# マハゼによる95Zrおよび95Nbの蓄積と排出


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# Accumulation and Excretion of $^{95}$Zr and $^{95}$Nb by Common goby (*Acanthogobius flavimanus*)

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In order to gain more information of $^{95}$Zr and $^{95}$Nb accumulation and excretion by marine fish, a laboratory study was undertaken using common goby. Radioactivity of $^{95}$Zr and $^{95}$Nb in the tissues or organs was simultaneously measured and readily distinguished by a high resolution detector, Ge (Li).

More than 60% of both total body burden of $^{95}$Zr and $^{95}$Nb was concentrated in the viscera (mainly digestive tracts) in spite of the small weight per cent (1.3%). The concentration factors of $^{95}$Zr and $^{95}$Nb on the 14th day after exposure were 34 and 42 for viscera; however, those were estimated to reach 70 and 83, respectively, if the fish were reared for a month under a constant level of radioactivity in sea water.

The turnover rate of viscera was 0.0081 for $^{95}$Zr and 0.0018 for $^{95}$Nb, and the biological half time was 85 days for $^{95}$Zr and 385 days for $^{95}$Nb, respectively.

In an elution pattern by gel filtration (Sephadex G–75) for viscera of common goby labelled by $^{95}$Nb, more than 80% of the total activity in the extracted solution exists with a relatively large molecular weight protein (probably more than 35,000). Although, $^{95}$Nb probably has no biological function in fish, it was apparently found to be organically bound in viscera of the fish.

Both $^{95}$Zr and $^{95}$Nb are among the more important fission products in radioavtive fallout and in the effluent discharged from nuclear fuel reprocessing plants. These nuclides are normally present together in effluent, but the proportions released vary, and separate measurement is necessary in the investigation of marine radioecology because of difference of their biological behaviour.

$^{95}$Zr and $^{95}$Nb can be simultaneously measured and readily distinguished by Ge(Li) detector.

$^{95}$Zr and $^{95}$Nb has been measured in water, biota and sediment as results of waste disposal[1–4]. The amount of fallout $^{95}$Zr in marine biota[5–7] has been measured in the study of the marine radioecology of $^{95}$Zr-$^{95}$Nb. Reports of its measurement in water, biota and sediment have been summarized by VOLCHOK *et al.*[8], DUTTON[9] and BRYAN *et al.*[10]. The concentration factors of $^{95}$Zr and $^{95}$Nb for edible marine plants, molluscs and invertebrates have been summarized by THOMPSON *et al.*[11]. However very few data of $^{95}$Zr and $^{95}$Nb have been reported for the sea water/fish system[7,12,13]. This may be due to higher accumulation in marine plants and molluscs than in fish.

In this paper, in order to gain more informations of $^{95}$Zr and $^{95}$Nb accumulation and excretion by marine fish, a laboratory study was undertaken using common goby.

## Materials and Methods

*Uptake Experiment*

Fifty five individuals of common goby (*Acanthogobius flavimanus*; average body weight: 38 g, average body length: 149 mm) were placed in an acrylic tank containing 125 liter of sea water with sufficient aeration. And 250 $\mu$Ci of $^{95}$Zr-$^{95}$Nb (as oxalate complex in 0.5% oxalic acid solution, from the Radiochemical Center of U.K.) was added into sea water. About 2 g/fish of nonactive food (soft parts of clam, *Gomphina melanaegis* from the coastal sea of near our Institute) was given once a day during the experimental period. The rearing sea water was changed every second day for maintenance of good environmental condition for the fish, and then 250 $\mu$Ci of $^{95}$Zr-$^{95}$Nb was newly added.

After exposure for 3, 7, 10 and 14 days, five fish were taken, narcotized with MS-222, weighed and three fish of them were dissected for preparation of head, muscle, skin with scales, fin, bone, liver, viscera (without liver), gill, blood and other samples. And the remaining two fish were homo-

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genized together by a laboratory disperser for the preparation of a whole body sample.

*Excretion Experiment*

In the excretion experiment following the uptake from water, the remaining fish of the uptake experiment were transferred to an acrylic tank containing 200 liter of nonactive sea water, and fed for 90 days after the commencement of the excretion experiment. The rearing sea water was frequently replaced with fresh sea water, and the radioactive substances excreted from the fish were removed.

At 2, 6, 14, 30, 57 and 90 days, every five fish were taken and treated in the same manner as the uptake experiment. The temperature of sea water was in the range of 15 and 17°C throughout these experimental periods.

In the case of the uptake and excretion experiments, the fish were dissected into 10 parts of tissues or organs as described above. The blood taken from three fish of them with a plastic syringe containing 0.2 m*l* of 5% citric acid solution was incorporated as one sample. The muscle sample was taken from the dorsal musculature. The skin sample consisted of a strip of skin taken from each side of the fish and freed of muscle. The fin sample was obtained by scissoring. The bone sample consisted of a portion of the spinal column scraped clean of muscle. The gill sample was taken from each side of the fish and the internal organs were taken whole.

The whole or a part of each tissue was placed in an acrylic resin test tube (13 mm$\phi$×70 mm) for measurement of radioactivity.

*Measurement of $^{95}$Zr and $^{95}$Nb Radioactivity in the Samples*

The $^{95}$Zr and $^{95}$Nb radioactivity in the samples were separately measured by use of the 1024 channels pulse height analyzer with 40 cm$^3$ Ge(Li) detector. Counting time was 2 or 3 minutes, depending on the magnitude of photopeaks of $^{95}$Zr and $^{95}$Nb in the smaples.

The radioactivity of $^{95}$Zr was calculated from the net counts integrated in both channel ranges from 422 to 430 and from 444 to 452, and the radioactivity of $^{95}$Nb was calculated from the net counts integrated in 7 channels around 456 channel of the energy photopeak of $^{95}$Nb.

*Accumulation and Excretion Experiment of $^{95}$Nb Alone*

The $^{95}$Nb radioactivity in the samples of the $^{95}$Zr-$^{95}$Nb experiments is the sum of $^{95}$Nb from the ambient water and $^{95}$Nb grown-in from $^{95}$Zr entered into the fish. Analysis of $^{95}$Nb in the samples, therefore, is very complicated. So an accumulation and excretion experiment of $^{95}$Nb alone was also carried out to compare with that of $^{95}$Zr-$^{95}$Nb.

Twenty common gobies were placed in an acrylic tank containing 50 liter of sea water with 120 $\mu$Ci of $^{95}$Nb (as oxalate complex, from the Radiochemical Center of U.K.).

After exposure for 3, 7, 10 and 14 days, every two fish were taken and each radioactivity of whole body was immediately measured with a whole body animal counter (Armac Scintillation Detector). And then, each fish was dissected for the preparation of various tissue or organ samples in the same manner as the $^{95}$Zr-$^{95}$Nb experiment. The radioactivity of $^{95}$Nb in the samples was measured with a well type scintillation counter (Aloka auto well gamma system).

In the case of excertion experiment of $^{95}$Nb, at 3, 8, 14, 30, 60 and 90 days after the start of the excretion experiment, every two fish were taken and treated in a similar manner as above.

*Observation of Binding of $^{95}$Nb and Protein by gel Filtration*

The $^{95}$Nb entered into the fish may be organically bound[14]. Therefore, it is intersting to observe which molecular weight portion of protein and peptide of fish $^{95}$Nb has strong affinity. So, gel filtration by use of Sephadex G-75 was carried out as follows: approximately three grams of the viscera of two common gobies at 14th day after the start of the uptake experiment were homogenized with 10 m*l* of 0.025 M tris-acetate buffer solution (pH: 8.4). Seven milliliters of the extracted solution separated by centrifugation (at 10,000 rpm, for 40 minutes) was applied to a Sephadex G-75 gel column (2 cm$\phi$×75 cm) equilibrated with the above mentioned buffer solution, which was also used as the developing solution.

The extraction ratio (total activity in extracted solution/total activity in 3 g of viscera) in this procedure was more than 86%. Each 1 m*l* of the fifty fractions taken in a fraction collector was placed in a polyethylene test tube and the radioactivity of $^{95}$Nb in it was measured with the same well type scintillation counter as mentioned above. The elution pattern of $^{95}$Nb bound to the protein was compared with that of $^{65}$Zn which has been

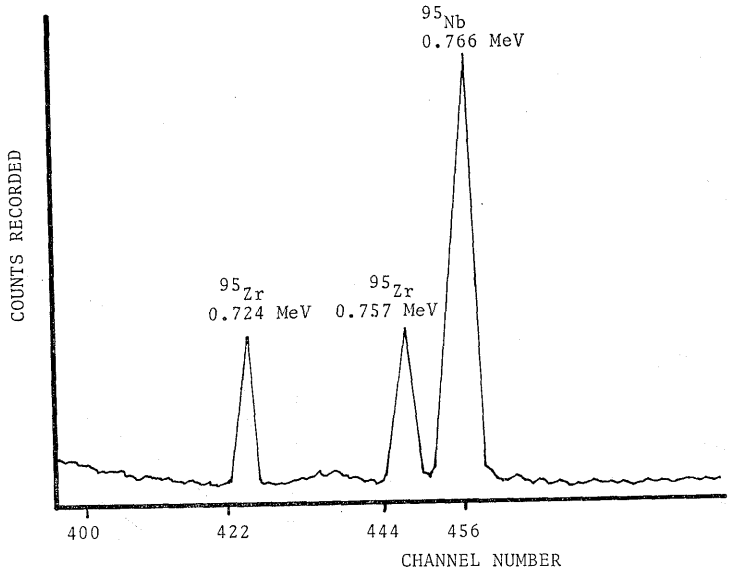


Fig. 1. Ge(Li) spectrum of the experimental sea water

well known as an essential element for animal organisms.

## Results and Discussion

*Uptake Experiment*

$^{95}Zr$ with a physical half time ($T_p$) of 65 days is associated with 2 gamma emitters at 0.724 and 0.757 MeV, and daughter, $^{95}Nb$ ($T_p$: 35 days) is also associated with a gamma photon at 0.766 MeV.

The $^{95}Zr$ and $^{95}Nb$ used for this experiment appears to be in transient equilibrium (with an activity ratio of 1: 2.2). The high resolution detector Ge(Li) enables the determinations of $^{95}Zr$ and $^{95}Nb$, and Fig. 1 shows a spectrum of the experimental sea water.

The accumulation patterns of $^{95}Zr$ and $^{95}Nb$ by several tissues and organs of common gobies are shown in Fig. 2(A) and (B). In this figure, it was found that both nuclides in all of tissues and organs except gill, fin and skin were accumulated relatively rapidly for the first week, and thereafter rather slowly. In the case of external tissue samples such as gill, fin and skin, the concentration of $^{95}Zr$ and $^{95}Nb$ became near the equilibrium concentrations with that of environmental sea water since 3 days after the start of this experiment. This may explain that $^{95}Zr$-$^{95}Nb$ is easily adsorbed by organic materials in sea water[1,15]. The digestive tracts constitute a large part such as 80–86% in weight of the viscera, and should be regarded as external organs rather than internal ones of the fish because marine fish has to drink considerable amount of sea water (8–55% of fish body weight/day) to keep the balance of the osmotic pressure between their bodies and evironmental sea water[16,17].

Table 1 shows a distribution (%) of $^{95}Zr$ and $^{95}Nb$ in the fish exposed for 14 days.

Table 1. Distribution (%) of $^{95}Zr$ and $^{95}Nb$ in common goby exposed for 14 days

| | Weight as % | $^{95}Zr$ | $^{95}Nb$ |
|---|---|---|---|
| Whole body (38 g) | 100.0 | 100.0 | 100.0 |
| Head | 21.1 | 15.0 | 9.6 |
| Muscle | 44.6 | 1.0 | 1.6 |
| Skin | 6.3 | 7.0 | 2.9 |
| Fin | 3.7 | 4.0 | 2.3 |
| Bone | 3.2 | 0.1 | 1.2 |
| Liver | 2.4 | 0.5 | 0.9 |
| Viscera | 1.3 | 63.0 | 67.0 |
| Gill | 1.8 | 5.5 | 1.6 |
| Blood | 5.0 | 0.9 | 4.4 |
| Others | 10.5 | 3.0 | 6.5 |

More than 60% of both total body burdens of $^{95}Zr$ and $^{95}Nb$ was concentrated in viscera in spite of the small weight per cent (1.3%). On the basis of this Table, it was found that the distribution of $^{95}Zr$ in external tissues such as gill, fin and skin was considerably larger than that in internal samples such as muscle, blood, liver and bone. Sastry

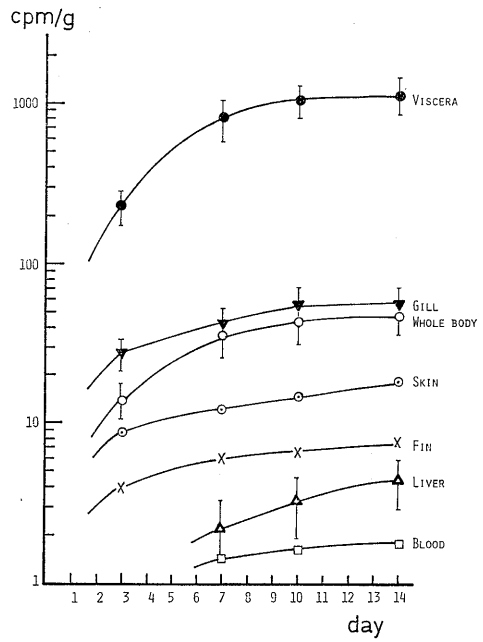


**Fig. 2 (A)** Uptake of $^{95}Zr$ by various tissues or organs of common goby.

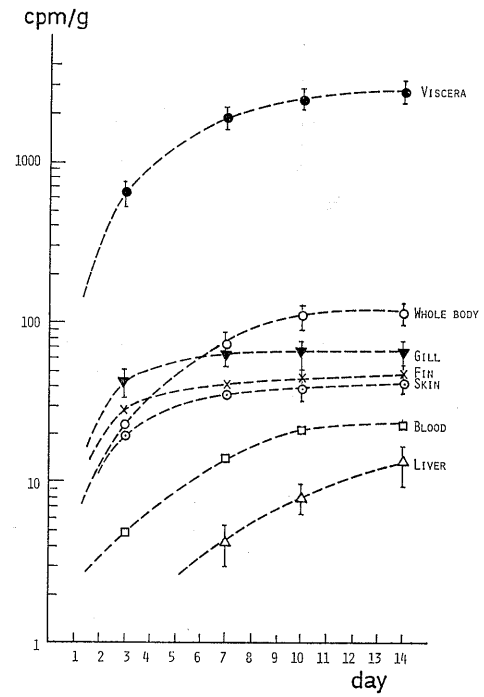


**Fig. 2 (B)** Uptake of $^{95}Nb$ by various tissues or organs of common goby.

*et al.*[18] who injected rats with equilibrium mixtures of $^{95}Zr$-$^{95}Nb$, demonstrated that bone preferentially concentrated $^{95}Zr$ and soft tissues concentrated $^{95}Nb$. Thus, $^{95}Nb$ rather than $^{95}Zr$ is probably organically bound to blood cell and soft tissues in fish.

The activity ratio (the ratio of each concentration of $^{95}Zr$ and $^{95}Nb$ in a fish or its tissues to that in the environmental sea water on a unit weight basis) at 14th day after exposure were calculated. The values of $^{95}Zr$ and $^{95}Nb$ were 34 and 42 for viscera, respectively, and approximately 1 for the both nuclides in whole body. In view of natural marine ecosystem, it is hardly conceivable that the radioactivity levels of $^{95}Zr$ and $^{95}Nb$ in sea water is kept for months under equilibrium or steady-state conditions because of relatively short half time of these radionuclides and an intermittent discharge of radioactive effluent from nuclear fuel reprocessing plants. Even if the radioactivity levels in sea water were kept for a month under steady-state conditions, the concentration factors of $^{95}Zr$ and $^{95}Nb$ were calculated to be 70 and 83 for viscera, 3 and 4 for whole body of the fish, respectively. The concentration factors of gill, skin and fin were almost similar to whole body of the fish, but those for liver, muscle and blood were appreciably lower than those for whole body of the fish. Thus, the concentration factors for $^{95}Zr$ and $^{95}Nb$ by the fish are relatively low and similar values for two elements of quite different valencies. And the similarity between the values of the nuclides was also obtained in the experiments with a porphyra (200–300 for $^{95}Zr$ and 400–450 for $^{95}Nb$)[19].

*Excretion Experiment*

Fig. 3 shows each excretion pattern of $^{95}Zr$ and $^{95}Nb$ in viscera of the fish. An approximately

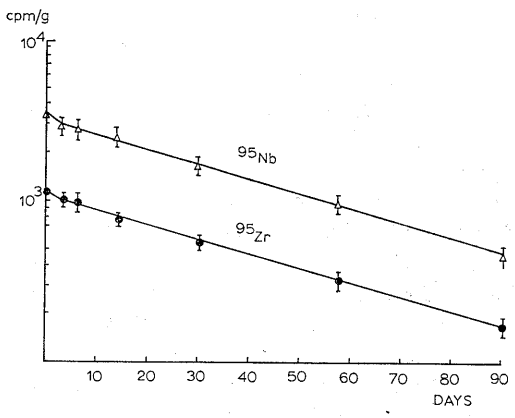


**Fig. 3.** Excretion of $^{95}Zr$ and $^{95}Nb$ by the viscera of common goby.

exponential relationship exists with a slope, loss coefficients, of 0.0187 for $^{95}$Zr and 0.0216 for $^{95}$Nb, although there is a rapid decrease during the first several days.

The $^{95}$Nb data on this line are indicated as the sum of $^{95}$Nb from the ambient water and $^{95}$Nb grown-in from $^{95}$Zr entered into the fish. However, as shown in Fig. 4, a quite similar loss

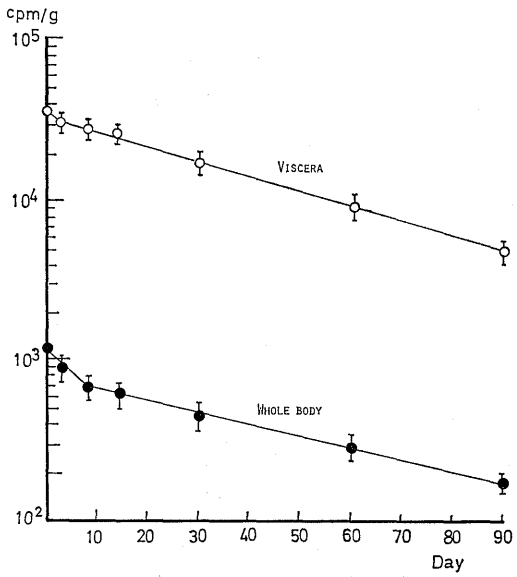


**Fig. 4.** Excretion of $^{95}$Nb by the viscera and whole body of common goby.

coefficient (0.0216) was obtained as a result of the excretion experiment of $^{95}$Nb alone. Thus, it was suggested that the $^{95}$Nb grown-in from $^{95}$Zr was negligibly small in the $^{95}$Zr-$^{95}$Nb experiment. Therefore, the effective and biological half times for $^{95}$Zr and $^{95}$Nb were calculated by using the formula; $1/T_{\text{eff}}=1/T_{\text{p}}+1/T_{\text{b}}$, where, $T_{\text{eff}}$ is the effective half time, $T_{\text{p}}$ is the physical half time and $T_{\text{b}}$ is the biological half time. Turnover rate ($\lambda$) is also calculated by using the $T_{\text{b}}$ as $\lambda=0.693/T_{\text{b}}$.

The turnover rate of viscera was 0.0081 for $^{95}$Zr and 0.0018 for $^{95}$Nb, and also the effective and biological half times were 37 and 85 days for $^{95}$Zr and 32 and 385 days for $^{95}$Nb, respectively. These figures were almost same in whole body of the fish (Fig. 5). The data for $^{95}$Nb were in good agreement with the results obtained from a single intraperitoneal injection on croaker ($T_{\text{eff}}=32.6$ days, $T_{\text{b}}=465$ days)[20].

Some factors concerning the accumulation and excretion of $^{95}$Zr and $^{95}$Nb by common goby are summarized in Table 2.

**Table 2.** Some factors concerning the accumulation and excretion of $^{95}$Zr and $^{95}$Nb by common goby

| | | $^{95}$Zr | $^{95}$Nb |
|---|---|---|---|
| Loss coefficient | | 0.00187 | 0.00216 |
| $T_{\text{eff}}$ (days) | | 37 | 32 |
| Turnover rate ($\lambda$) | | 0.0081 | 0.0018 |
| $T_{\text{b}}$ (days) | | 85 | 385 |
| CF* | Viscera | 70 | 83 |
| | Whole body | 3 | 4 |

* Concentration factor estimated when the fish were reared for a month under a constant level of the radioactivity in sea water.

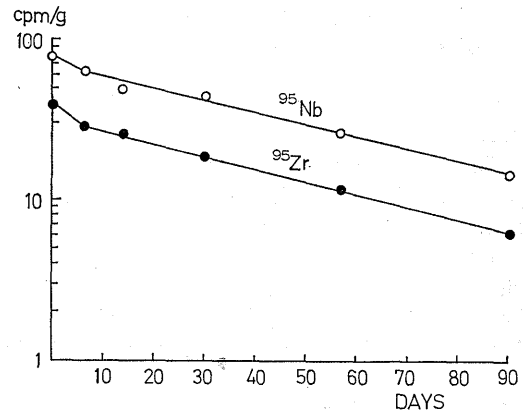


**Fig. 5.** Excretion of $^{95}$Zr and $^{95}$Nb by the whole body of common goby.

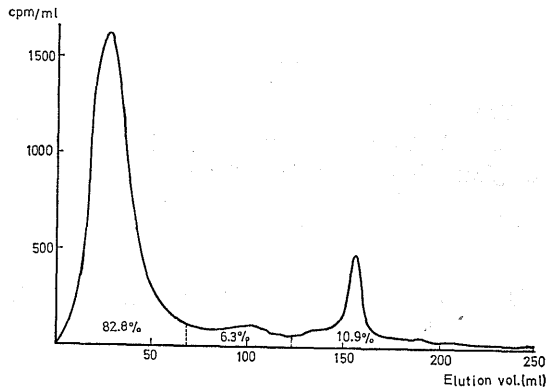


**Fig. 6.** Elution pattern by gel filtration for the viscera of common goby labelled by $^{95}$Nb.

*Observation of Binding of $^{95}$Nb and Protein by Gel Filtration*

Fig. 6 shows an elution pattern by gel filtration for the viscera of common goby labelled by $^{95}$Nb.

More than 80% of the total $^{95}$Nb activity in the extracted solution exists with a relatively large molecular weight protein (probably more than 35,000) and approximately 10% of the nuclide in

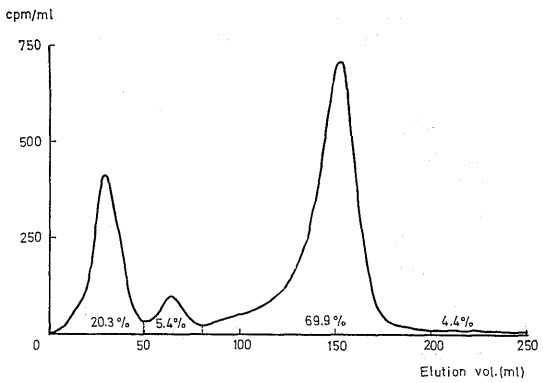


**Fig. 7.** Elution pattern by gel filtration for the viscera of common goby labelled by $^{65}Zn$.

it has the affinity for protein or peptide having molecular weight of about 2–3,000.

Fig. 7 shows an elution pattern of $^{65}Zn$ bound to the protein of the viscera sample treated similarly.

Nearly 70% of the total $^{65}Zn$ activity exsited with the protein of 2–3,000 of molecular weight. Although $^{95}Nb$ probably has no biological function in fish, it is apparently bound organically in the soft tissue of fish.

The evident discrepancy in the elution patterns of $^{95}Nb$ and $^{65}Zn$ cannot be clearly explained at present, but one of the reasons may be due to whether the nuclide is essential for fish.

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