The Effect of Zinc on the Ability of the Minnow, *Phoxinus phoxinus L.*, to Compensate for Torque in a Rotating Water-Current

by BENGT-ERIK BENGTSSON

The National Environment Protection Board, Brackish Water Toxicology Laboratory, Studsvik S-611-01 Nyköping, Sweden

Mortality is the most common parameter studied in toxicological bioassays. Although mortality studies are designed to extend over long periods of time, the sharp distinction between dead and living is unsatisfactory for the toxicologist's estimation of acceptable non-toxic levels for a particular substance. Accordingly, many studies in toxicology are supplemented by "General Observations" or short notes containing brief descriptions of some observed sublethal effects. In spite of the fact that these data are often far more interesting and perhaps of greater biological significance than the reported lethal limits, they are frequently neglected because of their non-numerical character. The full consequences of this observation suggest that mortality studies can be combined to advantage with an estimation of the performance or general condition of the survivors.

During a series of long-term experiments with minnows, Phoxinus phoxinus L., exposed to zinc nitrate the author was tempted to test the rotatory-flow principle described by LINDAHL and SCHWANBOM (1971). According to these authors sublethal toxic effects can be detected in living fish by subjecting them to a flow of water that is caused to rotate in a narrow tube around the direction of flow. The number of revolutions per minute with which the water rotates is raised from zero to a specified maximum while the behaviour of the fish is followed. The "critical r.p.m." is then defined as that at which the fish is unable to compensate for the torque of rotating flow and is forced to rotate.

A modified version of the above-mentioned apparatus was constructed at the author's laboratory. The present paper contains the results for some experiments performed with three categories of minnows, namely underyearlings, yearlings and mature fish following exposure to zinc nitrate for periods of 100 to 270 days.

MATERIAL AND METHODS

The fish used in this experiment (Phoxinus phoxinus L.) are identical with those used in a previous mortality study with zinc nitrate performed by the author (BENGTS-

Bulletin of Environmental Contamination & Toxicology, Vol. 12, No. 6 © 1974 by Springer-Verlag New York Inc.

SON in the press). The reader is referred to this earlier account for details of the chemical and physical characteristics of the experimental water environment, the practical procedures adopted to contain and feed the fish and the dosing arrangements.

The rotatory-flow apparatus used in the experiment was a simplified version of the apparatus described by LINDAHL and SCHWANBOM (1971). Since the principle of the device is identical with that of the prototype, it is described only briefly in the present paper. The testing tube and the propeller employed to produce the water flow constitute the submerged section. The upper section consists of two motors, one of which drives the propeller while the other supplies the rotation of the tube. The speed of this last motor is regulated by a servomotor to produce a linear increase in the number of revolutions per minute from zero to 150 in five minutes. A complete description of the apparatus can be obtained on request.

The underyearlings, yearlings and mature minnows were tested in tubes with an inner diameter of 16, 20 and 26 mm, respectively. The water velocity was varied from about one to three centimetres per sec., to optimize the accuracy of the measurements at different tube and fish sizes. It was, however, kept constant for the same test category, within which a comparison of critical r.p.m. was performed. Each individual was tested three times in clean water at a water temperature which was identical with that of the test aquaria. In all, the "performance" of 44 underyearlings, 96 yearlings and 45 mature fish was recorded in the rotatory-flow apparatus. The two categories of yearlings represent two different exposure groups, while in the mature category, the same individuals were studied on two different occasions, namely after 100 and after 270 days of exposure to zinc. Since different water velocities and water temperatures were employed in the two tests with the mature fish, the results are not entirely comparable. The instant at which the fish became incapable of compensating for the torque and was forced to rotate was recorded by means of a stop-watch and the critical r.p.m. was read from a nomogram of time versus rotational velocity.

A statistical analysis was made within each category of fish. Multiple comparisons among means were obtained by the posteriori Student-Newman-Keuls test as described in "Biometry" by SOKAL and ROHLF (1969). Data for exposure times and mean zinc concentrations are presented in table 1.

RESULTS

The fish did not acquire skill in compensating for the effect of the rotating water mass nor did they seem to be exhausted by the test. Those individuals most strongly affected by the toxic effects of zinc, however, exhibited swimming disability in the water current even before the tube had begun to rotate. The mean values, standard deviations and ranges of the critical r.p.m. for the 23 groups are presented in table 1.

TABLE 1

Category	Exposure data		Number of fish tested	Critical r.p.m.		
	duration (days)	mean Zn conc.(ppm)		mean	S.D.	range
Under-yearlings	108	control	19	101	5.6	92-112
	108	0.06	18	*93	11.0	73-102
	108	0.16	7	*80	10.0	65-96
Yearlings (I)	109	control	23	93	4.8	88-102
	109	0.06	21	94	5.2	86-102
	109	0.16	10	*80	22.1	28-100
	109	0.33	4	*43	20.4	14-62
	109	0.78	1	*59		
Yearlings (II)	150	control	10	97	4.0	90-104
	150	0.05	10	96	4.3	90-104
	150	0.13	8	96	4.3	89-104
	150	0.20	6	90	11.2	84-101
	150	0.30	3	*70	13.5	53-86
Adults (I)	100	control	10	101	6.1	90-109
	100	0.05	10	99	8.9	87-111
	100	0.13	10	98	8.9	77-107
	100	0.20	8	*70	27.0	22-108
	100	0.31	7	*56	24.2	6-79
Adults (II)	270	control	9	117	8.3	105-129
	270	0.05	10	121	7.3	109-129
	270	0.13	8	121	9.0	103-133
	270	0.20	8	108	26.6	43-133
	270	0.30	5	*95	19.2	65-123

In the table the mean values against which asterisks have been set represent significant decreases as compared with the respective values for the controls in each category.

For underyearlings the mean critical r.p.m. showed a significant decrease at a zinc level as low as 0.06 ppm and also at 0.16 ppm after 108 days of exposure.

After 109 days the yearlings exhibited a lower critical r.p.m. as compared to the control at zinc levels of 0.16, 0.33 and 0.78 ppm, whereas no effect was observed at 0.06 ppm. The yearlings were also seen to be less sensitive when tested after 150 days of exposure to zinc. Only the 0.30 ppm level produced a decreased critical r.p.m. A similar relationship seemed also to be valid for the adults. These exhibited a significant reduction of critical r.p.m. at levels 0.20 and 0.31 ppm after 100 days, whereas after a 270 day exposure only the 0.30 ppm level produced a reduction.

In a separate study (BENGTSSON 1974) a histological examination was performed on the adults. The results showed that eight of the 13 specimens remaining in the 0.20 and 0.30 ppm level were found to have suffered vertebral damage. The mean critical r.p.m. for these fish was significantly lower than that of the five remaining individuals, which showed no histological effects. The measured values for the two groups are 90.3 ±25.5 and 117 ±9.7 respectively.

DISCUSSION

The fish exposed to zinc give clear evidence for a positive correlation between age and ability to compensate for the rotating water-current. The results obtained for the underyearlings indicate that this category of minnow is highly sensitive to zinc. None of the parameters (mortality, growth, reproduction, activity and histology) studied in earlier investigations by the author (BENGTSSON 1974 and in the press) have given evidence of toxic effects at such a low level of zinc as is documented in the present study.

When the experiments were extended from 109 to 150 days for the yearlings and from 100 to 270 days for the adults, no suppression was observed in the ability to compensate for the torque below the 0.30 ppm level. On the contrary, there was an apparent recovery in the general condition of the remaining fish after a certain period of exposure. For the yearlings at least, this recovery was, however, solely related to selective mortality on the part of the fish which demonstrated visible symptoms of the chronic effects of zinc. Thus, after 150 days the remaining fish exhibited comparatively few external symptoms of zinc toxicity. There was no evidence of individual recovery of any significance. According to what is mentioned above, an observed decrease in the critical r.p.m. for a particular specimen may be used as an early indication of an incipient lethal toxic effect.

The reader may question whether or not the phenomenon of decreased critical r.p.m. is of ecological or bio-

logical significance. In this connection the data at present available from comparable investigations are too meagre to permit a firm conclusion to be drawn. From a (bio-)logical point of view, however, the display of a sub-lethal behavioural effect is certainly important. The normal behaviour of an animal allows it to function favourably in its environment. Since behaviour can be regarded as the integrated movements of the animal as a whole, any limitation of a particular aspect of its behaviour may well be hazardous to its survival. This remark is especially appropriate when damage to the integrating system itself (i.e. the nervous system) is involved. Thus LINDAHL and SCHWANBOM (1971) suggested that a reduction of the critical r.p.m. may be ascribed to disturbances in the function of the central nervous system as well as to other factors. This view is supported by the observations in this paper.

The vertebral damage observed in the adult fish (BENGTSSON 1974) ought to be sufficient cause for the observed decrease in critical r.p.m. Other effects, such as disturbed nerve functions, may however contribute to, or be, the main reason for the decrease observed in this investigation. Such an assumption is supported by the fact that some individuals exhibited a decreased critical r.p.m. in the absence of any detectable vertebral damage.

There is no doubt that further research into the above effect is both desireable and necessary in order to establish the exact ecological consequences of the causes underlying a reduction in critical r.p.m. Nevertheless, the rotatory-flow principle seems destined to become generally accepted and applied as a valuable addition to the facilities for performing quantitative toxicological bioassays.

ACKNOWLEDGEMENTS

The investigation was financially supported by the National (Swedish) Environment Protection Board, the National (Swedish) Board of Fisheries and the Section of Ecological Zoology, University of Umeå, where the work was performed. I want to thank Professor A. Lindroth and Mr. G. Borgström, University of Umeå, for valuable and skilful help in the design and construction of the test apparatus.

REFERENCES

BENGTSSON, B. E.: Oikos, in press (1974).

LINDAHL, P. E. and E. SCHWANBOM: Oikos 22, 210 (1971).

SOKAL, R. R. and F. J. ROHLF: Biometry. San Fransisco: W. H. Freeman and Company 1969.