海産魚2種の胚発生に及ぼすトリチウム水の影響

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Effects of Tritiated Water on the Embryonic Development of Two Marine Teleosts.

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Artificially fertilized eggs of common flounder, *Paralichthys olivaceus* and a species of puffer, *Fugu niphobles* were reared until hatching in tritiated sea water of various concentrations. No significant difference in their hatchability was observed between control and tritiated water groups of 10^{-8} to 10^{-2} Ci/*l*. However, a decrease in puffer egg hatchability was observed in tritiated water of very high concentrations, i. e., 1 and 10 Ci/*l*. Absorbed radiation dose was calculated to be 2160 rad in 180 hours for the 1 Ci/*l* group and 15600 rad in 130 hours for the 10 Ci/*l* group. The hatched larvae of the 10 Ci/*l* group were rather inactive, lying on the bottom of the tank, whereas the control larvae swam actively. Compared with control larvae, the 10 Ci/*l* larvae developed smaller stumpy-shaped bodies with swollen abdomens. Although the eye diameter of the 10 Ci/*l* larvae was found to be only about 57 per cent that of the controls, the body weights of both groups were nearly equal, possibly due to the larger amount of residual yolk in the 10 Ci/*l* larvae. These findings indicate that the morphological development of embryos reared in tritiated water of high concentration is retarded.

Tritium is being produced naturally by cosmic radiation. Past nuclear weapon tests have widely introduced additional amounts of this nuclide into the hydrosphere of the world. Therefore, the present level of tritium in terrestrial and surface ocean waters is approximately one order of magnitude higher than its natural level. The nuclear power industry, which is now growing rapidly, also produces significant amounts of tritium by reactor operation resulting in the coastal discharge of this nuclide from nuclear power plants and fuel reprocessing plants. Tritium being released from these nuclear facilities greatly exceeds other waste radioactivities in their liquid effluents because the removal of tritium from waste water is not economically feasible by the presently available technology. Therefore, it is inevitable that at least some local marine environments will continue to receive substancial amounts of tritium. The radiation dose to man of tritium discharged into coastal areas is not very significant compared with other nuclides found in the liquid effluents from these nuclear facilities because no remarkable bioconcentration of this nuclide is observed through marine food-chains related to human intake. However, there has been serious concern about the risk of biological effects of radionuclides present in environmental water on the embryonic development of aquatic organisms in view of the conservation of coastal fishery resources.

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The present investigation was undertaken to know the biological effect of tritiated water on the development of fertilized eggs of marine fishes and the results of some preliminary expertments are reported here.

Materials and Methods

Common flounder, *Paralichthys olivaceus* and a species of puffer, *Fugu niphobles,* were collected during their spawning season from the coastal sea near Kominato, Chiba prefecture. Eggs or sperms of these fishes were stripped into a plastic sylinder by gently pushing the ventral part of their bodies. After placement in a glass dish, eggs and sperms were mixed carefully by using a feather for a few minutes to promote their fertilization and then washed several times with natural sea water. In the case of flounder, the fertilized eggs were easily identified because the eggs floated on the water surface after successful fertilization. Fertilized eggs were then transferred into tritiated sea water of various concentrations.

Absorbed radiation dose to eggs due to tritium can be calculated by the following formula derived by a slight modification of ICRP's dose calculation method for internal organs.[1] Uniform distribution of tritium in eggs and no enrichment of tritium to eggs from water were assumed in this calculation. Therefore, the calculated dose is based on a model in which tritium concentration was equal both inside and outside the eggs.

$$D=51.2\ Q\cdot E_m\ \text{rad/day}$$

where D = absorbed beta dose rate to egg,

Q = microcuries per gram of water and egg,

E_m = mean energy of beta ray (0.0057 MeV for tritium).

Results

Hatchability experiment—1

Fertilized eggs of common flounder, *Paralichthys olivaceus,* were immersed in various concentrations of tritiated sea water ranging from 10^{-8}Ci/*l* to 10^{-2}Ci/*l* four hours after fertilization. At that time, fertilized eggs were at the 16–cell to 32–cell stage and water temperature was kept at 15±2°C. Hatching was observed from 72 hours to 96 hours after fertilization (68 to 92 hours after introduction into tritiated sea water). .Number of fertilized eggs used for each experimental group and percentage hatchability of each group are shown in Table 1. Radiation dose to embryo was calculated for 92 hours immersion in tritiated sea water assuming homogenous distribution of tritium within and outside of the embryo.

No significant difference in hatchability can be observed among control and all the tritiated water groups upto the tritium concentration of 10^{-2}Ci/*l*. It was considered that

Table 1. The hatchability of flounder eggs in different concentrations of tritiated water

tritium concentration (Ci/l)	accumulated dose (rad) in 92 hours	number of eggs	hatchability (%)
control	—	703	82.8
10^{-8}	10^{-5}	941	79.2
10^{-6}	10^{-3}	735	82.4
10^{-4}	0.11	890	88.5
10^{-2}	11	661	80.3

much higher concentration than in the above experiment should be necessary to detect any significant effect on the hatchability of these eggs.

Hatchability experiment—2

A species of puffer, *Fugu niphobles*, was used for the second experiment. Fertilized eggs at the two-cell stage (1 hour and 20 minutes after fertilization) were placed in tritiated sea water of 10^{-4}, 10^{-2} and 1 Ci/*l*. Hatching was observed from 140 to 180 hours after fertilization at the water temperature of 23°C. Calculated radiation dose to embryo during 180 hours and hatchability of each experimental group are tabulated in Table 2.

Table 2. The hatchability of puffer eggs in different concentrations of tritiated water

tritium concentration (Ci/l)	accumulated dose (rad) in 180 hours	number of eggs	hatchability (%)
control	—	911	41.6
10^{-4}	0.22	798	44.0
10^{-2}	22	779	41.2
1	2160	846	36.7

It was observed that hatchability of 1 Ci/*l* group seemed to be somewhat lower than that of control and the other two groups of lower level tritiated water.

Growth experiment

In order to detect the morphological abnormality due to tritium, fertilized eggs of puffer, *Fugu niphobles,* were immersed in tritiated sea water of extremely high concentration (10 Ci/*l*) about 19 hours after fertilization. The embryo was in its gastrula stage at this period. Well developing eggs were used for this experiment by selecting these eggs under a microscope from pooled eggs which had been fertilized one night before. Most of eggs hatched about 150 hours after fertilization (130 hours after immersion into tritiated water) at the water temperature of 27°C.

Although hatchability of tritiated water group was apparently lower than that of control group, the extent of hatchability decrease was not catastrophic considering the extremely high radiation dose to eggs as shown in Table 3. However, various differences

Table 3. Body weight and eye diameter of newly-hatched larvae of puffer

tritium concentration (Ci/l)	accumulated dose (rad) in 130 hours	number of eggs	hatchability (%)	body weight (μg) mean+s.d.	eye diameter (μ) mean±s.d.
control	—	403	97	72.2±4.9	244±13
10	15600	400	89	76.2±4.1	140±17

were observed between these two groups. Hatched larvae of control group were actively swimming in the middle layer of the culturing water, whereas these of 10 Ci/*l*-tritiated water group were quietly lying down on the bottom of the container. When examined under a microscope, the tritiated water group showed considerably smaller body size and stumpy shape with swelled abdomen compared with control group (Fig. 1). It was also observed that body surface colour was significantly darker in the control than in the tritiated water group, though it was not confirmed whether this was caused by different density of dermal melanophore or by different morphological states (with pigments concentrated or dispersed) of melanophore.

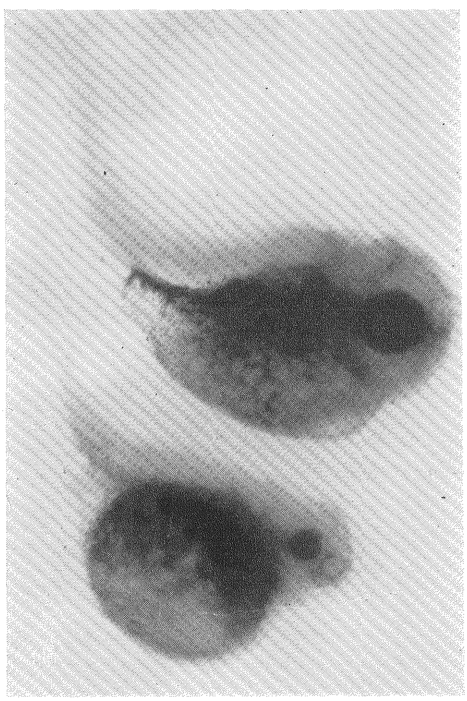

Fig. 1. Newly-hatched larvae of puffer.
upper: control larva
lower: larva hatched in tritiated water (10 Ci/*l*)

As body length of larvae could not be measured accurately because of their inconsistent posture, eye diameter was measured for twenty individuals of each group under a microscope as an comparative index of their morphological development. Mean value for eye diameter of tritiated water group was found as only about 57 per cent that of control group (Table 3).

On the other hand, twenty individuals of each group were weighed by using a Mettler M–5 balance after placed on a filter paper to remove the excess water. Tritiated water group showed slightly larger mean value of body weight than that of control, though the difference was not statistically significant.

Discussion

Only limited information is available on the effect of chronic exposure of aquatic organisms to tritiated water especially for marine fishes. BLAYLOCK *et al*[2] found no significant difference between the hatchability of carp eggs subjected to tritiated water of 7×10^{-2} Ci/*l* to 5×10^{-1} Ci/*l* and the control. STRAND *et al*[3] did not detect clear indication of hatchability decrease for rainbow trout eggs immersed in tritiated water of 10^{-5} Ci/*l* to 10^{-2} Ci/*l*. WALDEN[4] reported the results on eye diameter measurement for the hatched fry of three-spine stickleback (freshwater fish) and English sole (sea fish) subjected to tritiated water of 0.5, 1.0, and 2.0 Ci/*l* at their embryonic development. He observed significant reduction of eye diameter at tritium concentrations of 1.0 and 2.0 Ci/*l* but not at 0.5 Ci/*l* for both species of fish.

Results of the present investigation on flounder and puffer that did not show any significant decrease in hatchability at the tritium concentration of upto 10^{-2} Ci/*l* correspond to the above findings reported on freshwater fishes. Reduction of eye diameter reported by WALDEN can be calculated roughly as 10 per cent for 1 Ci/*l* and 20 per cent for 2 Ci/*l* whereas our puffer's eye diameter was reduced by about 40 per cent at 10 Ci/*l* of tritium concentration. It seems likely that effect of tritiated water on the hatchability and growth of marine fish embryo would become apparent at a very high concentration such as above 1 to 10 Ci/*l*. The present finding that no effect was observed in hatchability of eggs immersed in tritium water of 10^{-2} Ci/*l* (approximately 10 to 20 rad exposure) and below coincides with TEMPLETON's results[5] that no significant differenced at hatching was observed in plaice eggs chronically irradiated with ^{137}Cs source with total doses ranging from 0.6 to 500 R during the period from fertilization to hatching.

Smaller eye size and swelled abdomen of hatched larvae of the highly tritiated water (10 Ci/*l*) group indicated that morphological development of embryo was retarded during the time from fertilization until hatching, resulting in smaller body size and larger amount of remaining yolk in this group. It is reasonably understood that the under-developed embryo with larger amont of remaining yolk showed nearly equal body weight with more developed control group because the developing embryo should absorb necessary substances from their yolk before hatchning. A slight difference of body weight observed between the highly tritiated and control groups might be due to possible larger consumption of energy in control embryo during the development before hatching. However, it is not clearly verified by the present data.

POLIKARPOV and IVANOV[6] reported that ^{90}Sr in sea water increased number of abnormalities in hatched larvae of Black Sea fishes even at the concentration of 10^{-10} Ci/*l* and the percentage of abnormal larvae was as high as more than 80 per cent at ^{90}Sr concentration of 10^{-4} Ci/*l*. However, TEMPLETON[5] did not observe any significant increase in the mortality or in the production of abnormal larvae when eggs of brown trout and of plaice were reared from immediately after fertiltzation until hatching in water with ^{90}Sr over the concentration range 10^{-10} to 10^{-4} Ci/*l*. NELSON[7] found that a significant increase in abnormal larvae was detectable at the minimal concentrations of 10^{-4} Ci/*l* for ^{65}Zn (carrier free),

10^{-4} Ci/l for ^{51}Cr and 10^{-3} Ci/l for ^{90}Sr, when Pacific oyster larvae were reared for 48 hours following spawning in sea water containning either ^{65}Zn, ^{51}Cr, or ^{90}Sr. Although this kind of information is still insufficient and there have been some discrepancies between the results of different investigations, it seems likely that tritium is less hazardous to embryonic development of marine fishes than ^{90}Sr and some other radionuclides. More sensitive index of biological damage than hatchability or morphological difference may be useful for further investigation on the tritium effect on the embryonic development of marine fishes.

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