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A Re-Examination of the Toxicity Test for Water Pollutants

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ABSTRACT

In toxicity tests carried out in Japan, adult or young adult fish having relatively stable tolerance and relatively low sensitivity to various toxicants are usually used. However, in order to preserve the ecosystem, fish in the early stage of life, which are more sensitive to toxicants than adult or young adult fish, should be used as test organisms in the toxicity test. Medaka in the early stage of life were therefore exposed to 0.1 ppm Cd solution and their resistance to Cd toxicity was examined. As a result it was shown that resistance to Cd toxicity in medaka of early stage of life was steady from seven to twenty days after hatching. It is therefore suggested that medaka fry could be used as a test organism in toxicity tests.

Fish have very often been used in the usual toxicity tests for water pollutants and detailed methods for utilizing them are stated in JIS[4]. According to JIS, fish such as Cyprinida (carp), Cyprinodontida (medaka), and so on are generally considered suitable for use in toxicity tests, individuals less than 75 mm in length being more desirable. In most toxicity tests, adult or young adult fish of these species have usually been employed. It is known that fish at these stages of life have more powerful resistance to various toxicants in comparison with fish in the early stage of life[2,5,6]. Taking preservation of the ecosystem into consideration, however, it would be expected that fish in the early stage of life, which are more sensitive to water pollutants than fish in the adult or young adult stage, would be more suitable as test organisms in toxicity tests. However, it seems that in Japan fish in the early stage of life have not been sufficiently utilized in such toxicity tests. The present study was therefore carried out to examine whether such fish in the early stage of life could be utilized as test organisms in toxicity tests or not.

MATERIALS AND METHODS

Red medaka (red variety of *Oryzias latipes*), is small freshwater teleost fish, was used as the test organism because of the fact that it is easy to obtain, handle, and rear. As the water pollutant, cadmium (Cd) was chosen, as it is known to be one of the most toxic substances to fish and its characteristics are well documented. The present study was intended to investigate how the resistance of medaka fry to Cd toxicity changes with time.

Adult medaka were purchases from Ito Fish Farm, reared carefully in indoor glass aquariums, and made to spawn. The fertilized eggs were reared and hatched out under carefully managed conditions. The fry were fed on the primary bait for *Ayu (Plecoglossus altivelis)* (Oriental Yeast Co., Ltd; *Ayu* fish starter No. 0) until 7 days after hatching. Thereafter, the fish were fed on Tetramin. In the experiment, a large number of medaka fry harvested from one group of adult medaka were reared under the above-mentioned conditions and 12-15 individuals were then transferred to another small container at random one day before experimen-

tal exposure and were not fed at that time.

The exposure experiments were set up as follows; 0.1 ppm Cd solution was prepared using cadmium chloride ($CdCl_2$), 100 ml of which was added to each of several 100 ml beakers. Respective groups of 10 fish at ages 1, 2, 3, 5, 10, 20, 30, 40, 50, and 100 days after hatching were put into individual beakers and after 24 hr the numbers of survivals were counted. Special care was taken in the transfer of early fry from the rearing tank to the small container and from the small container to the 100 ml beakers.

Test solution pH was 6.9, total hardness (as $CaCO_3$) was 10.5 mg/liter, Cl^- was 6.5 mg/liter, and water temperature was 25°C. Judgement of death was defined as a lack of any perceptible heart beat.

RESULTS

The number of survivals against time of medaka fry — young adults which had been exposed to 0.1 ppm Cd solution for 24 hr are shown in Fig. 1. All of 10 fry survived at the first day after hatching, the number of survivals being gradually reduced to three and two of 10 fry for the second and third day after hatching, respectively. For the fifth day after hatching the number of survivals increased to 4 fry, then from the tenth to the twentieth day after hatching the number of survivals was 5 fry. From our another experiment, we obtained the results that the number of survivals of medaka fry which had been exposed to 0.1 ppm Cd solution for 24 hr was 5. This result therefore partially supports the data of the present study. The number of survivals increased from the thirtieth day after hatching, reached 10 fish at the fiftieth after hatching, and this number was the same on the one-hundredth day after hatching.

DISCUSSION

In the present study all of 10 fry survived for the first day after hatching. From this result, it is inferred that as medaka fry gradually adapted themselves to the given environment after hatching and absorbed sufficient nutrients from the yolk, the fry had the ability to resist Cd toxicity. For the second and third day after hatching the number of survivals was reduced. This was presumably because reserve nutrients present within the fry were gradually consumed and the fish at this time were unable to obtain sufficient bait. From the fifth to the twentieth day after hatching, the number of survivals increased and then remained steady at that level. It is inferred that the fry at this stage were able to adapt themselves to the given environment and take the bait, their resistance to Cd toxicity consequently increasing and becoming steady and the number of survivals corresponding to

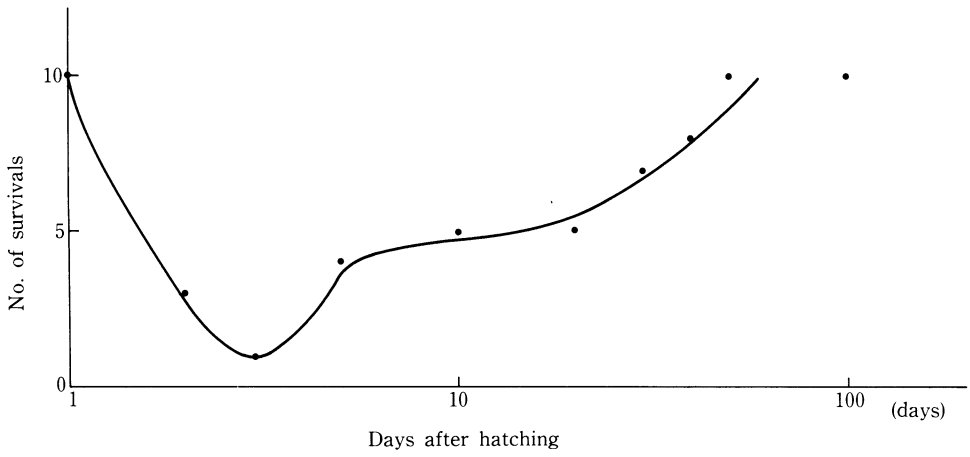

Fig. 1. Change in the number of survivals following exposure to 0.1 ppm Cd solution for 24 hr

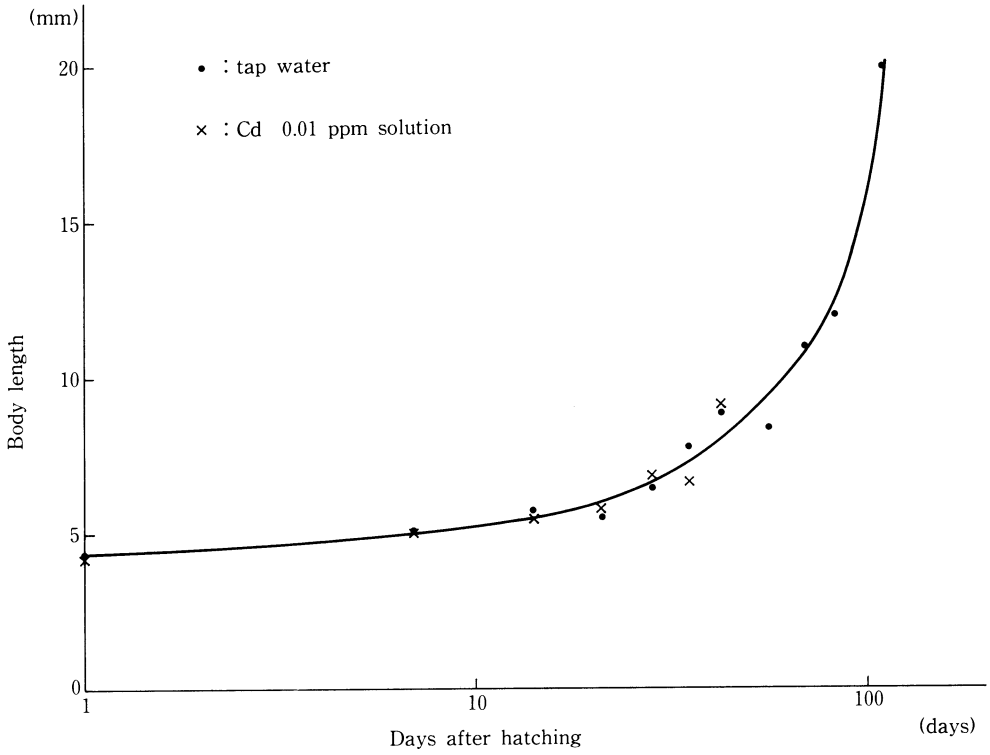

Fig. 2. Increase of body length with the passage of time. Growth of medaka raised in 0.01 ppm Cd solution was almost the same with that of medaka in tap water. (Hiraoka et al[3])

such changes. Hiraoka et al[3] has reported that medaka's body length increases remarkably from twenty-one days after hatching (Fig. 2), and it is supposed that such remarkable increase of body length also occurred in the case of the present study, although medaka's body length were not measured in this study. Resistance to Cd toxicity was thought to be gradually endowed as rapid growth proceeded, the number of survivals gradually increasing.

As a result of the present study three-day fry were most sensitive to Cd toxicity, but fry of this stage can not be utilized in toxicity tests because resistance to Cd toxicity in these fry are changeable before and after that stage. From seven to twenty days after hatching resistance to Cd toxicity is slightly greater than that at three days after hatching but such resistance is steady at this stage. Fujii et al[1] reported that in the case of continuous exposure experiments medaka fry are most sensitive to Cd toxicity from ten to twenty days after hatching. Also, as resistance to Cd toxicity is steady at this stage, the possibility arises that such medaka fry could be used in toxicity tests.

Adult or young adult fish are usually used in toxicity tests, having a degree of resistance to various kinds of toxicants at this stage of life. McKim[5] stated in his review that tests employing the embryo-larval and early juvenile life stages can be used to estimate the maximum acceptable toxicant concentration (MATC). Taking preservation of the ecosystem into consideration, it would be expected that fish of the stage which is most sensitive to water pollutants, that is, fish in the early stage of life, would be most useful in toxicity tests. As a result of the present study, it is suggested that medaka fry from the seventh to the twentieth day after hatching have such potential for use in toxicity tests. In the future more detailed studies will be carried out and establishment of more effective toxicity tests for preservation of the ecosystem will be expected.

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