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Bioaccumulation and distribution of ^{137}Cesium in the Humpback Grouper Fish (*Cromileptes altivelis*)

KETY MELINDA[1,♥], HENY SUSENO[2,♥♥], WAHYU PRIHATINI[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Pakuan University. Jl. Pakuan, Bogor 16143, West Java, Indonesia. Tel./Fax. +62-251-8312206, ♥email: ketymelinda@yahoo.com, wahyu_prihatini@yahoo.co.id

[2]Center for Technology of Radiation Safety and Meteorology, National Nuclear Energy Agency. Jl. Lebak Bulus Raya 49, South Jakarta, Indonesia. Tel./Fax. +62-21-7659409, ♥♥email: henis@batan.go.id

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Abstract. *Melinda K, Suseno H, Prihatini W. 2015. Bioaccumulation and distribution of ^{137}Cesium in the Humpback Grouper Fish* (Cromileptes altivelis). *Nusantara Bioscience 7: 180-184.* Humpback grouper fish (*Cromileptes altivelis*) is a highly valuable export commodity of food products. The high demand for this species needs to be supported by the intensive coastal aquaculture. However, the coastal aquaculture has been jeopardized by several threats, for an example radionuclide pollutant ^{137}Cesium (^{137}Cs) generated from nuclear reactor wastes. The pollutant accumulation in fish occurs because of continuing exposure to the pollutant-containing medium. This research was conducted from May to June 2015 at the Laboratory of Oceanic Radioecology, Center for Technology of Radiation Safety and Meteorology, National Nuclear Energy Agency, South Jakarta. This research observed the level of bioaccumulation and distribution of ^{137}Cs in *C. altivelis* bodies. Radionucleid ^{137}Cs of 2 Bq/mL concentration was inducted to two groups of fish, i.e. medium size (average body mass of 65.8 g) and small size (average body mass of 34.7 g). The calculation of concentration factor values at steady state (CF_{ss}) was done to determine the level of bioaccumulation ^{137}Cs in fish body. The distribution of ^{137}Cs in fish body was analyzed by calculating the percentage of accumulation ^{137}Cs in a certain organ to the total accumulation in fish body. The results showed that the value of CFss of medium size fish was 1.23 and CFss for small fish was 2.01. It means that small size fish accumulated ^{137}Cs almost twice as much as larger fish. The accumulation of ^{137}Cs in *C. Altivelis* was found mostly in its muscle (meat), which was 63.34%. These research findings provide reason for us to be more careful in consuming humpback grouper fish coming from ^{137}Cs polluted water due to the impact of biomagnifications of ^{137}Cs in fish.

Keywords: Bioaccumulation, ^{137}Cesium, concentration factor, *Cromileptes altivelis*

INTRODUCTION

Humpback grouper fish *Cromileptes altivelis* is one of reef fish species in Indonesia with high economic potential for consumable fish. The demand from domestic and foreign markets has increased over years. The export selling price for *C. altivelis* is about US $ 45 per kilogram (Jitunews 2015). The high demand of *C. altivelis* cannot be met only by harvesting naturally growing fish, but it also needs to be provided through aquaculture (BBAP Situbondo 2013). Floating net method is a commonly used in *C. altivelis* aquaculture and one of the locations of this aquaculture is at Jakarta Bay area.

The *C. altivelis* aquaculture in coastal area has been threatened by many things, for an example radioactive pollution of ^{137}Cesium (^{137}Cs). There has been a global debate about food material safety, including the safety level for radioactive exposure. In Japan, the safety level for ^{137}Cs contained in fish is 600 Bq/kg and in Europe, it is 1250 Bq/kg. However, in *Codex Guideline Level,* the standard for ^{137}Cs contamination in food is 1000 Bq/kg (Codex 2011). The nuclear-related research activities in Serpong Nuclear Area (SNA) potentially release products of fission reaction, including ^{137}Cs, to the environment. This release is commonly airborne which eventually will be deposited at soil and water. The SNA's neighboring Cisadane River, ending up in Jakarta Bay, has probably been exposed to ^{137}Cs. This radioactive may then be accumulated in bodies of animal species in that ecosystem (Suseno 2013).

C. altivelis is a predatory fish which might accumulate heavy metals due to biomagnification along the food chain. Heavy metals cannot be destroyed during metabolism. Therefore, when heavy metal contaminated fish is eaten by bigger predatory fish, the metal will be accumulated inside predatory fish bodies. Heavy metal accumulation occurs since there is continuous contact between fish and the polluted medium. Heavy metals enter fish bodies via three ways, i.e., food, gill and diffusion on skin surface (Sahetapy 2011).

Suseno (2013) studied bioaccumulation of ^{137}Cs in freshwater snail (*Pila ampullacea*) and found that concentration factor of ^{137}Cs in *P. ampullacea* was 13.2 – 27.6 mL/g. The level of accumulation in *P. ampullacea* was influenced by its body mass but it was not dictated by water pH. Malek et al. (2004) reported that in fish species of *Silurus asotus*, 75% ^{137}Cs accumulation was found in its muscle and the accumulation level was higher in juvenile than in adult individuals. The same pattern was also found in fish species of *Oncorhynchus mykiss* (Baudin et al. 2000).

MATERIALS AND METHODS

This research was conducted from May to June 2015 at the Laboratory of Oceanic Radioecology, Center for Technology of Radiation Safety and Meteorology, National Nuclear Energy Agency, South Jakarta, Indonesia.

Procedures

Acclimatization

Acclimatization process was run for seven days in an aquarium filled with 200 liters of clean seawater. The aquarium was installed with several filters, skimmer, and aerator. During the acclimatization, *C. altivelis* was fed twice a day (morning and afternoon) with mosquito fish.

Bioaccumulation and depuration of ^{137}Cs

After acclimatization, fish samples were separated into two groups of three fish based on body mass. The average body mass of the fish of each group was 65.8 g and 34.7 g. Each group of fish samples was put into separate aquariums containing 50 liters of clean seawater that had been exposed to 2 Bq/mL of ^{137}Cs (Retno 2011). The fish was left for Cs uptake process for eleven days. One fish of each group was set as a control, put in separate aquarium containing clean seawater without ^{137}Cs exposure.

After the uptake phase, the depuration phase took place for nine days. Depuration is the pollutant releasing process from fish bodies by putting them in aquarium containing clean seawater without ^{137}Cs exposure. The seawater was replaced every day. The concentration of ^{137}Cs left in fish bodies (retention value) was measured and recorded every day using NaI(Tl) detector.

Distribution of ^{137}Cs

The identification of bioaccumulation distribution locations of ^{137}Cs was conducted by dissecting fish bodies and taking their organs. Fish body organs examined included brain, heart, liver, viscera, gill, and muscles. Each organ was separated and kept in vials. The concentration of ^{137}Cs was measured using a gamma HPGA spectrophotometer.

Data analyses

The value of concentration factor (CF)

The concentration data of ^{137}Cs in fish bodies during uptake and depuration phases were analyzed in order to get the CF value. This number was then applied to observe the presence of pollutants in the environment and the response of the species to those pollutants. The measurement of CF value used the formula below:

$$CF = \frac{\text{Concentration of radioactive tracer (Bq)/fish weight (g)}}{\text{Concentration of radioactive tracer (Bq)/water volume (mL)}}$$

(Whicker and Schultz 1982)

The up taking rate of ^{137}Cs

The up taking rate of ^{137}Cs by fish body was measured by applying the formula below:

$$\frac{dC_t}{d_t} = k_u C_w - k_e C_t$$

Where: C_t = Concentration of ^{137}Cs in fish body at a certain time t; C_w = Concentration of ^{137}Cs in the environment; k_u = uptake constant value of ^{137}Cs (per day); k_e = release constant of ^{137}Cs (per day); t = time (day) (Louma et al. 2005)

The value of steady state concentration factor

The value of steady-state CF (CF_{ss}) is a ratio between k_u and k_e. This stage occurs when the quantity of uptake of ^{137}Cs from the environment into the fish body is equal to the quantity of release of ^{137}Cs out of the fish body. If during the uptake phase of ^{137}Cs, the steady state has not achieved yet, the value of CF*ss* can be calculated using the formula below:

$$CFss = \frac{ku}{ke}$$

The value of CF_{ss} was then used for modelling, i.e. the estimation of CF value at a certain time t, by using this formula:

$$CF_t = CF_{ss}\,(1 - e^{\,ke.t})$$

If the ^{137}Cs up taking process stopped, and $k_u C_w = 0$, the release rate (depuration) was calculated using the formula below:

$$\frac{dC_t}{d_t} = -k_e C_t$$

Where: C_t = Concentration of ^{137}Cs in fish body at a certain time t; k_e = release constant of ^{137}Cs (per day); t = time (day) (Louma et al. 2005)

Distribution of ^{137}Cs

The analysis of the distribution of ^{137}Cs accumulation in fish body was done by calculating the ratio of ^{137}Cs concentration in one organ to the total concentration of ^{137}Cs in fish body (Malek et al. 2004)

RESULTS AND DISCUSSION

Bioaccumulation of ^{137}Cs in *Cromileptes altivelis*

The results of the measurement of concentration factor (CF) values during ^{137}Cs uptake phase of two groups of fish samples are shown in Figure 1, in which smaller fish had higher CF value than larger fish. The slope of uptake phase (k_u) in first fish group (average body mass of 65.8 g) was 0.09, and the slope was 0.18 for second group of fish (average body mass of 34.7 g). It means that larger fish accumulated ^{137}Cs as much as 0.09 of ^{137}Cs concentration in water. On the other hand, small fish accumulated ^{137}Cs twice as much as larger fish did during the same period of time.

Depuration of ^{137}Cs in *Cromileptes altivelis*

When the uptake phase finished, the depuration phase took place. The results showed that the depuration rate (k_e) of first group of fish (body mass of 65.8 g) was 0.08, and the second group (body mass of 34.7 g) was 0.09 (Figure 2). The rates of ^{137}Cs release from bodies of the two fish groups did not differ significantly.

The estimation of steady-state concentration factor (CF_{ss})

Modeling analysis by using the CFss value was applied to estimate when the steady state is achieved if those fish are continuously exposed to pollutants. The values of CFss of first and second fish groups were 1.23 and 2.01. It means that the medium size fish group accumulated ^{137}Cs as much as 1.23 of ^{137}Cs concentration in water. On the other hand, smaller fish accumulated more ^{137}Cs, which was about 2.01 times as much as ^{137}Cs concentration in water. If their exposure to the ^{137}Cs continues, the steady state would be achieved on the 5th day for medium size fish (Figure 3) and on the 33th for smaller fish (Figure 4).

Distribution of ^{137}Cs in *Cromileptes altivelis* body

The results of the analysis of distribution of ^{137}Cs accumulation in organs of fish samples are shown in Figure 5. The highest percentage of ^{137}Cs accumulation in *Cromileptes altivelis* was found in muscle tissues, which was about 63.34%.

Discussion

The ^{137}Cs contamination is dangerous for ecosystem since it can concentrate on food chain. ^{137}Cs also has similar chemical properties to K^+ ion, in which it can be easily accumulated in organism's bodies (Cahyana 2012). This research showed that different-size *C. altivelis* had different level of ^{137}Cs accumulation, in which smaller fish accumulated twice as much ^{137}Cs as medium-size fish. This difference might be due to the difference in metabolism intensity. Smaller fish are juveniles that have higher metabolism rate (Torres et al. 2012), including ^{137}Cs metabolism. Therefore, the accumulation through the respiratory system might be at higher rate. Naturally, fish body will excrete useless compounds entering the body. However, the quantity and the excretion rate of those compounds may not always be equal to their entering rate. Therefore, the concentration of ^{137}Cs in the fish body increased (Figure 1). This progressive increase of a certain compound in concentration in organism is called bioaccumulation (Fisher 2002).

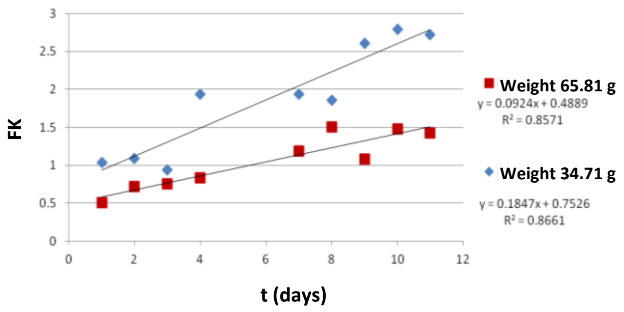

Figure 1. The gradient relationships between the uptake of ^{137}Cs and exposure time

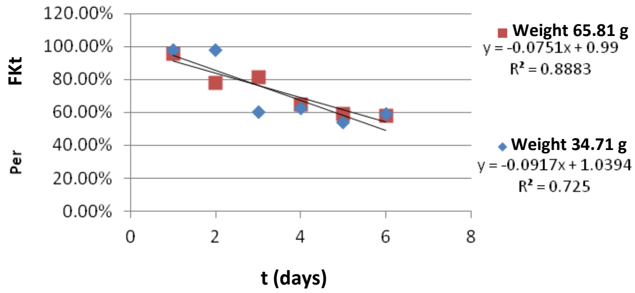

Figure 2. The gradient relationships between the rate of ^{137}Cs release and exposure time

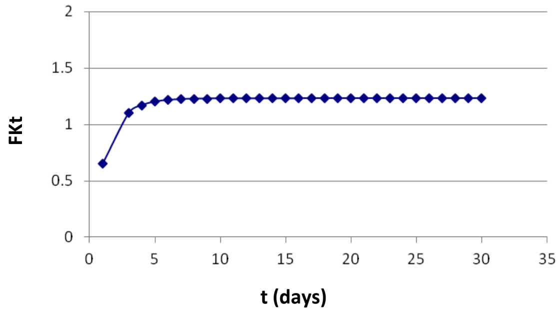

Figure 3. The estimation of CF_{ss} of ^{137}Cs in *Cromileptes altivelis* with average body mass of 65.8 g

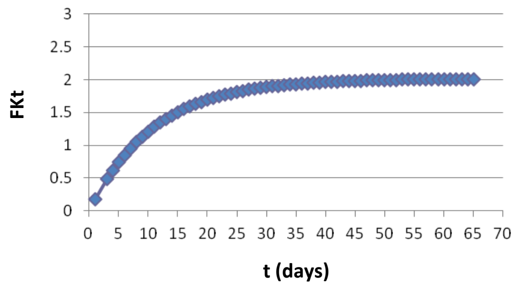

Figure 4. The estimation of CF_{ss} of ^{137}Cs in *Cromileptes altivelis* with average body mass of 34.7 g

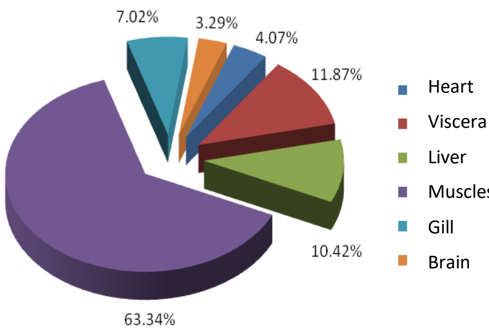

Figure 5. The distribution of ^{137}Cs accumulation inside the body of *Cromileptes altivelis.*

The rate of bioaccumulation differs between organisms. Green snail *Perna viridis* (body mass < 20 g) was able to accumulate ^{137}Cs as much as 1.45 time of its concentration in water (Retno 2011). Golden snail *Pomacea canaliculata*, with average size of 3.9 cm, could even accumulate ^{137}Cs as much as 43.49 of its concentration in water (Yandra et al. 2013). Biomagnification of ^{137}Cs may occur through the food chain in estuary ecosystem. Predatory fish are on the top of trophic level, and they are consumable fish. Therefore, the monitoring of their radioactive level is critical (Doi et al. 2012).

Based on the modeling analysis of steady-state concentration factor (CFss), smaller fish took a longer time to reach a steady state. The accumulation of ^{137}Cs in medium size *C. altivelis* (body mass of 65.8 g) reached a steady state on the 5^{th} day after the exposure (Figure 3). On the other hand, the steady state of ^{137}Cs accumulation in small fish (body mass of 34.7 g) did not occur until 33^{th} after exposure (Figure 4). Smaller fish (juveniles) have more intensive metabolism and are more able to regulate ^{137}Cs through metabolism, excretion, and detoxification (Kojadinovic 2007). Those physiological characteristics make smaller fish more tolerant to the rise of the ^{137}Cs concentration in their bodies. Consequently, it takes a longer time for smaller fish to achieve the steady state of the ^{137}Cs concentration accumulation than larger fish. The ability of organism to accumulate contaminating compounds is also influenced by species-specific capacity (Cardoso et al. 2009). In juveniles of *Silurus asotus* (body mass of 14-25 g), it was reported that this species achieved a steady state after 1.5 days of ^{137}Cs exposure (Malek et al. 2004). At the steady state, the fish excrete and detoxicate ^{137}Cs to balance it in the body, so its radiation effects could be tolerated (Barka 2012). As the smaller fish are more tolerant of ^{137}Cs accumulation and the ratio of ^{137}Cs per body mass is higher than in larger fish, smaller fish are more poisonous and more dangerous to be consumed by human.

The result of this research showed that the rate of ^{137}Cs released from fish bodies did not differ significantly between both groups of fish. The release of pollutant compounds is not dictated by body mass of the organism, but it is more influenced by the volume per time of the water flowing in habitat where those organisms live (Retno 2011). In this research, the rate of water flow and the volume were exactly similar for both groups of fish. The accumulation of ^{137}Cs was higher in smaller fish than in larger fish, but the rate of ^{137}Cs release was similar. This result suggests that smaller fish are even more dangerous to be eaten. Consuming fish harvested from contaminated estuaries may allow the accumulated ^{137}Cs to be transferred into human bodies.

Every type of metals has different behavior when it is accumulated and distributed in organism bodies. Cesium is accumulated more in muscles (Malek et al. 2004) and on the other hand, Strontium tends to be accumulated in bones. Fish muscle (the meat) is the biggest part of fish body mass and it is the consumable part for human.

This study found that the highest accumulation of ^{137}Cs in *C. altivelis* body occurred in muscles, i.e., 63.34% (Fig. 5). This finding is similar to results of previous studies (Baudin et al. 2001; Zhao et al. 2001; Malek et al. 2004). It is believed that high distribution of ^{137}Cs in the muscles is due to the similarity of chemical properties between ^{137}Cs and K^+ ions. Consequently, ^{137}Cs can replace the position of K^+ ions in the cells and accumulate in fish body. K^+ ions play important roles in protein synthesis, triggering muscle irritability and glycolysis to provide energy for cell activities (Fried and Hademenos 2005). K^+ ions are mostly found in muscles as they are important in managing muscle contraction. The replacement of K^+ ions by ^{137}Cs can disturb structures and functions of muscles, which is related to nervous system. High concentration of ^{137}Cs in the body will cause hyperirritability and cramps due to excessive loss of K^+ ions in nervous system (Fried and Hademenos 2005). Muscles are the targeted tissues for ^{137}Cs, because it can compete with K^+ ions in binding site of essential proteins (Hampton et al. 2004). The replacement of K^+ ions by ^{137}Cs, even a little, will affect cells and fish body tissues, as the cesium cannot replace K^+ ion function to activate several enzymes. ^{137}Cs will then be toxic for fish body (Gilbert and Pessala 2012).

The high accumulation of ^{137}Cs in *C. altivelis* body also occurred in viscera (11.87%) and liver (10.42%). This is also believed to be due to the similarity of chemical characteristics between ^{137}Cs and K^+ ions. Intestinal tissues and liver absorb much potassium since it is important for carbohydrate metabolism to convert glucose into glycogen in the liver (Haas 2000). The distribution of ^{137}Cs in *C. altivelis* gill was about 7.02%. This result agrees with Zhao et al. (2001) finding in *Lutjanus argentimaculatus*, which found that accumulation of ^{137}Cs in gill was less than 10%. Gill is the first organ to have direct and continuous contact with ^{137}Cs in the environment. However, the absorbed compound will be transported directly to other parts of fish body.

Although the concentration of contaminants in the environment is low, they can still affect organism body tissues. The effect of heavy metal contamination is not only driven by the concentration of those heavy metals, but also their roles in body metabolism. The magnitude of the effect of that contamination is also influenced by the quantity of those heavy metals that can be absorbed and excreted by the body (Jakimska et al. 2011).

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