

研究論文

**002 Bioaccumulation and Retention of Some Radionuclides
by Developing Eggs and Larvae of Rainbow Trout***

Yuichiro KIMURA,** Yoshihide HONDA**
and Yasushi NISHIWAKI

(Received October 2, 1978)

The experimental methodology employed in radiobiological studies with developing fish eggs and larvae is discussed on the basis of the experimental results of uptake, accumulation and loss of radionuclides by developing eggs and larvae of rainbow trout. The period of uptake by the organisms lasted until the isotopic equilibrium is nearly reached, and the concentration factors at equilibrium were estimated on the basis of the exponential model together with the rates of uptake and turnover rates. The localization of radionuclides in the eggs was investigated by means of fixation with the use of formalin solution. It was noted that the substantial fractions of some radionuclides were released into formalin solution during fixation even in a refrigerator.

Finally, the radiation dose to developing fish embryos in contaminated water was estimated on the basis of a dosimetry model.

INTRODUCTION

The additions of artificial radioactivity to the aquatic environment either through nuclear weapons tests or the release of low level radioactive wastes from nuclear facilities have been relatively small as compared with the background levels of natural radioactivity in the aquatic environment. However, if we consider rapid development of nuclear industries in the future, it is extremely important for protection of man and his environments against man-made radioactive materials to elucidate radioecological concentration processes in the environment. Since it has been suggested that developing fish eggs constitute one of the most sensitive components of the marine ecosystem [1, 2], it is of particular interest for evaluation of potential environmental radiation damage to obtain relevant informations on the accumulation of radionuclides by developing fish eggs as well as radiation effects on their embryogenesis. In relation to these problems, experimental studies on the accumulation of radionuclides by fish eggs, the assessment of radiation dose to developing fish embryos and the effects of radionuclides in water on fish embryogenesis have been carried out by many workers [1-32]. However, the informations on the radioecological concentration processes during development of fish eggs

*The synopsis of this paper was presented to the meeting of the Advisory Group on Methodology of Radiation Effects Experiments on Aquatic Organisms and Ecosystems for the Evaluation of Effects of Releases of Radionuclides into Aquatic Environment organized by the IAEA, and held in Vienna, Austria, 21-25 November 1977.

**Department of Nuclear Reactor Engineering, Faculty of Science and Technology,

and growth of larvae are not necessarily enough because of the difficult problems involved in hatching and rearing fish larvae under laboratory conditions. In addition the incompatibility of the experimental results of radioecological studies conducted at different laboratories has been often pointed out. Regarding this problem, recently the pertinent reviews have been published in the reports of the meetings on radiobiological studies with aquatic organisms organized by the IAEA (33, 34). The authors present methods and some of the results of the experimental studies on the uptake, accumulation and retention of some radionuclides by eggs and larvae of rainbow trout as well as on the localization of radionuclides in the eggs and the radiation dose to the developing fish embryos (28-30).

EXPERIMENTAL METHODS

Radionuclides

The chemical and physico-chemical forms of the isotope as well as the specific activity may be considered the factors of most important consideration (35). The initial chemical forms and the specific activity of radionuclides used in the experiments were as follows:

^{60}Co : CoCl_2 , specific activity 128 mCi/mg,

^{131}I : NaI , carrier free,

^{137}Cs : CsCl , carrier free,

^{144}Ce : CeCl_3 , carrier free,

^{106}Ru : Ru chloro complex and three different chemical forms of RuNO complexes, namely, RuNO-nitro complex, RuNO-nitrato complex and RuNO-binuclear complex; the final concentrations of Ru carrier in rearing waters were less than 10^{-6} M. The radionuclides were added to rearing waters at the concentrations of about 1-10 $\mu\text{Ci/l}$.

Fish eggs and larvae

The artificially fertilized eggs of rainbow trout, *Salmo gairdneri irideus* were obtained from Samegai Trout Hatchery, Shiga Prefecture in Japan. The eggs put in polyethylene bags were transported under ice cold conditions by car immediately after fertilization. The diameter of egg was 5.7 mm on an average. The development of fish egg is illustrated in Fig. 1.

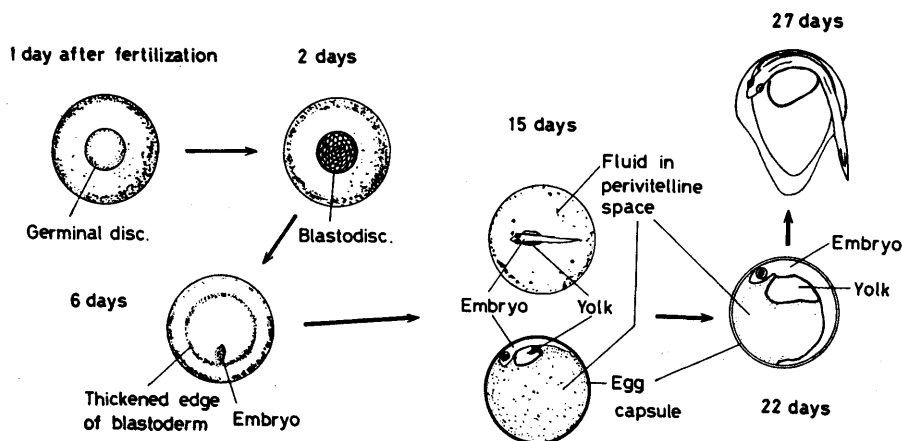


Fig. 1 Development of fish egg

The larvae used in the experiments, as shown in Plate 1, were advanced fry which were about 10 days old after hatching in our laboratory. The average body length and weight of the advanced fry were 1.5 cm and 0.1 g, respectively.

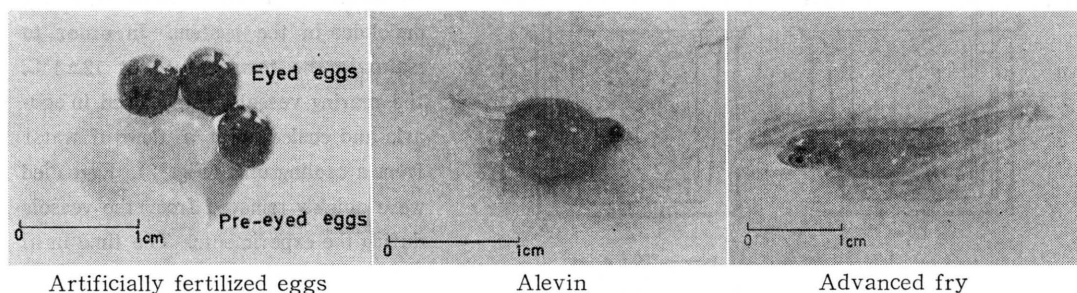


Plate 1 Development and growth of rainbow trout

Rearing method for eggs

As for the laboratory rearing of fish from fertilized eggs, great advances have been made in recent years in relation to the development of cultural fishery. Various experimental methods have been used for the experiments with fish eggs and larvae rearing in the past. Although similar experimental methods may be applicable to the eggs of marine and fresh water fish, special consideration would be necessary for pelagic, adhesive or demersal eggs under different environmental conditions [36-41]. Pentreath [42] described two simple containers that have been successfully used for studies on the accumulation of radionuclides by pelagic plaice eggs and by demersal herring eggs, respectively. For the purpose of radiobiological studies with developing fish eggs, many of the experiments have been conducted in closed system, in which the effective control of water temperature and oxygenation as well as water quality including the prevention of microbial growth is of great importance [18, 33]. However, Brown [5] studied the accumulation of ^{90}Sr and ^{90}Y by eggs and alevins of the Atlantic salmon and sea trout on glass racks in a light-tight partitioned Perspex tank through which the experimental water flowed. In this case the water was continuously contaminated with an equilibrium mixture of carrier free ^{90}Sr and ^{90}Y in the form of nitrate to give a specific activity for both isotopes of $\approx 5 \times 10^{-6} \mu\text{Ci/ml}$ at a flow rate of 900 ml/min. For the purpose of radiobiological studies the simple apparatus may, in general, be considered better and easier to handle. However, whether to use the closed system or the flowing system in the experiment simulating the natural conditions should be decided after careful consideration of the environmental conditions where the eggs to be used for the experiments may exist in nature. In our studies with fertilized eggs of rainbow trout' non-flowing and non-circulating water system was adopted. Except for the case of ^{106}Ru chloro complex, the use of antibiotics and other chemicals to control bacterial and saprolegnia growth on eggs was not adopted in our experiments because of the possible influence of the chemicals on the uptake process as described later.

The rearing vessels made of plastics held 30 liters of aged, filtered tap water which was prepared by successive filtration using ORPET filter (Organo Co. Ltd. in Japan) and Millipore HAWP filters of pore size of $0.45 \mu\text{m}$. Analytical data of the filtered tap water were as follows: Na 12.5 ppm, Ca 2.7 ppm, Cl 24.4 ppm, total dissolved solids 124 ppm, electric conductivity $160 \mu\text{mho/cm}$ at 12°C , pH 7.2. As shown in Fig. 2, the rearing vessel had an incubation basket made of polyethylene for settling the eggs and was equipped with an aeration apparatus. Viable artificially fertilized eggs, 1 day after fertilization (blastodisc stage) were transferred to the basket in a group of about 2,500 after the radioactive concentration of rearing water reached approximately constant level. In this apparatus, the eggs

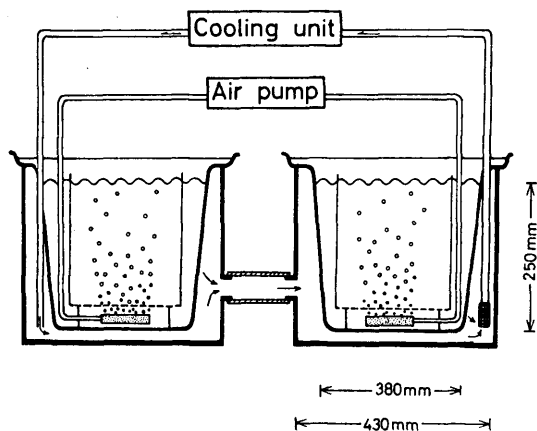


Fig. 2 Experimental vessels

accumulation, the contaminated eggs (eye pigmentation stage) in a group of about 1,200 were reared in non-active water in the same way as mentioned above to observe the elimination of radionuclides. The rearing water was frequently changed to prevent a buildup of contaminants.

Rearing method for advanced fry

The advanced fry were reared in plastic vessels with 20 liters of river water contaminated with each radionuclide at the radioactive concentrations of about 1–10 $\mu\text{Ci}/\text{l}$ and were equipped with the aeration apparatus similar to that for eggs (Fig. 2). Analytical data of the river water were as follows: Na 3.2 ppm, k 0.3 ppm, Ca 31.0 ppm, Cl 4.5 ppm, total dissolved solids 138 ppm (loss on ashing 21.7%, residue after ignition 78.3%), total suspended matter 0.2 ppm, electric conductivity 178 $\mu\text{mho}/\text{cm}$ at 12°C, pH 7.6.

The advanced fry were transferred to the vessels in a group of about 200 fishes after radioactive concentration of the water reached approximately constant level. In the elimination experiments, non-active rearing waters were frequently changed to prevent a buildup of contaminants. The advanced fry were reared without feeding throughout the experimental periods.

Fractionation of radionuclides in rearing water

To elucidate physical state of the radionuclides in rearing water, the fractionation of the radionuclides was carried out by filtration of 5 ml of rearing water using Millipore HAWP (0.45 μm pore size) or RAWP (1.2 μm pore size) filters.

Measurement of activity

The samples of 6 eggs, 6 advanced fry, 1 ml of rearing water, each filter retained radionuclide and 1 ml of filtrate were taken into polyethylene test tubes for radioactive assay at appropriate time intervals. The biological samples were blotted lightly with a filter paper and weighed. The volume of the sample put in a polyethylene tube was limited to a certain level in order to assure a constant geometry on counting. The activity of individual sample made up in triplicate was measured in the fresh condition using Auto-well gamma system Model JDC-752 (Aloka Co. Ltd. Japan). The calculated mean values of activities for samples showed a standard deviation of about 10% or less.

Estimation of parameters, rate of uptake, turnover rate and concentration factor

As is often pointed out whenever the uptake and loss experiments in radioecology are conducted, one of the principal aims of the experiments is to estimate biological parameters such as rate of up-

can be easily recovered together with the basket for the purpose of measurement of radioactivity without significantly disturbing the distribution of radionuclides in the system. In order to maintain the temperature at $12 \pm 1^\circ\text{C}$, the rearing vessels were placed in aquaria and cooled with a flow of water from a cooling unit (Fig. 2). Eggs died were quickly removed from the vessels during the experiments. The time until hatching was about 30 days at the temperature of 12°C. After about 17 days, attaining an apparent equilibrium of

take, turnover rate and concentration factor, etc., and these parameters should be useful to predict the accumulation of the radionuclide from water by an organism at the levels of environmental radioactive contamination due to man's nuclear activities.

Although some proposals have been attempted for the modeling of the dynamics of radionuclide distribution in the ecosystem, the exponential model [43] was assumed to fit the experimental data and the parameters, rate of uptake(u) and turnover rate(β) as well as concentration factor were calculated using the least squares method. The concentration factor for each radionuclide in the developing eggs and advanced fry was then estimated as the ratio u/β and compared with the average of observed values (the ratio of the activity in organism to that in environmental water of the same weight) at apparent equilibrium. The numerical calculations were performed using YHP Model-30 personal computer system.

RESULTS AND DISCUSSION

Fractionation of the radionuclides in rearing water

It has been pointed out that the physico-chemical state of the isotopes in water might affect the

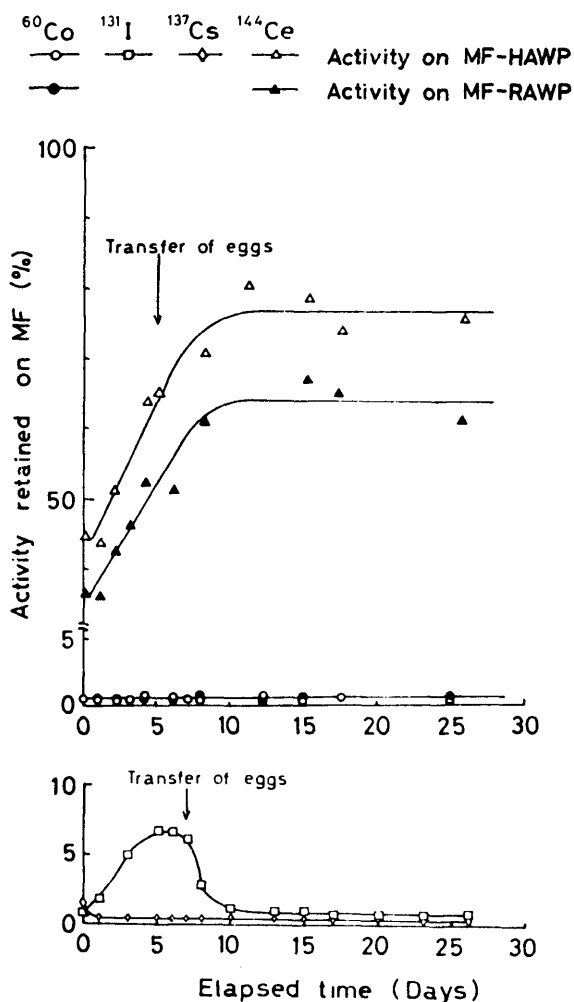


Fig. 3 ^{60}Co , ^{131}I , ^{137}Cs and ^{144}Ce retained on Millipore filters from rearing waters for eggs of rainbow trout [29]

uptake and elimination processes by aquatic organisms. As can be seen in Fig. 3 and 4, a remarkable difference between ^{144}Ce and the other radionuclides was observed.

The activity of ^{144}Ce retained on the filters increased with time and reached an equilibrium state in about 15 days. The retention patterns of ^{144}Ce by both HA and RA types of the filters were quite similar to each other. The ^{144}Ce retained on both HA and RA filters at equilibrium were about 75% and 65% respectively, while those of ^{60}Co , ^{106}Ru , ^{131}I and ^{137}Cs were less than 10%. Among three different chemical forms of RuNO complexes, nitrate complex was somewhat more retained on the filters than nitro and binuclear complexes. As already reported [44, 45], these results indicate that most of ^{60}Co , ^{131}I and ^{137}Cs in fresh water are of quite soluble form, while some of ^{106}Ru especially in RuNO- nitrate complex and more than 50% of ^{144}Ce in fresh water are of particulate form. The results obtained in rearing water for the advanced fry were similar to those for the eggs.

Uptake of the radionuclides by eggs and advanced fry

The uptake and accumulation of the

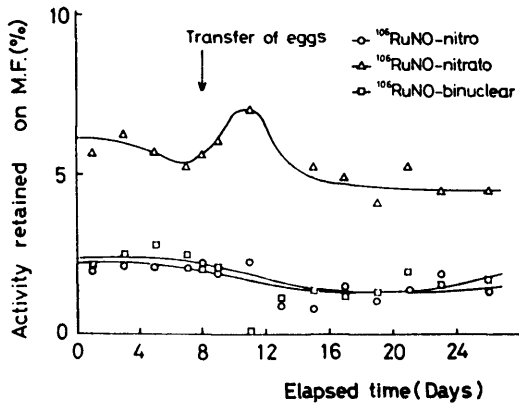


Fig. 4 $^{106}\text{RuNO}$ complexes retained on Millipore filters from rearing waters for eggs of rainbow trout [29]

potassium permanganate. In the uptake experiments of ^{106}Ru chloro complex by the eggs, the activity in non-treated pre-eyed eggs reached an equilibrium state more slowly than that in treated eyed eggs with malachite green and potassium permanganate. The average concentration factors for ^{106}Ru in non-treated and treated whole eggs with the chemicals were 30 and 75, respectively. These results indicate that there might be some effects of chemicals on the uptake and accumulation of ^{106}Ru by the eggs.

radionuclides by the eggs are shown in Figs. 5, 6 and 7, and those by the advanced fry are shown in Figs 8 and 9. The uptake curves were fitted on the basis of the exponential model [43]. The uptake and accumulation of ^{60}Co and ^{137}Cs by the eggs were quite rapid as shown in Fig. 5. The maximum levels of ^{60}Co and ^{137}Cs in the eggs were reached in 1 day, while those of the other radionuclides in the eggs were attained somewhat slowly. The time required to reach an apparent equilibrium state for ^{131}I , ^{144}Ce and ^{106}Ru was several days or more as shown in Figs 5, 6 and 7. In order to control bacterial and saprolegnia growth on eggs, the eyed eggs are treated with malachite green and

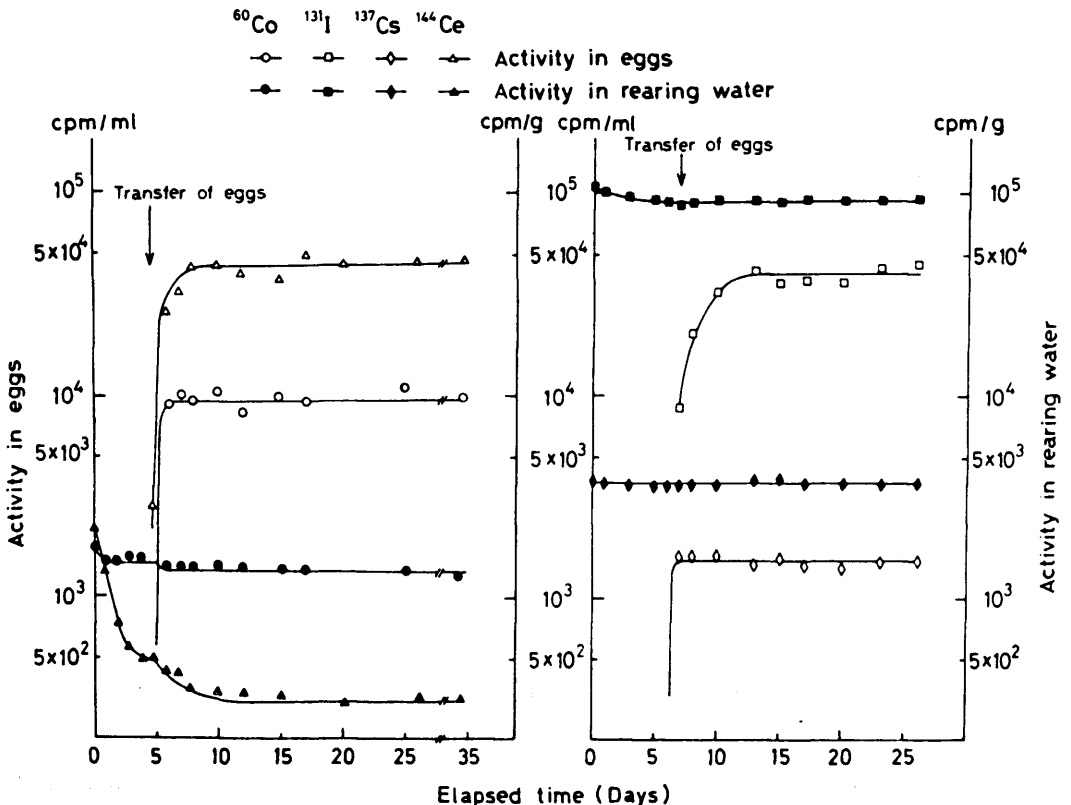


Fig. 5 Uptake of ^{60}Co , ^{131}I , ^{137}Cs and ^{144}Ce by eggs of rainbow trout [29]

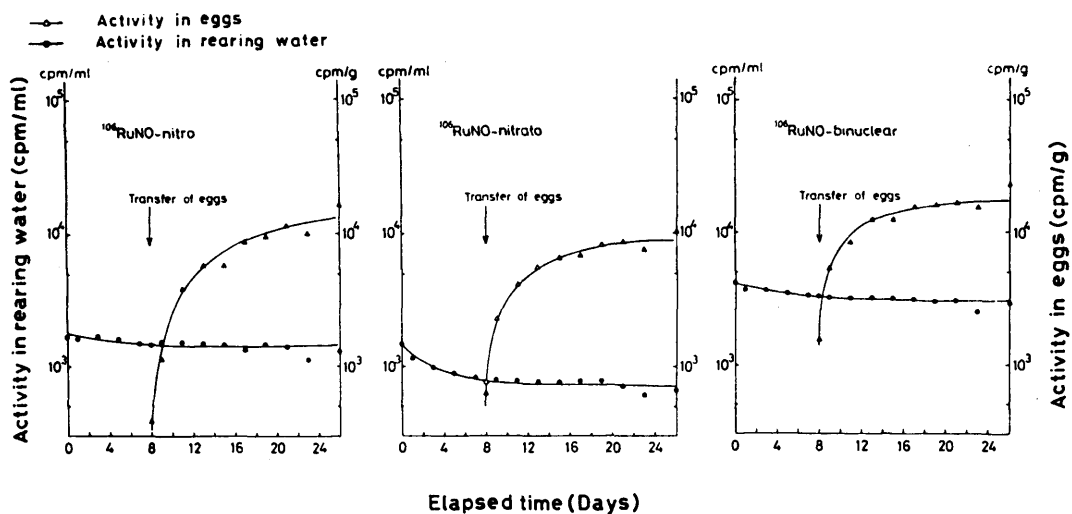


Fig. 6 Uptake of ¹⁰⁶RuNO complexes by eggs of rainbow trout [29]

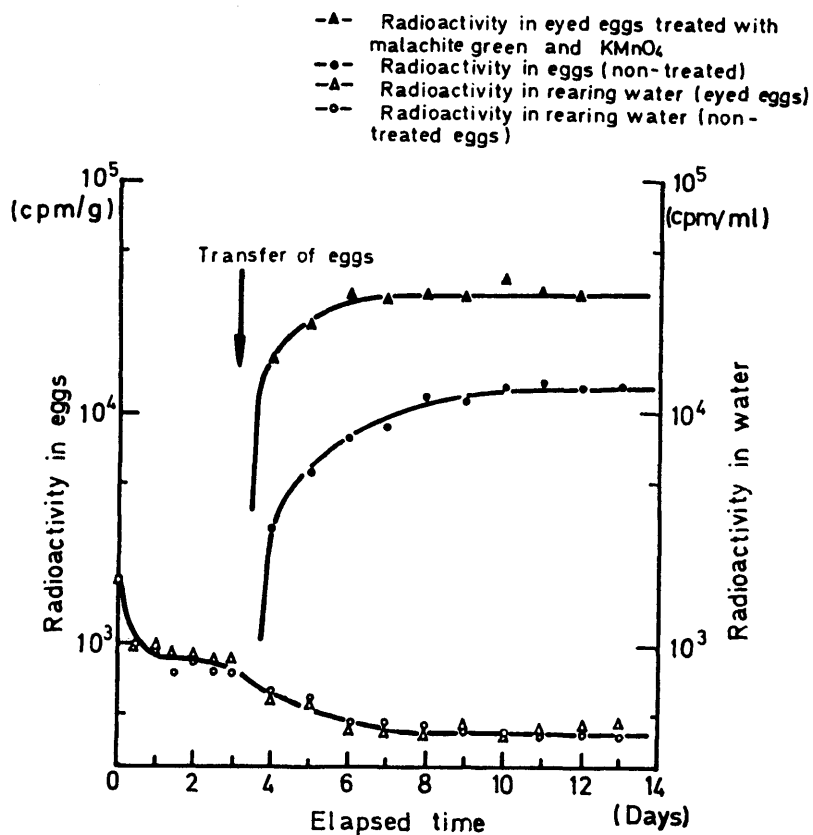


Fig. 7 Uptake of ¹⁰⁶Ru chloro complex by eggs of rainbow trout [28]

The parameters, rate of uptake(α) and turnover rate(β) calculated from the fitted curves of uptake based on the exponential model [43] are summarized together with the concentration factors for the radionuclides by the eggs and the advanced fry in Tables 1 and 2, respectively.

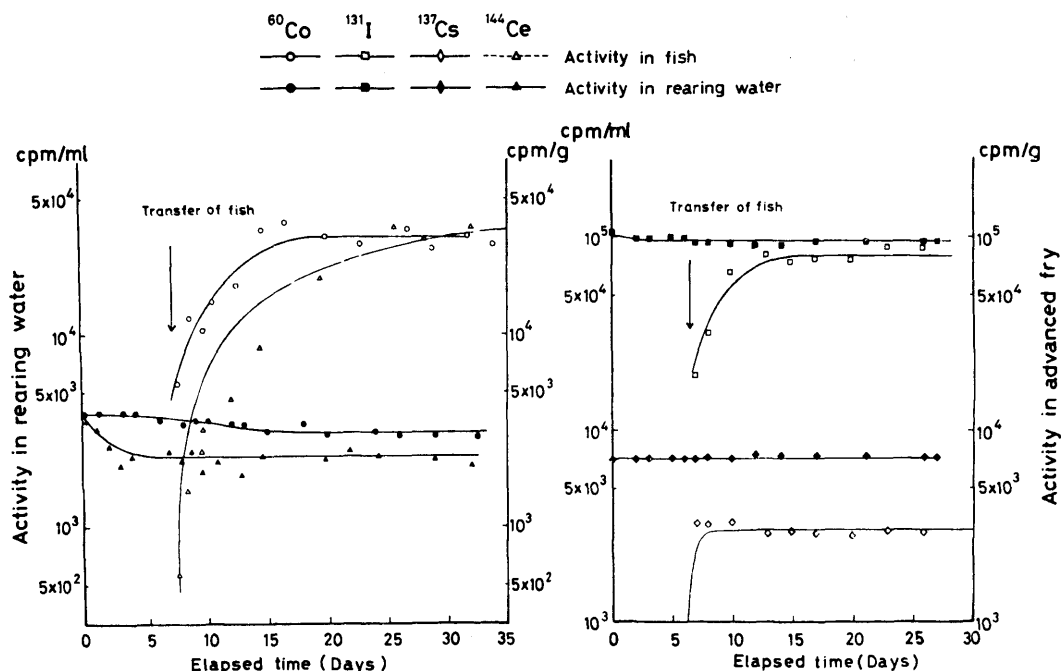


Fig. 8 Uptake of ^{60}Co , ^{131}I , ^{137}Cs and ^{144}Ce by advanced fry (early stage) of rainbow trout [30]

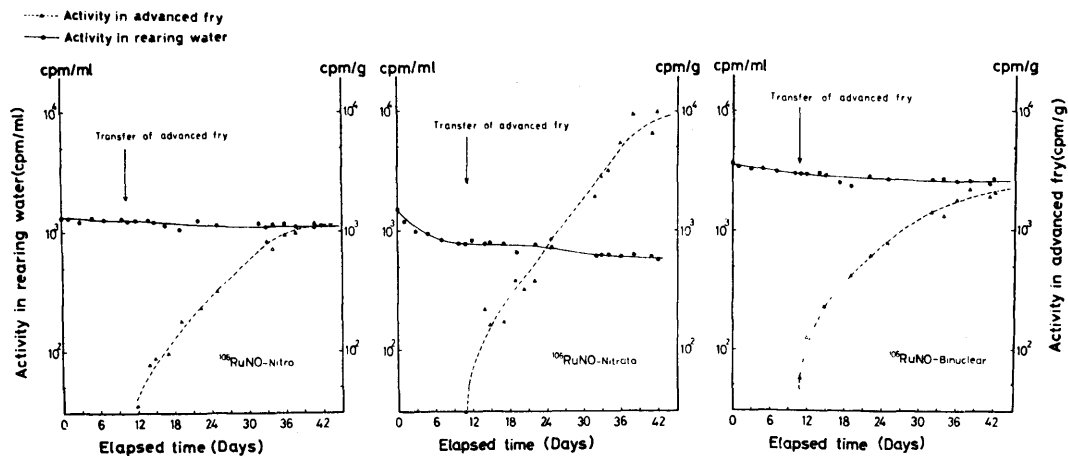


Fig. 9 Uptake of $^{106}\text{RuNO}$ complexes by advanced fry (early stage) of rainbow trout [30]

As can be seen in these tables, the concentration factors estimated as the ratio u/β for each radionuclide are quite similar to those observed. The average values of observed concentration factors at apparent equilibrium for ^{60}Co , ^{131}I , ^{137}Cs , ^{144}Ce , ^{106}Ru -nitro, -nitrato and -binuclear complexes in the whole eggs were 7.0, 0.4, 0.4, 120, 11.0, 14.5 and 6.8, respectively. Among the radionuclides tested, the highest concentration factor of 146 for ^{144}Ce and the lowest of 0.4 for ^{131}I and ^{137}Cs were obtained as shown in Table 1. The concentration factors for ^{144}Ce , ^{106}Ru and ^{60}Co in the eggs showed the same order of magnitude reported for the eggs of the Black Sea haddock [1] and turbot [22]. Although the

Table 1 Rate of uptake, turnover rate and concentration factor for radionuclides in eggs of rainbow trout [29]

Radionuclide	Rate of uptake(u)	Turnover rate(β)	Concentration factor	
			Estimated (u/ β)	Observed (average)
^{60}Co	22.2	3.0	7.4	7.0
^{131}I	0.29	0.67	0.4	0.4
^{137}Cs	1.02	2.5	0.4	0.4
^{144}Ce	113.8	0.78	146.0	120.0
$^{106}\text{RuNO}$ -nitro	0.99	0.10	9.9	11.0
$^{106}\text{RuNO}$ -nitrate	2.26	0.18	12.5	14.5
$^{106}\text{RuNO}$ -binuclear	1.14	0.18	6.3	6.8

Table 2 Rate of uptake, turnover rate and concentration factor for radionuclides in advanced fry of rainbow trout [30]

Radionuclide	Rate of uptake(u)	Turnover rate(β)	Concentration factor	
			Estimated (u/ β)	Observed (average)
^{60}Co	2.04	0.20	10.2	11.0
^{131}I	0.27	0.31	0.9	0.8
^{137}Cs	2.11	5.00	0.4	0.4
^{144}Ce	1.00	0.05	19.2	16.0
$^{106}\text{RuNO}$ -nitro	—	—	—	1.0*
$^{106}\text{RuNO}$ -nitrate	—	—	—	12.5*
$^{106}\text{RuNO}$ -binuclear	—	—	—	0.9*

* : On 31st day

— : Could not be estimated

concentration factor for ^{137}Cs obtained in our experiments was somewhat lower than the values of 1-2 in the Atlantic salmon eggs reported by Kosheleva [21] and the value of 9 in the eggs of turbot reported by Ivanov [22]. Kosheleva [21] pointed out that the low ^{137}Cs concentration factor was due to a low potassium content in the eggs. Among the different chemical forms of ^{106}Ru complexes, the highest value of 30 for ^{106}Ru chloro complex [28] and the lowest value of 6.3 for ^{106}Ru -binuclear complex [29] were obtained. Although the development period of fish eggs can be affected by the temperature of surrounding water, the period of uptake by marine teleost eggs may not last until the organism reaches isotopic equilibrium because of the relatively short development period. Therefore, the concentration factors for fish eggs are not always those at equilibrium. However, as was early described, since the duration until hatching of the eggs of rainbow trout was about 30 days at the temperature of 12°C, the values of the concentration factors for the radionuclides in the eggs of rainbow trout reported in this paper were those at equilibrium. On the other hand, the uptake and accumulation of $^{106}\text{RuNO}$ complexes by the advanced fry were much slower and no apparent equilibrium was observed for 31 days exposure, accordingly, theoretical curves of uptake based on the exponential model [43] were not obtained (Fig. 9).

Comparing the results listed in Table 1 with those in Table 2, a remarkable difference in concentration factor for ^{144}Ce may be noticed. The concentration factor for ^{144}Ce in the eggs was much higher than that in the advanced fry. This higher concentration factor for ^{144}Ce in the eggs might be

attributed to larger surface area in the eggs than in the advanced fry per unit weight. The advanced fry at early stage of growth (about 10 days old after hatching) have their yolk-sacs and the development of digestive organ is not yet enough, therefore the transfer of radionuclides through the alimentary tracts by the advanced fry is considered to be quite small. Taking account of this physiological feature of the advanced fry, among the three radionuclides ^{60}Co , ^{131}I and ^{137}Cs which were of quite soluble form in rearing water, it is pointed out that the rate of uptake and turnover rate of ^{60}Co by the eggs were higher than those by the advanced fry, in spite of the fact that the similar trend was not necessarily observed for ^{131}I and ^{137}Cs . Brown [5] reported that the accumulation factor for $^{90}\text{Sr} + ^{90}\text{Y}$ by sea trout, and salmon alevins and fry increased with time until 77 or 87 days after hatching. In our studies [30], the concentration factor for ^{60}Co and ^{137}Cs by the fry of rainbow trout also increased with growth of the fish. However, this trend was not necessarily observed for ^{131}I , ^{144}Ce and $^{106}\text{RuNO}$ complexes.

Elimination of the radionuclides by eggs and fry

The elimination patterns of the radionuclides by the eggs and advanced fry are shown in Fig. 10-13.

As can be seen in these figures, the elimination of ^{106}Ru complexes by the eggs was much slower than that of the other radionuclides. The whole egg retention of ^{106}Ru was more than 50% for 12 days until hatching. It is also found that more than 99% of ^{137}Cs was eliminated in about 10 days. Comparing the elimination patterns of ^{60}Co , ^{131}I and ^{137}Cs by the eggs with those of ^{106}Ru and ^{144}Ce until hatching, it might be interesting to note the possible effects of the physico-chemical characteristics of the radionuclides in environmental water. The biological half-lives of 0.3 days for ^{60}Co , 0.4 days for ^{131}I , 0.7 days for ^{137}Cs , as short-lived component and 3.0 days for ^{137}Cs as long-lived one and also of 7.0 days for ^{144}Ce in the eggs were estimated from graphical analysis of each elimination curve of the radionuclides by the eggs.

On the other hand, except for the elimination of ^{137}Cs , the retention curves of ^{60}Co , ^{131}I , ^{144}Ce and $^{106}\text{RuNO}$ complexes by the advanced fry consisted of the initial short component and the following long one. The rapid loss of ^{137}Cs from the eggs might be attributed to relatively loose fixation of ^{137}Cs by the egg membrane, low uptake of ^{137}Cs by the embryo and also low content of potassium in the egg. The initial elimination of $^{106}\text{RuNO}$ complexes by the advanced fry was larger than that by the eggs, while that of ^{60}Co by the advanced fry was rather small. These results indicate that a simple relationship between the physico-chemical characteristics of radionuclides in water and their elimination modes by eggs or advanced fry is not necessarily observed.

Localization of radionuclides in eggs

The knowledge on the localization of radionuclides at organ, tissue and subcellular levels not only contributes to the development of kinetics model based on the information about metabolic routes but also to the development of dosimetry models. However, for many instances of fish eggs, discrete tissue dissection is difficult, even with the use of micromanipulators, either because of small size of the organisms or because cross contamination often may occur under fresh conditions. In order to overcome such technical difficulty, the special techniques such as fixation with the use of chemical or physical agents (e.g., thermal fixation) and autoradiography including the preparation of egg section [18.32] have been required. To examine the distribution of radionuclides in eggs, the eggs taken from aquaria were fixed in 4% formalin solution for several days in a refrigerator. The fixed eggs were separated into the egg capsule (egg membrane), embryo and yolk with perivitelline fluid. The results are shown in Table 3.

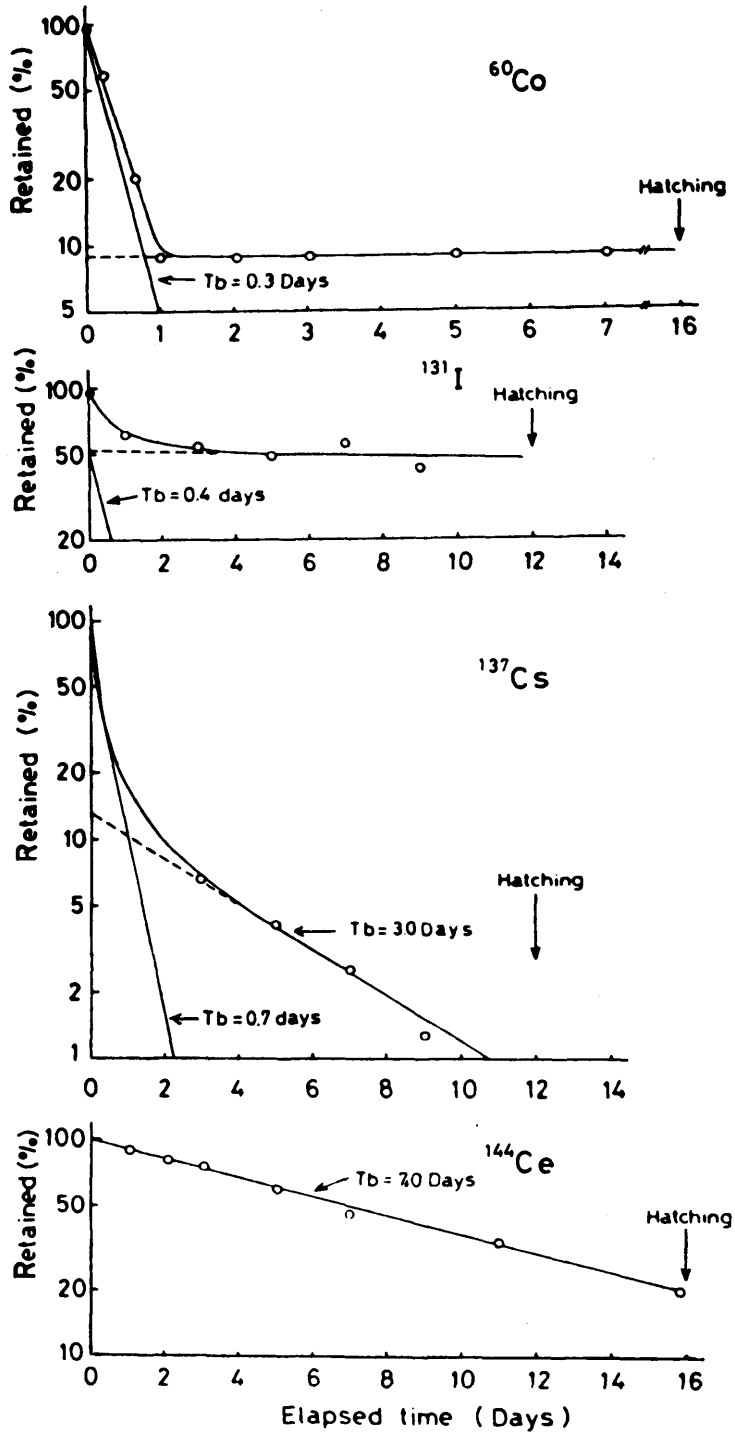


Fig. 10 Elimination of ^{60}Co , ^{131}I , ^{137}Cs and ^{144}Ce by eggs of rainbow trout [29]

It was noted that the substantial fractions of ^{131}I and ^{137}Cs were released into the formalin solution during fixation. In the uptake experiments, distribution of the radionuclides in embryos was relatively

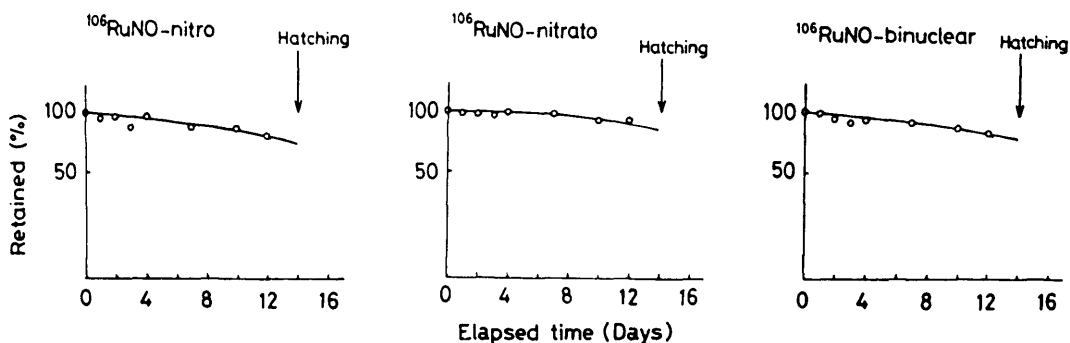


Fig. 11 Elimination of $^{106}\text{RuNO}$ complexes by eggs of rainbow trout [29]

Table 3 Distribution of ^{60}Co , ^{131}I , ^{137}Cs , ^{144}Ce , ^{106}Ru in eggs of rainbow trout [29]

Nuclide	Days after fertilization	4 % HCHO*	Egg Capsule	Embryo	Yolk & Perivitelline fluid
^{60}Co	19	6.3	52.6	5.0	36.2
^{131}I	19	31.4	31.3	8.2	29.1
^{137}Cs	19	62.5	3.9	3.3	30.3
^{144}Ce	19	0.4	13.8	4.5	81.3
$^{106}\text{RuNO-nitro}$	21	1.0	86.9	1.4	10.7
$^{106}\text{RuNO-nitrato}$	21	2.0	81.8	1.9	14.3
$^{106}\text{RuNO-binuclear}$	21	1.2	85.5	2.4	10.9

*The eggs were fixed in 4 % HCHO solution.

Each value is the average of 18 eggs.

small even in the case of ^{60}Co , ^{131}I and ^{137}Cs which showed mainly soluble form of the radionuclides in rearing water. Ichikawa et al. [13] also reported that ^{60}Co was adsorbed onto the egg membrane of *Oryzias Latipes*, and was not accumulated in the embryo. These results are presumably due to the fact that the vitelline membrane has been generally believed to be impermeable [46]. Although the significant distribution of ^{60}Co , ^{131}I , ^{137}Cs and ^{144}Ce was observed in yolk with perivitelline fluid, it would be considered that most of the radionuclides were probably associated with perivitelline fluid because of its permeability of the egg capsule [46]. Kosheleva [21] reported that ^{144}Ce was for the most part localized in the egg membrane of the Atlantic salmon egg and ^{137}Cs in the embryo tissue. However, in our experiments considerable part of ^{144}Ce was localized in the perivitelline fluid, and ^{137}Cs in the embryo tissue was rather small [29]. The further studies are required to elucidate the possible reasons for this discrepancy. In the case of $^{106}\text{RuNO}$ complexes, the most part of ^{106}Ru in the eggs was associated with egg capsule as already reported for ^{106}Ru chloro complex by the authors [28]. No remarkable difference in the distribution of the radionuclides in the eggs was observed between the uptake and elimination experiments [29].

Estimation of radiation dose to the developing embryos

The radiation doses received by the developing embryos due to the radioactivities distributed in both the eggs and the surrounding waters were estimated on the basis of a dosimetry model described by Woodhead [18]. The radiation doses thus calculated ranged from 2.7 rad for ^{60}Co to 45.2 rad for $^{106}\text{RuNO-binuclear}$ complex, as shown in Table 4.

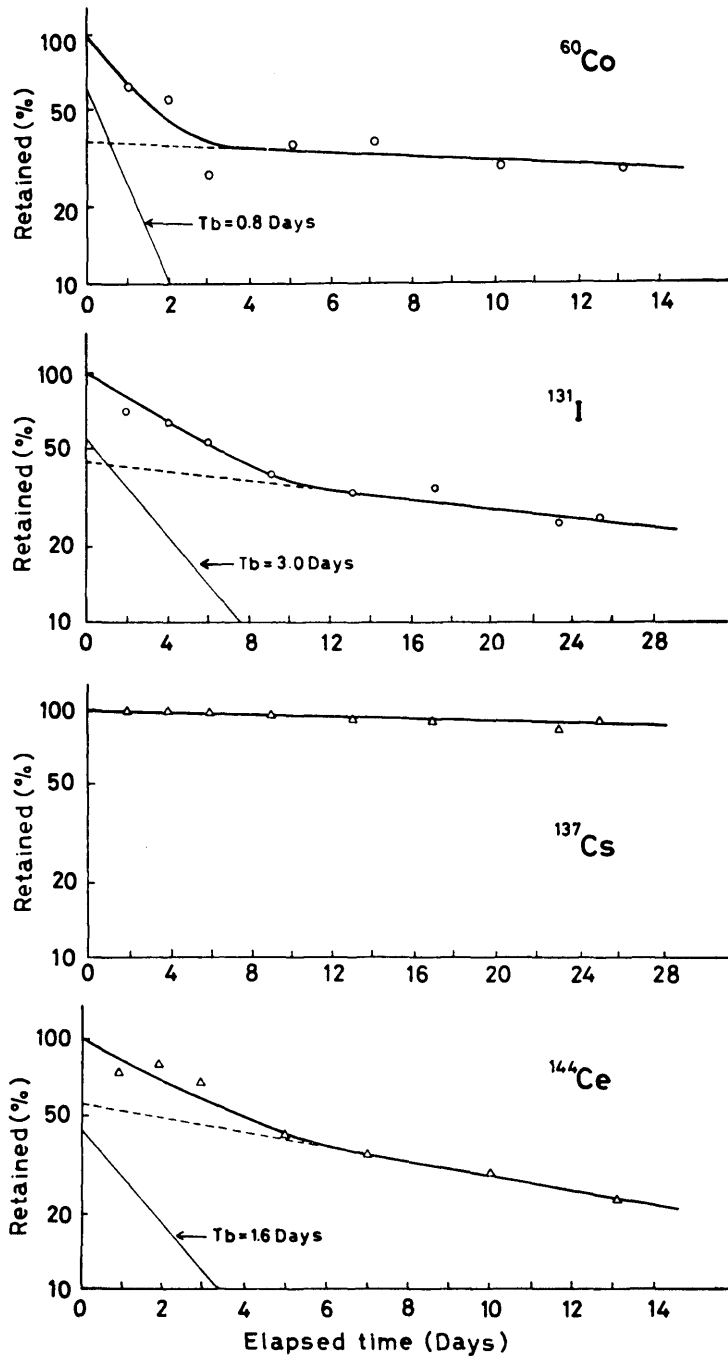


Fig. 12 Elimination of ^{144}Ce , ^{131}I , ^{60}Co and ^{137}Cs by advanced fry (late stage) of rainbow trout [30]

No positive correlation between the mortality or hatching rate of the eggs and the radiation doses received by the developing embryos of rainbow trout was observed for 20 days, during which the eggs were kept in the contaminated waters after fertilization.

Table 4 Estimates of the dose rate to developing embryos of rainbow trout

Nuclide	Dose Rate from β -rays				Dose Rate from γ -rays				Grand-total ($\frac{\text{rad}}{20\text{days}}$)
	From uniformly active egg capsule $\times 10^{-2}(\frac{\text{rad}}{\text{hour}})$	From uniformly active embryo and perivitelline fluid $\times 10^{-4}(\frac{\text{rad}}{\text{hour}})$	From water $\times 10^{-3}(\frac{\text{rad}}{\text{hour}})$	Sub-total ($\frac{\text{rad}}{20\text{days}}$)	From uniformly active egg capsule $\times 10^{-4}(\frac{\text{rad}}{\text{hour}})$	From uniformly active embryo and perivitelline fluid $\times 10^{-6}(\frac{\text{rad}}{\text{hour}})$	From water $\times 10^{-4}(\frac{\text{rad}}{\text{hour}})$	Sub-total ($\frac{\text{rad}}{20\text{days}}$)	
^{60}Co	0.13	2.53	0.14	0.81	13.20	36.90	26.60	1.93	2.74
^{131}I	1.46	7.31	6.34	10.40	3.17	7.52	190.00	9.28	19.68
^{137}Cs	0.23	1.02	1.20	1.73	0.85	1.72	66.10	3.21	4.94
^{144}Ce	0.26	60.80	0.09	4.21	0.22	5.27	0.43	0.03	4.24
$^{106}\text{RuNO-nitro}$	5.70	13.90	2.94	29.44	3.05	1.68	5.93	0.43	29.87
$^{106}\text{RuNO-nitrato}$	3.56	12.20	1.55	18.42	1.91	1.48	3.14	0.24	18.66
$^{106}\text{RuNO-binuclear}$	8.39	22.80	6.30	44.39	4.50	2.76	12.70	0.83	45.22

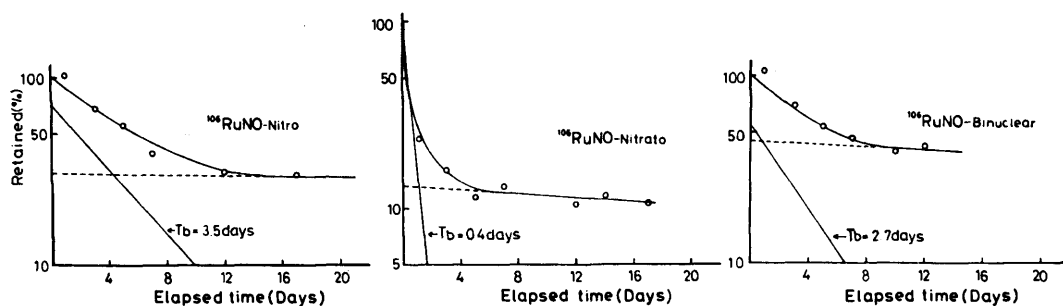


Fig. 13 Elimination of $^{106}\text{RuNO}$ complexes by advanced fry (late stage) of rainbow trout [30]

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Assistant Professor O. Horibe and Mr. Y. Mizumoto, Kinki University, for their assistance in theoretical fitting of the uptake curves by using YHP Model-30 computer system. The authors also thank Dr. T. Ishiyama, Radiation Center of Osaka Prefecture, for the preparation and supply of ^{106}Ru -binuclear complex for the experiments.

REFERENCES

- [1] POLIKARPOV, G. G., Radioecology of Aquatic Organisms, North Holland Publishing Co., Amsterdam (1966) 314 pp.
- [2] WOODHEAD, D. S., The biological effects of radioactive waste, Proc. R. Soc., Ser. B **177** (1971) 423-437.
- [3] POLIKARPOV, G. G., IVANOV, V. N., The effect of ^{90}Sr - ^{90}Y on the developing anchovy eggs, Vopr. Ikhiol. **1** (320) (1961) 583. (Transl. UKAEA Reactor Group Inform. Ser. 166(w).), Cited from IAEA Technical Report Series No. 172.
- [4] POLIKARPOV, G. G., IVANON, V. N., The harmful effect of ^{90}Sr - ^{90}Y in the early development of the red mullet, the green wrasse, the horse mackerel and the Black Sea anchovy, Dokl. Akad. Nauk SSSR **144** 1 (1962) 219, cited from IAEA Technical Report Series No. 172.
- [5] BROWN, V. M., The accumulation of Strontium-90 and Yttrium-90 from a continuously flowing natural water by eggs and alevins of the Atlantic Salmon and Sea Trout, UKAEA Rep. No. PG-288 (1962) 16 pp.
- [6] BROWN, V. M., TEMPLETON, W. L., Resistance of fish embryos to chronic irradiation, Nature **203** (1964) No. 4951, 1257-1259.
- [7] TEMPLETON, W. L., Resistance of fish eggs to acute and chronic irradiation, Disposal of Radioactive Wastes into Seas, Oceans and surface Waters, IAEA, Vienna (1966) 847-860.
- [8] NEWCOMBE, H. B., MCGREGOR, J. F., Major congenital malformations from irradiations of sperm and eggs, Mutation Res., **4** (1967) 663-673.
- [9] MCGREGOR, J. F., NEWVOMBE, H. B., Major malformations in trout embryos irradiated prior to active organogenesis, Radiat. Res., **35** (1968) 282-300.
- [10] SCHROEDER, J. H., X-ray-induced mutations in the poeciliid fish, *Lebistes reticulatus* PETERS, Mutation Res., **7** (1969) 75-90.
- [11] HIYAMA Y., SHIMIZU, M., SUYAMA, I., Radiation effect on development of marine fish eggs (in Japanese), Nuclear Safety Research Association of Japan, Annual Progress Report for the

- year of 1967 (1969) 1-3.
- [12] HIYAMA, Y., SHIMIZU, M., SUYAMA, I., Effect of ^{90}Sr on development of marine fish eggs and sea urchin eggs (in Japanese), Nuclear Safety Research Association of Japan, Annual Progress Report for the year of 1968 (1970) 1-4.
- [13] ICHIKAWA, R., ETOH, H., UENO, A., Effect of ^3H , ^{60}Co and ^{65}Zn on development of fresh water fish eggs (in Japanese), Nuclear Safety Research Association of Japan, Annual Progress Report for the year of 1969 (1970) 1-8.
- [14] ICHIKAWA, R., ETOH, H., SUYAMA, I., UENO, A., Effect of ^{90}Sr on development of eggs of *Fugu niphobles* (in Japanese), Nuclear Safety Research Association of Japan, Annual Progress Report for the year of 1970 (1971) 4-7.
- [15] ICHIKAWA, R., ETOH, H., SUYAMA, I., UENO, A., Effect of radionuclides on development of fish eggs (in Japanese), Nuclear Safety Research Association of Japan, Annual Progress Report for the year of 1971 (1972) 1-13.
- [16] ICHIKAWA, R., SUYAMA, I., UENO, A., Effect of radionuclides on development of fish eggs (in Japanese), Nuclear Safety Research Association of Japan, Annual Progress Report for the year of 1972 (1973) 1-5.
- [17] AKITA, Y., SHIROYA, T., Radiation effects on development of eggs of marine organisms, Report on the Influence of the Low Concentrations of Radionuclides in Water to Aquatic Organisms (in Japanese), Nuclear Safety Research Association of Japan (197).
- [18] WOODHEAD, D. S., The assessment of the radiation dose to developing fish embryos due to the accumulation of radioactivity by the egg, *Radiat. Res.*, 43 (1970) 582-597.
- [19] TEMPLETON, W. L., NAKATANI, R. E., HELD, E., Radioactivity in the marine environment (1970), Chap. 9, NAS-NRC, Washington, D. C.
- [20] FEDROV, A. F., Radiation doses for some types of marine biota under present day conditions, *Radioactivity in the sea*, IAEA Publ. No. 4, IAEA, Vienna (1964).
- [21] KOSHELEVA, V. V., Accumulation of radioactive isotopes by the developing eggs of the Atlantic salmon, Effect of ionizing radiation on the organisms, AEC-tr-7418, USAEC, Washington, D. C. (1971) 7-15.
- [22] IVANOV, V. N., Accumulation of radionuclides by roe and pro-larvae of Black Sea fishes, *Marine Radioecology* (POLIKARPOV, G. G., Ed), AEC-tr-7299, USAEC, Washington, D. C. (1970).
- [23] PODYMAKHIN, V. N., Radiometric and dosimetric characteristics of experiments for determining the influence of radioactivity of a water medium on the development of eggs of the Atlantic salmon, Effect of ionizing radiation on the organisms, AEC-tr-7418, USAEC, Washington, D. C. (1971) 113-133.
- [24] MCGREGOR, J. F., NEWCOMBE, H. B., Dose-response relationships for yields of major eye malformations following low doses of radiation to trout sperm, *Radiat. Res.*, 49 (1972) 155-169.
- [25] NEWCOMBE, H. B., MCGREGOR, J. F., Increased embryo production following low doses of radiation to trout spermatozoa, *Radiat. Res.*, 51 (1972) 402-409.
- [26] MCGREGOR, J. F., NEWCOMBE, H. B., Decreased risk of embryo mortality following low doses of radiation to trout sperm, *Radiat. Res.*, 52 (1972) 536-544.
- [27] ETOH, H., Effect of internal exposure on fish, *Radioactivity and Fish* (in Japanese), (N, EGAMI Ed.), Koseisha-Koseikaku, Tokyo (1973), 298-316.
- [28] HONDA, Y., KIMURA, Y., TAMURA, Y., TANAKA, C., Uptake of ^{106}Ru by eggs and fry of rainbow trout, *J. Radiat. Res.*, 13 (1972) 95-99.
- [29] KIMURA, Y., HONDA, Y., Uptake and elimination of some radionuclides by eggs and fry of rain-

- bow trout(I), *J. Radiat. Res.*, **18** (1977) 170-181.
- [30] KIMURA, Y., HONDA, Y., Uptake and elimination of some radionuclides by eggs and fry of rainbow trout(II), *J. Radiat. Res.*, **18** (1977) 182-193.
- [31] NISHIWAKI, Y.; KIMURA, Y., HONDA, Y., SAKANOUÉ, M., KOBAYASHI, A., Studies on radioecological concentration processes in the aquatic environments, —Uptake and retention of Am-241 by fish—, Proc. of the IVth International Congress of International Radiation Protection Association held in Paris (1977) Vol. 2, 641-644.
- [32] TILL, J. E., FRANK, M. L., Bioaccumulation, distribution and dose of ^{241}Am , ^{244}Cm and ^{238}Pu in developing fish embryos, Proc. of the IVth International Congress of International Radiation Protection Association held in Paris (1977) Vol. 2, 645-648.
- [33] IAEA, Design of Radiotracer Experiments in Marine Biological Systems, Technical Reports Series No. 167, Vienna (1975), 289 pp.
- [34] IAEA, Effects of Ionizing Radiation on Aquatic Organisms and Ecosystems, Technical Reports Series No. 172, Vienna (1976), 119 pp.
- [35] SHIMIZU, M., Procedures for radioecological studies with molluscs, Design of Radiotracer Experiments in Marine Biological Systems, Technical Reports Series No. 167, IAEA, Vienna (1975) 121-136.
- [36] SORGELOOS, P., PERSONNE, G., Three simple culture devices for aquatic invertebrates and fish larvae with continuous recirculation of the medium, *Mar. Biol.* **15** (1972) 251-254.
- [37] HOUDE, E. D., RAMSEY, A. J., A culture system for marine fish larvae, *Prog. Fish Cult.* **33** (1971) 156-157.
- [38] SHELBORNE, J. E., The artificial propagation of marine fish, *Advances in Marine Biology*, **2** (RUSSELL, F. S., Ed.), Academic Press, London and New York (1964) 1-83.
- [39] SHELBORNE, J. E., Marine fish cultivation: priorities and progress in Britain, *Marine Aquaculture* (McNEIL, W. J., Ed.), Oregon State University Press (1968) 15-36.
- [40] BLAXTER, J. H. S., "Development: eggs and larvae", *Fish Physiology*, **3** (HOAR, W. S., RANDALL, D. J., Eds.), Academic Press, London and New York (1970) 177-252.
- [41] MAY, R. C., An Annotated Bibliography of Attempts to Rear the Larvae of Marine Fishes in the Laboratory, US Dept. Commerce, NOAA Tech. Rep. NMFS SSRF-632 (1971).
- [42] PENTREATH, R. J., Radiobiological studies with marine fish, Design of Radiotracer Experiments in Marine Biological Systems, Technical Reports Series No. 167, IAEA, Vienna (1975) 137-170.
- [43] HIYAMA, Y., SHIMIZU, M., Uptake of radioactive nuclides by aquatic organisms: The application of the exponential model, *Environmental Contamination by Radioactive Materials*, IAEA, Vienna (1969) 463-476.
- [44] ROBERTSON, D. E., Influence of the physico-chemical forms of radionuclides and stable trace elements in seawater in relation to uptake by the marine biosphere, *Marine Radioecology* (2nd ENEA Seminar, Hambrug, 1971), OECD, Paris (1971) 21-93.
- [45] HONDA, Y., Physico-chemical behavior of radionuclides in seawater, *Fundamental Aspects of Marine Radioecology*(I) (in Japanese), Proc. 2nd NIRS Seminar, Chiba (1975) 27-35.
- [46] HAYES, F. R., The growth, general chemistry and temperature relation of salmonoid eggs, *Quart. Rev. Biol.* **24** (1949) 4.