLUTIONS									Li_2CO_3 SOLUTIONS								
INTENDED Li^+ EXPOSURE CONC. (mg/L)	0.0	0.25	0.50	1.1	2.2	4.4	8.7	17.5	35.0	70.0	0.25	0.50	1.1	2.2	4.4	8.7	17.5	35.0	70.0
MEASURED Li^+ CONC. (mg/L) • MEAN	0.02	0.37	0.84	1.67	3.26	4.33	8.74	17.9	37.4	46.1	0.21	0.53	1.15	2.33	4.60	9.41	17.8	35.2	70.3
• ± c.i. [a]	---	±0.02	±0.07	±0.04	±0.39	±0.05	±0.22	±0.84	±1.06	±1.74	±0.03	±0.02	±0.11	±0.08	±0.09	±0.86	±0.33	±0.96	±1.52
• (n) [b]	(12)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(5)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(10)
TEST DURATION (DAYS/DATES)	32 TO HATCHING -- 66 TO BUTTON-UP / FEBRUARY 23 TO APRIL 30, 1979																		
TEMPERATURE (RANGE, °C)	10.2 - 10.7 TO HATCHING / 10.2 - 12.5 TO BUTTON-UP																		
DISSOLVED OXYGEN (RANGE, mg/L)	9.9-10.9	9.6-10.8	10.0-10.9	9.5-10.9	10.2-10.9	10.0-10.9	10.0-10.8	10.1-10.8	9.9-10.9	9.5-10.9	9.9-10.8	8.8-10.9	8.3-10.9	9.7-10.9	10.3-10.8	10.1-11.0	9.2-10.9	8.0-10.9	10.4-10.8
pH (RANGE)	7.2-7.8	7.2-7.7	7.4-7.7	6.9-7.6	7.3-7.7	7.4-7.7	7.3-7.7	7.4-7.9	7.4-7.9	7.3-7.9	7.3-7.9	6.9-8.1	7.3-8.0	6.9-8.0	7.5-8.1	7.6-8.3	7.3-9.2	7.7-9.6	8.7-10.2
TOTAL ALKALINITY (RANGE, mg/L AS $CaCO_3$)	56-63										57-65	60-67	63-72	72-80	88-97	118-137	182-194	302-323	551-580
TOTAL HARDNESS (RANGE, mg/L AS $CaCO_3$)	64 - 70																		

EXPERIMENTAL RESULTS

EGG INTEGRITY (VIABLE EGGS / TOTAL EGGS)	829/832	422/422	392/393	410/413	412/413	[517/528]	330/404	379/437	396/451	365/451	444/449	570/577	463/463	493/493	[383/424]	301/341	387/472	278/337	432/466
FERTILIZATION (FERTILIZED EGGS / VIABLE EGGS)	817/829	417/422	387/392	400/410	410/412	514/517	325/330	377/379	391/396	357/365	440/444	568/570	459/463	491/493	381/383	295/301	379/387	273/278	[77/432]
EMGRYOGENESIS (EGGS COMPLETING EMBRYOGENESIS / FERTILIZED EGGS [c])	771/817	397/417	373/387	381/400	404/410	488/514	312/325	353/377	[341/391]	291/357	429/440	548/568	436/459	472/491	368/381	[269/295]	294/379	247/273	0/77
HATCHABILITY (EGGS HATCHING / EGGS COMPLETING EMBRYOGENESIS [c])	743/771	390/397	369/373	366/381	388/404	[451/488]	119/312	85/353	69/341	71/291	410/429	539/548	432/436	462/472	[314/368]	57/269	84/294	90/247	-0-
SAC-FRY SURVIVAL (FRY SURVIVAL TO BUTTON-UP / EGGS HATCHING [c])	673/743	343/390	325/369	330/366	[258/388]	258/451	4/119	0/85	0/69	0/71	364/410	472/539	409/432	[383/462]	88/314	0/57	0/84	0/90	-0-

[a] STATISTICAL CONFIDENCE INTERVAL ABOUT THE MEAN AT $\alpha = 0.05$
[b] NUMBER OF OBSERVATIONS
[c] SOME EGGS WERE SUBSAMPLED AND PRESERVED OR LOST DURING ANALYTIC PROCEDURES

Alkalinity and hardness (expressed as ppm $CaCo_3$) were determined titrametrically by methods described in Standard Methods (APHA et al. 1971). Both alkalinity and hardness exist within narrow ranges in Columbia River water (56 to 63 mg/L and 64 to 70 mg/L, respectively).

Trout eggs bioassays were performed in both LiF and Li_2CO_3 solutions. Upper concentrations of Li^+ from LiF were limited to $\leq$80 mg/L due to its saturation in Columbia River water. However, we were able to prepare Li_2CO_3 solutions with Li^+ concentrations of 100 and 1000 mg/L.

All expressions of toxicity are based on the measured concentrations in Tables A1 through A3, and are expressed as mg of Li^+/L.

1.1.2 Biological Parameters

Egg integrity (I) was defined by the ratio

$$I = \frac{\Sigma i - b}{\Sigma i},$$

where Σi is the total number of eggs incubated and b is the number of eggs with broken protective membranes (macroscopically observable). All broken eggs were removed from experimental incubators.

Fertilization success (F) was determined using the equation

$$F = \frac{\Sigma f}{\Sigma i - b},$$

where Σf is the total number of fertilized eggs. This includes both the eggs entering embryogenesis (obviously fertile and not disturbed) and fertile eggs dying before embryonic formation. At this stage of our experiments, dead eggs were immediately preserved and later examined for evidence of fertilization. This was done microscopically by identifying early formations of blastodiscs in successfully fertilized eggs (Leitritz and Lewis 1976).

Completion of embryogenesis (E) was determined with the equation

$$E = \frac{\Sigma e}{(\Sigma f) - e_s} \quad ,$$

where Σe is the total number of eggs completing embryogenesis, and e_s is the number of eggs sub-sampled prior to completion of embryogenesis.

Hatchability (H) was determined using the ratio

$$H = \frac{\Sigma h}{\Sigma e} \quad ,$$

where Σh is the total number of eggs hatching successfully.

Sac-fry survival (S) is defined by the equation

$$S = \frac{\Sigma s}{(\Sigma h) - s_s} \quad ,$$

where Σs is the total number of sac fry that survived to their "button-up" stage and s_s is the number of sac fry sub-sampled and preserved. The term "button-up" is a commonly accepted expression that depicts the sealing of a ventral integument where the yolk sac had once protruded (Leitritz and Lewis 1976). This marks the beginning of fryhood.

It was not possible to maintain these sac fry in the recirculating test chambers where they had undergone embryogenesis (Figure A1). Sac fry require a continuous passage of much larger volumes of water for development to occur. This was provided by passing Columbia River water through hatchery troughs. Sac fry were transferred to these stream conditions after hatching. Experimental quantities of Li^+ were not added to these troughs. The required large volumes of single-passing river water prohibited direct Li^+ bioassays here. However, parameter S expresses the ability of rainbow trout fry to button-up after they had been exposed to Li^+ contamination during previous developmental stages.

1.2 JUVENILES

Juvenile rainbow trout (~10 cm in length) were tested for survival in continuously-flowing solutions of Li^+. Statistical interpretations of egg and fry bioassay results suggested that Li_2CO_3 was consistently more toxic than LiF to developmental stages of trout (Table A4). Thus, we tested acute responses of juveniles to only Li_2CO_3 to allow for more tests of a single compound and greater numbers of test concentrations. Single-pass deliveries of specific Li_2CO_3 concentrations were maintained by proportional diluters based on the design of Mount and Brungs (1967).

In the three final tests, five Li^+ concentrations plus control were delivered to replicated exposure aquaria at a rate of 0.1 L/min (±0.01 L/min). Each exposure-aquarium replicate had a volume of 30 L, producing a hydraulic retention time of five hours. Experimental concentrations of Li^+ and controls were replicated twice, and each replicate held 10 or 25 juvenile trout.

Experimental fish were exposed to test solutions of Li^+ for 30 days in Tests 1 and 2, and 10 days in Test 3. Survival ratios are reported for exposure durations of 10 and 30 days. Intended exposure concentrations of Li^+ are shown in Tables A5 to A7. In Test 1, the highest intended concentration of Li^+ (1.0 mg/L) was monitored twice weekly. Lower test concentrations were proportionally diluted from this 1.0 mg/L stock solution. More-intensive monitoring of Tests 2 and 3 involved analyzing each experimental concentration of Li^+ once or twice weekly. Experimental Li concentrations were analyzed by the same methods described in Appendix 1.1.1.

Temperatures in the exposure aquarium were maintained with a 10°C water bath and monitored continuously using a YSI temperature recorder. Observations of pH, dissolved oxygen, total alkalinity and total hardness were made twice weekly using methods described in Appendix 1.1.1.

TABLE A4. Statistical answers to two questions regarding the toxicity of LiF and Li_2CO_3: 1) Are the treatment results of either compound different from controls?, and 2) Does either compound exhibit greater toxicity in any test?

PARAMETER	TEST NUMBER	STATISTICAL HYPOTHESIS (FOR DETECTING SIGNIFICANT DIFFERENCE, S, AT α = 0.05)			
		CONTROL = TREAT. AVE.		LiF TREAT. = Li_2CO_3 TREAT.	
		LiF TREAT.	Li_2CO_3 TREAT.	LiF = Li_2CO_3	MORE-TOXIC COMPOUND [a]
RAINBOW TROUT:					
• EGG INTEGRITY	1	S	S	NOT S	
	2	S	S	S	Li_2CO_3
	3	S	S	NOT S	
• EGG FERTILIZATION	1	NOT S	S	S	Li_2CO_3
	2	NOT S	NOT S	NOT S	
	3	NOT S	S	S	Li_2CO_3
• EMBRYOGENESIS	1	S	S	S	Li_2CO_3
	2	S	S	S	
	3	S	S	S	Li_2CO_3
• HATCHABILITY	1	S	S	S	Li_2CO_3
	2	S	S	S	Li_2CO_3
	3	S	S	S	Li_2CO_3
• SAC-FRY SURVIVAL	1	S	S	S	Li_2CO_3
	2	S	S	S	Li_2CO_3
	3	S	S	NOT S	Li_2CO_3
COLUMBIA RIVER PERIPHYTON:					
• BIOMASS	PRE-1 [b]	S	S	S [c]	LiF
	PRE-2	S	S	NOT S	
	PRE-3	S	S	NOT S	

[a] THE LITHIUM COMPOUND (LiF OR Li_2CO_3) SHOWING GREATER TOXICITY
[b] PRELIMINARY TEST RUNS (15 DAYS) CONDUCTED PRIOR TO FIRST TEST RUN (MAY 30, 1979)
[c] SIGNIFICANT ONLY AT Li^+ CONCENTRATIONS > 78 mg/L

TABLE A5. Experimental conditions and results of the first test where juvenile trout were exposed to elevated Li^+ concentrations. Results producing LRCTs are enclosed in boxes.

TEST 1:

PARAMETERS	CONTROL	Li_2CO_3 SOLUTIONS				
EXPERIMENTAL CONDITIONS						
INTENDED Li^+ EXPOSURE CONC. (mg/L)	0.0	0.06	0.12	0.25	0.5	1.0
MEASURED Li^+ CONC. (mg/L) • MEAN	< 0.01	0.05	(0.12)[b]	(0.25)[b]	0.54	1.35
• ± c.i.[a]	---	---			---	±0.24
• (n)[c]	(3)	(1)			(1)	(8)
TEST DURATION (DAYS / DATES)	30 / SEPTEMBER 7 - OCTOBER 9, 1978					
TEMPERATURE (RANGE, °C)	10.0 TO 10.6					
DISSOLVED OXYGEN (RANGE, mg/L)	9.3 TO 10.9					
pH (RANGE)	6.8 TO 8.1					
TOTAL ALKALINITY (RANGE, mg/L AS $CaCO_3$)	56-63	56-63	56-65	56-65	58-67	64-74
TOTAL HARDNESS (RANGE, mg/L AS $CaCO_3$)	64 - 70					
EXPERIMENTAL RESULTS						
SURVIVAL RATIO @ 10 DAYS $\left(\frac{\text{FISH SURVIVING}}{\text{TOTAL FISH}}\right)$	$\frac{20}{20}$	$\frac{20}{20}$	$\frac{20}{20}$	$\frac{20}{20}$	$\frac{20}{20}$	$\boxed{\frac{16}{20}}$
SURVIVAL RATIO @ 30 DAYS $\left(\frac{\text{FISH SURVIVING}}{\text{TOTAL FISH}}\right)$	$\frac{20}{20}$	$\frac{20}{20}$	$\frac{20}{20}$	$\frac{20}{20}$	$\frac{20}{20}$	$\boxed{\frac{1}{20}}$

[a] STATISTICAL CONFIDENCE INTERVAL ABOUT THE MEAN AT $\alpha = 0.05$
[b] ASSUMED CONCENTRATION, NO Li^+ ANALYSES PERFORMED
[c] NUMBER OF OBSERVATIONS

TABLE A6. Experimental conditions and results of the second test where juvenile trout were exposed to elevated Li^+ concentrations. Results producing LRCTs are enclosed in boxes.

TEST 2:

EXPERIMENTAL CONDITIONS

PARAMETERS	CONTROL	Li_2CO_3 SOLUTIONS				
INTENDED Li^+ EXPOSURE CONC. (mg/L)	0.0	0.25	0.5	1.0	2.0	4.0
MEASURED Li^+ CONC. (mg/L) • MEAN	< 0.01	0.23	0.49	1.03	1.98	3.80
• ± c.i. (a)	---	±0.10	±0.09	±0.22	±0.88	±1.23
• (n) (b)	(3)	(3)	(3)	(3)	(2)	(2)
TEST DURATION (DAYS/DATES)	30/AUGUST 14 - SEPTEMBER 13, 1979					
TEMPERATURE (RANGE, °C)	9.9 TO 10.4					
DISSOLVED OXYGEN (RANGE, mg/L)	9.4 TO 10.9					
pH (RANGE)	7.2 TO 8.7					
TOTAL ALKALINITY (RANGE, mg/L AS $CaCO_3$)	56-63	57-65	59-67	62-72	77-84	75-99
TOTAL HARDNESS (RANGE, mg/L AS $CaCO_3$)	64 - 70					

EXPERIMENTAL RESULTS

SURVIVAL RATIO @ 10 DAYS $\left(\frac{\text{FISH SURVIVING}}{\text{TOTAL FISH}}\right)$	$\frac{50}{50}$	$\frac{50}{50}$	$\frac{49}{50}$	$\boxed{\frac{13}{50}}$	$\frac{0}{50}$	$\frac{0}{50}$
SURVIVAL RATIO @ 30 DAYS $\left(\frac{\text{FISH SURVIVING}}{\text{TOTAL FISH}}\right)$	$\frac{50}{50}$	$\frac{50}{50}$	$\frac{48}{50}$	$\boxed{\frac{0}{50}}$	$\frac{0}{50}$	$\frac{0}{50}$

(a) STATISTICAL CONFIDENCE INTERVAL ABOUT THE MEAN AT $\alpha = 0.05$
(b) NUMBER OF OBSERVATIONS

TABLE A7. Experimental conditions and results of the third test where juvenile trout were exposed to elevated Li^+ concentrations. Results producing LRCTs are enclosed in boxes.

TEST 3:

PARAMETERS	CONTROL	Li_2CO_3 SOLUTIONS				
EXPERIMENTAL CONDITIONS						
INTENDED Li^+ EXPOSURE CONC. (mg/L)	0.0	0.25	0.5	1.0	2.0	4.0
MEASURED Li^+ CONC. (mg/L) • MEAN	< 0.01	0.36	0.64	1.32	2.20	4.01
• ± c.i.[a]	---	±0.12	±0.10	±0.21	±0.11	±0.14
• (n)[b]	(9)	(9)	(8)	(8)	(5)	(3)
TEST DURATION (DAYS/DATES)	10/NOVEMBER 4-14, 1979					
TEMPERATURE (RANGE, °C)	9.8 TO 10.4					
DISSOLVED OXYGEN (RANGE, mg/L)	9.0 TO 10.7					
pH (RANGE)	7.1 TO 8.8					
TOTAL ALKALINITY (RANGE, mg/L AS $CaCO_3$)	56-63	58-66	60-68	64-74	71-80	84-93
TOTAL HARDNESS (RANGE, mg/L AS $CaCO_3$)	64 - 70					
EXPERIMENTAL RESULTS						
LARGE FISH[c] SURVIVAL RATIO $\left(\frac{\text{FISH SURVIVING}}{\text{TOTAL FISH}}\right)$	50/50	50/50	50/50	**6/50**	0/50	0/50
SMALL FISH[d] SURVIVAL RATIO $\left(\frac{\text{FISH SURVIVING}}{\text{TOTAL FISH}}\right)$	50/50	50/50	**46/50**	4/50	0/50	0/50

(a) STATISTICAL CONFIDENCE INTERVAL ABOUT THE MEAN AT $\alpha = 0.05$
(b) NUMBER OF OBSERVATIONS
(c) 13.28 g ± 1.53 g (MEAN WET WEIGHT ± c.i. AT $\alpha = 0.05$), LENGTH ≈11 cm
(d) 8.45 g ± 1.08 g (MEAN WET WEIGHT ± c.i. AT $\alpha = 0.05$), LENGTH ≈9 cm

2.0 EXPERIMENTS WITH COLUMBIA RIVER BIOTA

2.1 THE EXPERIMENTAL SYSTEM

Columbia River periphyton and midge larvae (Chironomus sp.) were exposed to continuous solutions of Li^+ to relate their responses to specific concentrations. These experiments focused on the ability of test organisms to sustain their populations in the elevated Li^+ concentrations of our exposure containers. Tests were performed under direct sunlight and at ambient temperatures of Columbia River water. Each exposure container received a continuous supply (single-pass) of raw river water which served two purposes. Raw river water both supplied the "seed" for experimental periphyton populations and diluted the Li^+ stock solution to appropriate test concentrations.

Continuous deliveries of specific Li^+ concentrations were supplied by the serial-dilution system shown in Figure A2. All hardware components are made of glass, lucite and flexible plastic tubing. The Li^+ stock concentrate was serially diluted with river water to desired concentrations, and delivered to exposure containers at a rate of 0.1 L/min (±0.01 L/min). Internal flow adjustments necessary to calibrate the diluter were made daily throughout each test by changing positions of the delivery arms (Figure A3).

Exposure containers were made of plastic and had 2-L capacities (Figure A3). Their hydraulic retention time (V/Q) was 20 min. Ambient river temperatures were maintained in exposure containers with a river-water bath. Experimental temperatures were monitored with a YSI probe and recorder. Observations of pH, total alkalinity and total hardness were made twice weekly during experiments using techniques described in Appendix 1.1.1.

Preliminary scoping tests were performed using both LiF and Li_2CO_3 solutions. Statistical interpretations of these results (Table A4) showed that both compounds were similarly toxic, except LiF was more toxic at concentrations >78 mg/L. Since Li_2CO_3 was frequently more toxic than LiF to developing trout, we elected to use only Li_2CO_3 in the final tests. This allowed us to expand the number of test concentrations and their replication.

DIAGRAM OF SERIAL DILUTION DELIVERY SYSTEM

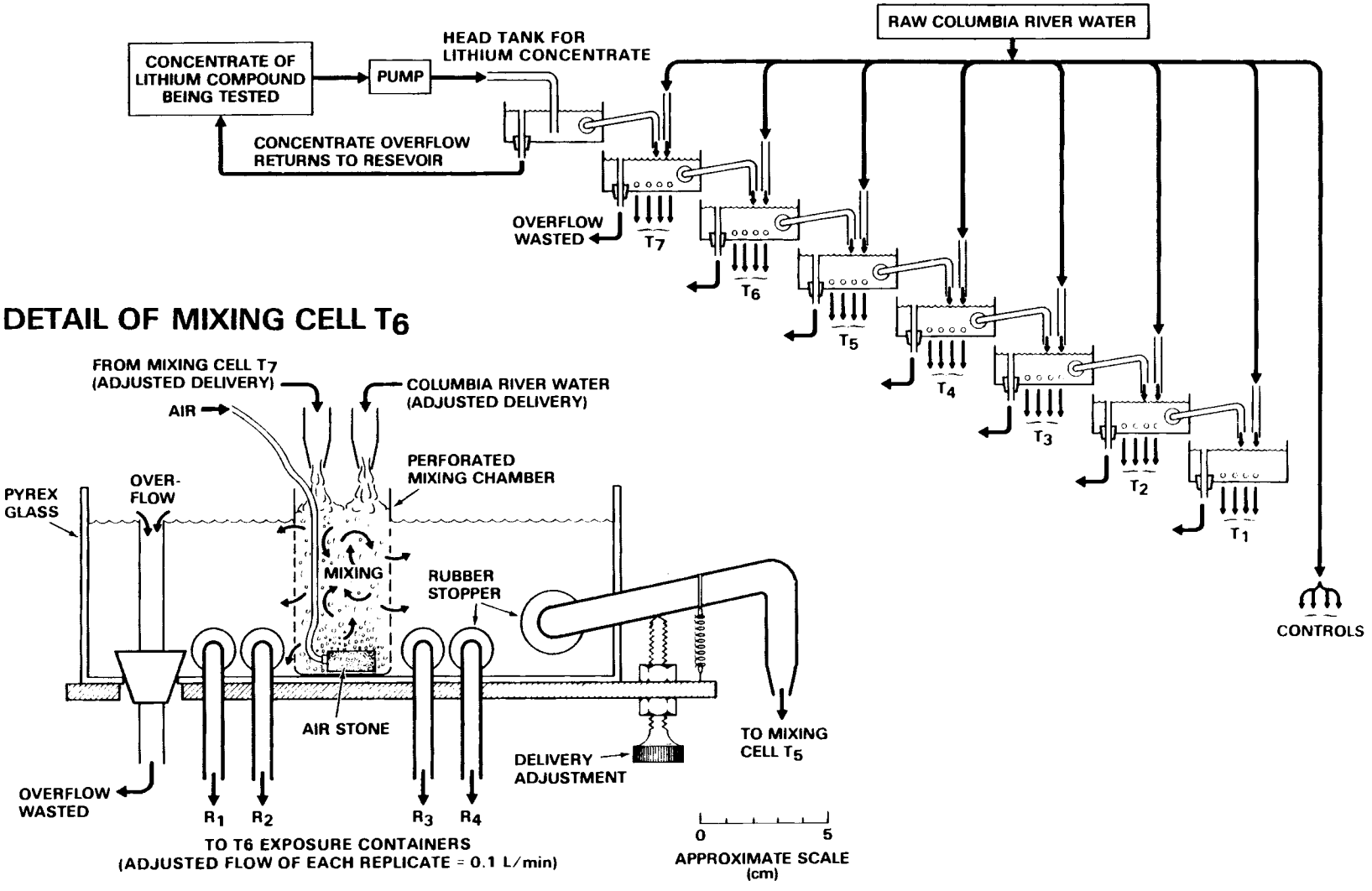

FIGURE A2. The serial dilution system used to deliver elevated Li^+ concentrations to Columbia River biota.

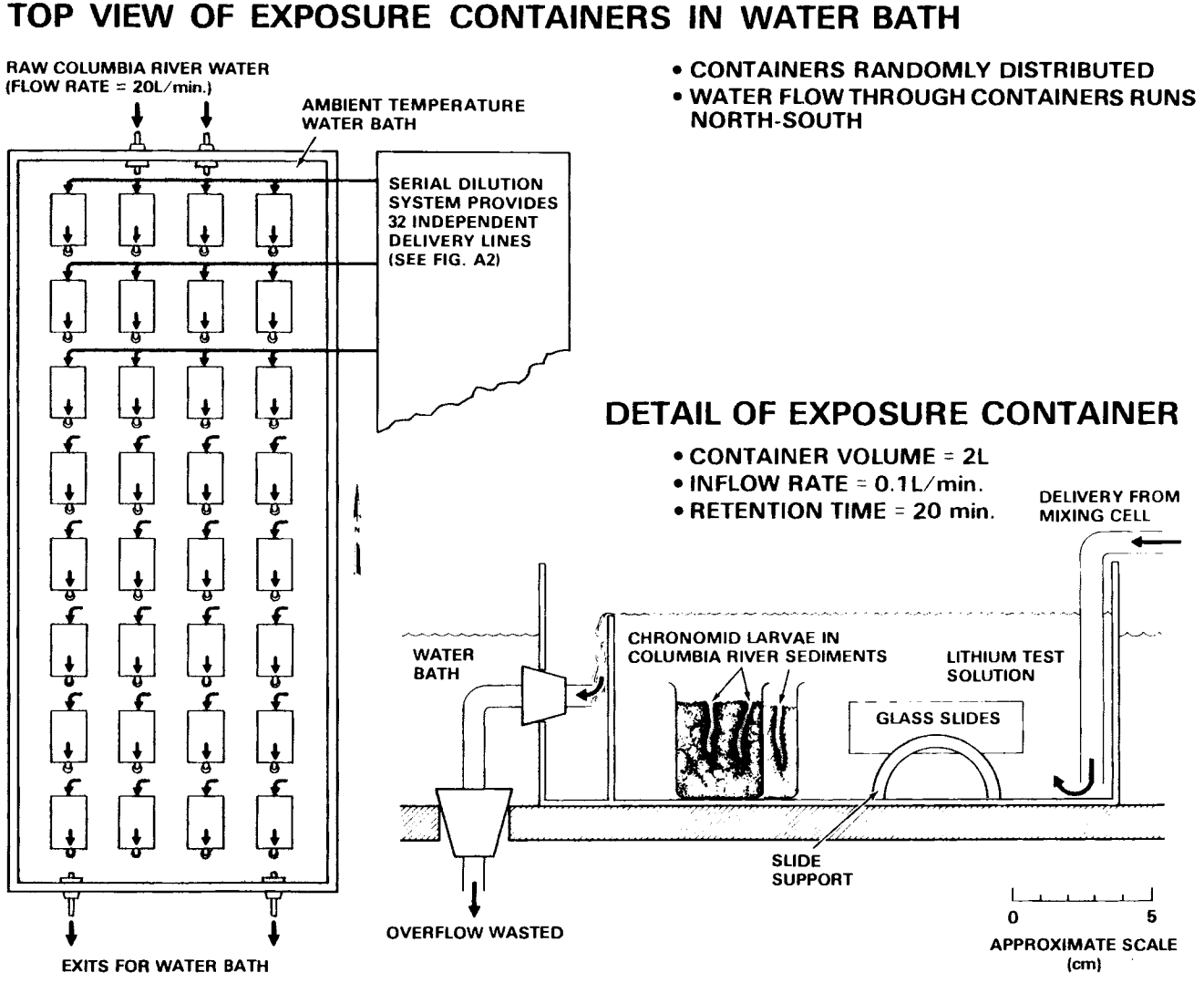

FIGURE A3. Experimental array and detail of exposure containers used to test Columbia River biota in elevated Li^+ concentrations.

In our four final tests, we exposed Columbia River organisms to seven Li^+ concentrations plus control for 15 days (Tables A8 to A11). Test populations of periphyton colonized naturally in our exposure containers during experimental periods. Chironomid larvae were collected from McNary reservoir and acclimated in laboratory aquaria containing McNary sediments for 10 days. Larvae-exposure modules (Figure A3) were prepared and again acclimated in laboratory aquaria for 3 days prior to each test run.

The seven test concentrations and control were allocated to the water table (Figure A3) in a randomized block design where the treatment levels were randomly assigned to the eight exposure containers within each pair of consecutive rows. The test concentrations and controls were replicated four times.

Analysis of variance for a two-way classification was used to analyze biomass data (Tables A8-A11). Subsequent analyses using Fisher's protected LSD (least significant difference) test was employed to determine the LRCT values. Because of unequal treatment variances, Friedman rank sum test for a two-way layout was used to analyze the photosynthetic-rate data (Tables A8-A11). No significant block effects were found. Wilcoxon rank sum tests were then used to compare survival rates of treatment versus control groups of larvae (Tables A8-A11) for insect habitation.

2.2 MEASUREMENTS OF BIOLOGICAL PARAMETERS

Three biological parameters were measured that define a quantitative association between Li^+ concentrations and the inhabitability of our exposure containers. To account for the habitation of periphyton, we measured the total biomass and photosynthetic rate of algae growing in the exposure containers on the fifteenth day. To measure the habitation of insect larvae, we counted numbers of chironomids remaining in the exposure modules after 14 days.

Measurements of net photosynthetic rates were made from dawn to noon on the last day of testing. Water flow was stopped during these periods of measurement. We used Verduin's technique (1964) that related pH changes in exposure containers with changes in CO_2 concentrations. For each experimental

TABLE A8. Experimental conditions and results of the first test where periphyton and insect larvae were exposed to elevated Li^+ concentrations. Results producing LRCTs are enclosed in boxes.

TEST 1:

EXPERIMENTAL CONDITIONS

PARAMETERS	CONTROL	Li_2CO_3 SOLUTIONS						
INTENDED Li^+ EXPOSURE CONC. (mg/L)	0.0	0.1	0.25	0.62	1.5	4.0	10.0	25.0
MEASURED Li^+ CONC. (mg/L) • MEAN	<0.02	0.088	0.26	0.68	1.71	4.24	10.3	25.7
• ± c.i.[a]	---	±0.02	±0.04	±0.11	±0.27	±0.69	±1.18	±2.23
• (n)[b]	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(14)
TEST DURATION (DAYS/DATES)	14 / MAY 30 - JUNE 13, 1979							
TEMPERATURE (RANGE, °C)	8.9 - 15.4							
pH (RANGE)	8.1-9.3	8.1-9.2	8.3-9.1	8.4-9.2	8.6-9.2	9.2-9.4	9.5-9.6	9.5-9.9
TOTAL ALKALINITY (RANGE, mg/L AS $CaCO_3$)	56-63	56-64	58-65	60-69	66-77	82-98	122-146	225-264
TOTAL HARDNESS (RANGE, mg/L AS $CaCO_3$)	64 - 70							

EXPERIMENTAL RESULTS

BIOMASS (mg, ASH-FREE DRY WT) • MEAN	242.70	225.68	206.11	196.65	203.88	161.24	151.77	134.77
• ± c.i.[c]	±41.05	±20.05	±13.68	±9.22	±32.77	±32.46	±26.41	±27.36
• (n)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
PHOTOSYNTHETIC RATE (mgC/L · DAY) • MEAN	4.25	9.47	6.84	7.52	3.58	2.33	0.37	NOT
• ± c.i.[c]	±1.74	±0.32	±3.17	±1.55	±0.45	±1.96	±0.68	DETER-
• (n)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	MINED
INSECT HABITATION (LARVAE REMAINING / TOTAL LARVAE EXPOSED)	NOT DETERMINED							

[a] STATISTICAL CONFIDENCE INTERVAL ABOUT THE MEAN AT $\alpha = 0.05$
[b] NUMBER OF OBSERVATIONS
[c] SPECIFIC c.i.'s MAY NOT REFLECT THE RESULTS OF ANOVA PROCEDURES FOR LRCT DETERMINATIONS, WHICH USED POOLED VARIANCE ESTIMATES

TABLE A9. Experimental conditions and results of the second test where periphyton and insect larvae were exposed to elevated Li^+ concentrations. Results producing LRCTs are enclosed in boxes.

TEST 2:

PARAMETERS	CONTROL	Li_2CO_3 SOLUTIONS						
EXPERIMENTAL CONDITIONS								
INTENDED Li^+ EXPOSURE CONC. (mg/L)	0.0	0.1	0.25	0.62	1.5	4.0	10.0	25.0
MEASURED Li^+ CONC. (mg/L) • MEAN	<0.02	0.06	0.16	0.46	1.41	3.49	9.78	27.70
• ± c.i. [a]	---	±0.02	±0.05	±0.10	±0.34	±0.56	±0.82	±2.07
• (n) [b]	(10)	(10)	(10)	(10)	(10)	(10)	(14)	(14)
TEST DURATION (DAYS/DATES)	14/JUNE 25 - JULY 8, 1979							
TEMPERATURE (RANGE, °C)	15.2 TO 21.7							
pH (SINGLE OBSERVATION ON DAY-14)	7.9-8.3	7.9-8.3	7.8-8.3	8.4-8.9	8.8-9.0	9.1-9.2	9.3-9.5	9.6-9.9
TOTAL ALKALINITY (RANGE, mg/L AS $CaCO_3$)	56-63	56-63	56-64	59-67	64-76	77-92	120-139	240-277
TOTAL HARDNESS (RANGE, mg/L AS $CaCO_3$)	64 - 70							
EXPERIMENTAL RESULTS								
BIOMASS (mg, ASH-FREE DRY WT) • MEAN	0.20	0.19	0.21	0.19	0.18	0.15	0.13	0.11
• ± c.i. [c]	±0.02	±0.02	±0.03	±0.02	±0.02	±0.04	±0.02	±0.02
• (n)	(4)	(4)	(4)	(4)	(4)	(3)	(4)	(4)
PHOTOSYNTHETIC RATE (mg C/L · DAY) • MEAN	5.23	1.99	2.76	2.40	2.18	1.09	0.0	0.0
• ± c.i. [c]	±0.51	±0.35	±1.99	±0.13	±0.49	±1.32	---	---
• (n)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
INSECT HABITATION (LARVAE REMAINING / TOTAL LARVAE EXPOSED)	48/160	47/160	48/160	43/160	13/160	2/160	0/160	8/160

[a] STATISTICAL CONFIDENCE INTERVAL ABOUT THE MEAN AT $\alpha = 0.05$
[b] NUMBER OF OBSERVATIONS
[c] SPECIFIC c.i.'s MAY NOT REFLECT THE RESULTS OF ANOVA PROCEDURES FOR LRCT DETERMINATIONS, WHICH USED POOLED VARIANCE ESTIMATES

TABLE A10. Experimental conditions and results of the third test where periphyton and insect larvae were exposed to elevated Li^+ concentrations. Results producing LRCTs are enclosed in boxes.

TEST 3:

EXPERIMENTAL CONDITIONS

PARAMETERS	CONTROL	Li_2CO_3 SOLUTIONS						
INTENDED Li^+ EXPOSURE CONC. (mg/L)	0.0	0.1	0.25	0.62	1.5	4.0	10.0	25.0
MEASURED Li^+ CONC. (mg/L) • MEAN [a]	< 0.02	0.10	0.27	0.61	1.62	4.24	10.8	28.1
• ± c.i. [b]	---	±0.05	±0.14	±0.22	±0.46	±0.78	±0.75	±1.7
• (n)	(8)	(8)	(8)	(8)	(8)	(8)	(15)	(11)
TEST DURATION (DAYS / DATES)	15 / AUGUST 9-23, 1979							
TEMPERATURE (RANGE, °C)	18.6 TO 24.0							
pH	7.8-8.3	7.9-8.3	8.2-8.3	8.5-8.6	9.1-9.2	9.0-9.3	9.8-9.9	10.2-10.3
TOTAL ALKALINITY (RANGE, mg/L AS $CaCO_3$)	56-63	56-63	57-66	59-69	64-78	81-99	128-146	246-278
TOTAL HARDNESS (RANGE, mg/L AS $CaCO_3$)	64 - 70							

EXPERIMENTAL RESULTS

BIOMASS (mg, ASH-FREE DRY WT) • MEAN	0.22	0.20	0.21	**0.16**	0.15	0.15	0.17	0.13
• ± c.i. [c]	±0.02	±0.01	±0.04	**±0.04**	±0.03	±0.01	±0.03	±0.02
• (n)	(4)	(4)	(4)	**(4)**	(4)	(4)	(4)	(4)
PHOTOSYNTHETIC RATE (mg C/L · DAY) • MEAN	13.23	7.89	10.63	11.75	6.60	**1.12**	0.0	0.0
• ± c.i. [c]	±2.32	±1.43	±5.03	±5.60	±1.40	**±1.03**	---	---
• (n)	(4)	(4)	(4)	(4)	(4)	**(4)**	(4)	(4)
INSECT HABITATION (LARVAE REMAINING / TOTAL LARVAE EXPOSED)	14/160	7/160	7/140	**4/160**	1/160	0/160	0/120	0/120

[a] STATISTICAL CONFIDENCE INTERVAL ABOUT THE MEAN AT $\alpha = 0.05$
[b] NUMBER OF OBSERVATIONS
[c] SPECIFIC c.i.'s MAY NOT REFLECT THE RESULTS OF ANOVA PROCEDURES FOR LRCT DETERMINATIONS, WHICH USED POOLED VARIANCE ESTIMATES

TABLE A11. Experimental conditions and results of the fourth test where periphyton and insect larvae were exposed to elevated Li^+ concentrations. Results producing LRCTs are enclosed in boxes.

TEST 4:

EXPERIMENTAL CONDITIONS

PARAMETERS	CONTROL	Li_2CO_3 SOLUTIONS						
INTENDED Li^+ EXPOSURE CONC. (mg/L)	0.0	0.1	0.25	0.62	1.5	4.0	10.0	25.0
MEASURED Li^+ CONC. (mg/L) • MEAN	<0.02	0.06	0.17	0.44	1.21	3.32	9.38	27.8
• ± c.i.[a]	---	±0.03	±0.06	±0.12	±0.22	±0.36	±0.32	±1.24
• (n)	(9)	(9)	(9)	(9)	(9)	(9)	(15)	(15)
TEST DURATION (DAYS / DATES)	15 / SEPTEMBER 11-25, 1979							
TEMPERATURE (RANGE, °C)	17.0 TO 21.3							
pH (RANGE)	7.5-8.3	7.9-8.3	7.8-8.3	7.8-8.3	8.1-8.3	8.8-8.9	9.2-9.3	9.7-9.8
TOTAL ALKALINITY (RANGE, mg/L AS $CaCO_3$)	56-63	56-63	57-65	58-67	63-73	77-89	121-133	247-272
TOTAL HARDNESS (RANGE, mg/L AS $CaCO_3$)	64 - 70							

EXPERIMENTAL RESULTS

BIOMASS (mg, ASH-FREE DRY WT) • MEAN	0.70	0.54	0.65	**0.43**	0.33	0.38	0.26	0.13
• ± c.i.[c]	±0.45	±0.13	±0.05	**±0.07**	±0.06	±0.17	±0.07	±0.04
• (n)	(4)	(4)	(4)	**(4)**	(4)	(3)	(4)	(4)
PHOTOSYNTHETIC RATE (mg C/L · DAY) • MEAN	15.98	19.1	22.6	24.0	25.8	30.1	20.6	**8.2**
• ± c.i.[c]	±5.66	±3.31	±2.59	±1.45	±1.80	±3.15	±0.99	**±2.9**
• (n)	(4)	(4)	(4)	(4)	(4)	(4)	(3)	**(3)**
INSECT HABITATION (LARVAE REMAINING / TOTAL LARVAE EXPOSED)	19/160	15/160	11/160	**7/160**	3/160	0/160	0/160	0/160

[a] STATISTICAL CONFIDENCE INTERVAL ABOUT THE MEAN AT $\alpha = 0.05$
[b] NUMBER OF OBSERVATIONS
[c] SPECIFIC c.i.'s MAY NOT REFLECT THE RESULTS OF ANOVA PROCEDURES FOR LRCT DETERMINATIONS, WHICH USED POOLED VARIANCE ESTIMATES

concentration of Li_2CO_3, and the control, we determined changes in pH induced by the titration of 0.02 N NaOH into 100 ml of test water. Resulting changes of CO_2 were calculated stoichiometrically from these data. This association was developed graphically to allow for the interpolation of CO_2 consumption by the photosynthetic process. Thus, the difference of pH from dawn to noon infers the amount of photosynthesis expressed as mg C/L.

Photosynthetic rates were calculated and expressed as mg C/L per day. Rate calculations involved insolation data recorded daily at Battelle's meteorological station to establish a solar-day curve from dawn to dusk. This curve is a graphic relationship between time, in minutes of the solar day, and insolation in Langley's ($cal/cm^2/min$). From this we determined the total insolation (ΣI) received on the day of the test, and the insolation received during the period of pH measurement (I_m). Ratios of these quantities were then used to convert photosynthesis into a daily rate with the equation

$$P_r = P \frac{\Sigma I}{I_m} ,$$

where P is a measure of photosynthesis in mg C/L and P_r is the net photosynthetic rate is mg C/L/day.

Periphyton biomass was measured by removing all of the algae from the water, walls and floors of exposure containers and drying the samples at 105°C in tarred crucibles.

After dry weights were determined on a Mettler Model H64 microbalance, the samples were ash-fired at 500°C to permit complete combustion. The resulting ash weights were subtracted from dry weights to obtain a measurement of biomass (organic mass) expressed in mg.

Standard glass microscope slides were also used as substrates for periphyton growth. These were placed in exposure containers (Fig. A3) to measure the diversity of diatom populations as a response to Li^+ concentrations. Although these samples have been collected on day 14, preserved, labeled and stored; diatom-diversity results will not be analyzed and discussed in this document. There are two reasons for this.

First, the use of "species diversity" or taxonomic diversity as an indicator of organizational complexity and community structure has been the subject of much debate in the 1970's. The consenses on this matter among leading theoretical ecologists has caused the diversity notion to be largely abandoned (Emery 1981). Second, the time and expense of diatom sample preparation, taxonomic identification and interpretation of results fell beyond the resources of this research program.

The habitation of insects in our exposure containers was observed using the midge larvae from McNary Reservoir sediments. Test chironomids were held in experimental Li^+ concentrations within exposure modules (Figure A3). Each exposure module held 20 chironomids that were well-burrowed into 200 ml of native sediments prior to testing. Two modules were placed in each replicate exposure container. Thus, each test concentration plus control exposed 160 larvae in the four replicates.

To measure habitation, we counted the number of larvae remaining after 14-day experimental periods. The ratio of larvae remaining to total numbers initially exposed formed a measure of habitation. This is only a relative measure, however, since it cannot be interpreted as direct evidence of toxic challenge. Chironomid larvae could have left because of metamorphic emergence of natural preference to downstream conditions. There were few dead larvae discovered in the experimental sediments. Nevertheless, the parameter proved to be sensitive to Li^+ and was also interpretable statistically.

PNL-3640
UC-11

DISTRIBUTION

No. of Copies

OFFSITE

A. A. Churm
DOE Patent Division
9800 S. Cass Avenue
Argonne, IL 60439

Helen McCammon, Director
Ecological Research Division, EV-34
Office of Health and Environmental Research, GTN
U.S. Department of Energy
Washington, D.C. 20545

R. L. Watters
Ecological Research Division, EV-34
Office of Health and Environmental Research, GTN
U.S. Department of Energy
Washington, D.C. 20545

27 Technical Information Center

No. of Copies

ONSITE

DOE Richland Operations Office

H. E. Ransom

Pacific Northwest Laboratory

C. D. Becker
C. E. Cushing
R. M. Emery (15)
K. E. Harding (4)
D. C. Klopfer (5)
T. L. Page
T. M. Poston
J. R. Skalski (5)
J. A. Strand
W. L. Templeton
B. E. Vaughan (20)
D. G. Watson
Technical Information (5)
Publishing Coordination (2)