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Bioaccumulation and retention kinetics of cesium in the Milkfish *Chanos chanos* from Jakarta Bay

Wahyu Retno Prihatiningsih [a,b], Heny Suseno [a,*], Neviaty P. Zamani [b], Dedy Soedharma [b]

[a] Marine Radioecology Group, Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency, Jl. Lebak Bulus Raya No. 49, Kotak Pos 7043 JKSKL, Jakarta Selatan 12070, Indonesia

[b] Marine Technology and Sciences, Bogor Agricultural University, Gd. Marine Center lt. 3 FPIK Kampus IPB Darmaga, Bogor, West Java, Indonesia

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ABSTRACT

Laboratory radiotracer experiments were conducted to study the uptake, assimilation, and retention of cesium (^{137}Cs) in milkfish (*Chanos chanos*) from Jakarta Bay. In this study, we have examined the bioaccumulation and distribution of ^{137}Cs in *C. chanos* obtained from ^{137}Cs-labeled seawater and ^{137}Cs-labeled *Artemia sp*. feeding. The uptake of ^{137}Cs via seawater displayed a one-compartment model suggesting that the concentration factors of ^{137}Cs within the milkfish (weight 2.46–9.86 g) at a steady-state period were between 10.66 and 3.98 mL g^{-1} after 10 days of exposure. The depuration rate was observed to be low, with only 22.80–49.14% of ^{137}Cs absorbed by *C. chanos*, which was absent 6 days after exposure. By contrast, depuration occurred quickly for radiolabeled food uptake, reaching 20% of retention within 10 days after exposure. Muscles and viscera of the milkfish exhibited the highest degree of end uptake and end depuration of ^{137}Cs from seawater and feeding.

1. Introduction

Jakarta Bay is ecologically degraded due to high load of biological, chemical, and radioactive pollutants from both marine and inland areas (Rengganis, 2012; Rinawati et al., 2012; Arifin, 2004; Williams et al., 2000). These pollutants were discharged from upland regions transported by 13 rivers flowing into Jakarta Bay, including the Cisadane River (Koropitan et al., 2008; Arifin, 2004). At present, Indonesia has a research power reactor with an output power of 30 MW, and is planning to establish another HTGR-type research power reactor with a capacity of 10 MW at Serpong, near Jakarta (BATAN, 2014). Radionuclides such as ^{137}Cs may potentially be released from the reactor and other nuclear facilities and transported by the Cisadane River into Jakarta Bay, thereby affecting the fishery activities such as capture fishery and aquaculture, which contribute to huge quantities of biota culture (e.g., milkfish, green mussels, shrimps, and seaweeds). Unfortunately, these biotas can accumulate ^{137}Cs. At present, there has been only limited number of studies conducted on the effect of nuclear facilities on bioaccumulation of ^{137}Cs in Indonesia. A study on bioaccumulation of ^{137}Cs by *Perna viridis* at Jakarta Bay was conducted by Suseno in 2004. A similar study was conducted on freshwater snail *Pila ampullaceal* concerning the potential release of ^{137}Cs into the Cisadane River (Suseno and Prihatiningsih, 2014. However, these studies only described the accumulation processes of ^{137}Cs from seawater. Hedouin et al. (2010) investigated metal bioaccumulation of *Isognomon isognomum* and *Malleus regula* exposed to Ag, Cd, Co, Cr, and Zn from three different contamination pathways (seawater, food, and sediment). The biokinetic approach has successfully demonstrated the mechanism of bioaccumulation by marine biotas for both nonradioactive contaminants using radiotracer and radioactive contaminants such as ^{134}Cs, ^{137}Cs, and ^{241}Am (Metian et al., 2007; Rowan, 2013; Bustamante et al., 2006; Baudin et al., 2000).

The milkfish *Chanos chanos* is the sole existing species of the family Chanidae that is widely cultured in Jakarta Bay. Biologically, milkfish is a euryhaline fish species that is extremely resistant to water quality changes. They can absorb and accumulate various contaminants from the environment (Takarina et al., 2012; Palanikumar et al., 2012; Chou et al., 2006). According to the report of the Ministry of Marine and Fisheries of Indonesia in 2010, milkfish is regarded as a high-value food item and the fourth largest aquaculture commodity being cultivated in the country, with seaweed, Nile tilapia, and shrimps being the first three. The rate of production of the milkfish *C. chanos* is approximately 291.300 ton/year. The aquaculture production of this species increased from 212.883 to >291.000 ton from 2006 to 2009, contributing to >6% of the total national seafood production (KKP, 2010). In addition, the demand for milkfish in Indonesia has been increasing every year, with an average increase of 5.96% from 2005 to 2009 (GAIN Report, 2010). Therefore, research on bioaccumulation of ^{137}Cs is required to protect human health from the effects of the research reactor. In order to investigate and better understand the bioaccumulation of ^{137}Cs, this study assesses the uptake and depuration kinetics of *C. chanos* from Jakarta Bay

* Corresponding author.
E-mail address: henis@batan.go.id (H. Suseno).

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that were exposed to ^{137}Cs through seawater and food. It also investigates the distribution of ^{137}Cs in the milkfish body.

2. Materials and methods

2.1. Organisms tested

C. chanos (fish length 3.2–5.8 cm) were collected from a fish farm in Tanjung Pasir, Jakarta Bay, Indonesia, in September 2014. Then, they were shipped to the Marine Radioecology Laboratory, Center for Technology of Radiation Safety and Metrology in South Jakarta, where they were acclimated to laboratory conditions (open circuit, 1000-l aquarium, water renewal: 30% h^{-1}; T: 27 ± 0.5 °C; salinity: 35‰; pH: 8.0 ± 0.1) for 2 months before the experiment. During this period, *C. chanos* were fed LAJU (LJA-3) commercial pellets daily.

2.2. Radiotracers and counting

The uptake and depuration kinetics of ^{137}Cs were determined by high specific activity radiotracers purchased from Polatom, Poland (^{137}Cs as CsCl, $t_{½}$ = 30 years). Activity of the tracers was measured non-destructively using 661.66-keV NaI gamma detector (type 3428, Bicron) equipped with a multichannel analyzer and computer installed with (Genie, 2000) spectra analysis software from Canberra Ind. Standards 300 Bq activity and appropriate fish phantom geometry were used to determine the activity by comparison. Measurements were corrected by considering efficiency and physical radioactive decay. The counting time was adjusted to obtain a propagated counting error <5% (Rodriguez y Baena et al., 2006).

2.3. Seawater exposure

Six samples of similar-sized *C. chanos* were placed in a 10-l closed-circuit constantly aerated glass aquarium (T: 27 ± 0.5 °C; salinity: 35) and exposed to ^{137}Cs radiotracers dissolved in 0.45-μm filtered seawater for 10 days, according to the method described by Metian et al. (2007). In addition, four different-sized *C. chanos* were placed in four 10-l glass aquariums. Six samples of *C. chanos* were placed in the same condition for dissection at the end of uptake and six other specimens in 10-l open-circuit constantly aerated glass aquarium for dissection at the end of depuration (T: 27 ± 0.5 °C; salinity: 35). Nominal activity of ^{137}Cs radiotracer was 2 kBq/l. In terms of stable metal concentration, these additions corresponded to Cs (5.8 pmol l^{-1}), which are one to three orders of magnitude lower than those naturally found in seawater. The seawater was changed and spike was renewed daily during the first week, and then every alternate day to maintain constant activity in the seawater. The activity of the radiotracers in the seawater was checked daily, before and after each spike renewal, to calculate their time-integrated activities (Rodriguez y Baena et al., 2006). Immediately before each renewal of seawater and spike, the *C. chanos* were fed *Artemia sp.* in clean, unspiked seawater (30 min). They were then radiocounted for 15 min. At the end of the 10-day exposure period, six samples of *C. chanos* were selected for the dissection experiment. The viscera and remaining muscle parts were separated, weighed, and radioanalyzed to assess the distribution of ^{137}Cs within the body. The remaining 24 samples were then placed in clean, unspiked conditions (flowing and filtered seawater, flux: 50 l h^{-1}; T: 27 ± 0.5 °C; salinity: 35‰; daily feeding on *Artemia sp.*) for 6 days to determine the depuration of the radiotracers in the whole body. Six individuals of this species were collected at the end of the depuration period and dissected to assess radiotracer distribution between organs.

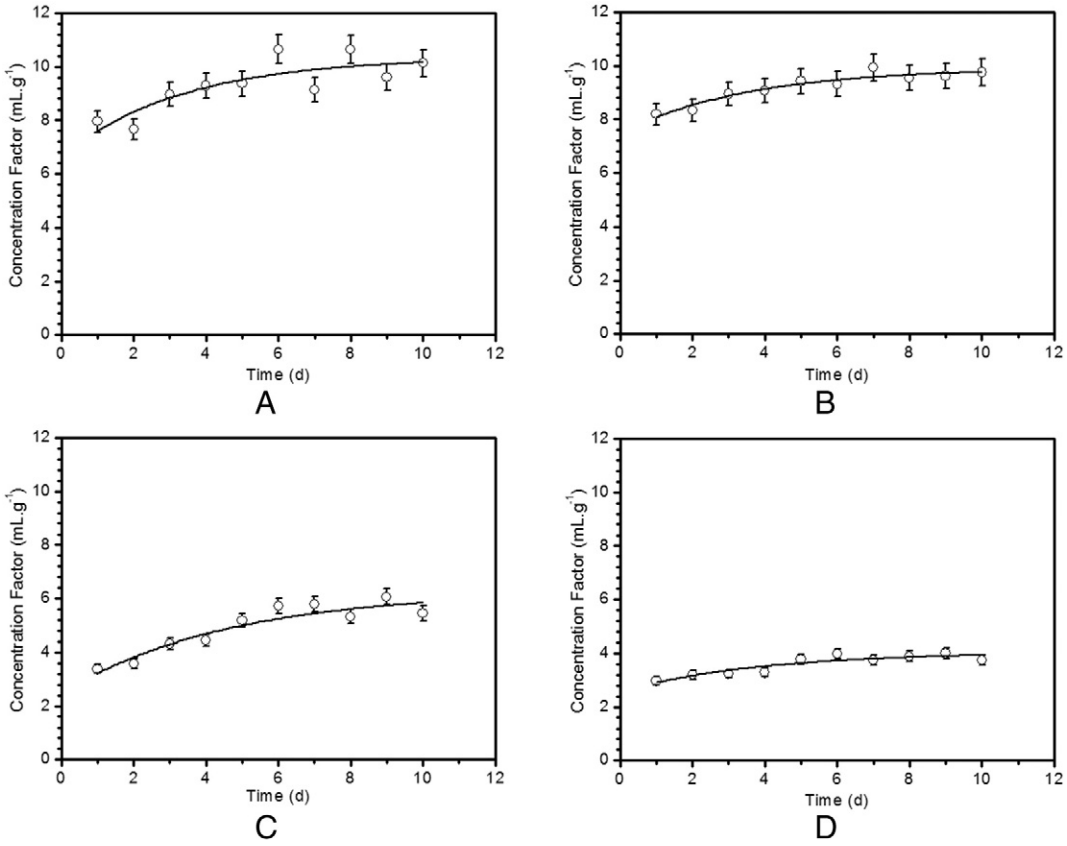

Fig. 1. Uptake kinetics of ^{137}CS in *Chanos chanos* (A. 2.46 g, B. 5.29 g, C. 8.32 g, D. 9.86 g) exposed to ^{137}CS for 10 days via seawater (mean concentration factors, CF ± SD, n = 6 per body size).

Table 1
Whole-body uptake and depuration kinetic parameters of ^{137}Cs in *Chanos chanos* following seawater and food exposure experiments: (seawater) exposed to waterborne radionuclides (n = 6) for 10 days, followed by 6 days of depuration (n = 6); (food) after a 2-h feeding on radiolabeled *Artemia sp.* for ^{137}Cs followed by 6 days of depuration (n = 6).

Chanos chanos group	n	Body Size (g)	Sea water					Food			*BAF*
			CF_{ss} (mL g^{-1})	k_u (d^{-1})	k_e (d^{-1})	R^2	$T_{1/2b}$	*AE* (%)	k_e (d^{-1})	R^2	
1	6	2.46	10.66 ± 0.53	1.23 ± 0.06	0.71 ± 0.04	0.6 ± 0.03	0.97 ± 0,05	36.98 ± 1.85	0.26 ± 0.01	0.73 ± 0.04	10.78 ± 0.54
2	6	5.29	9.93 ± 0.50	0.88 ± 0.04	0.55 ± 0.03	0.59 ± 0.03	1.26 ± 0.06				
3	6	8.32	5.78 ± 0.29	0.65 ± 0.03	0.49 ± 0.02	0.57 ± 0.03	1.41 ± 0.07	35.24 ± 1.76	0.26 ± 0.01	0.73 ± 0.04	5.87 ± 0.29
4	6	9.86	3.98 ± 0.20	0.34 ± 0.02	0.46 ± 0.02	0.44 ± 0.02	1.51 ± 0.07				

2.4. Food exposure

Artemia sp. was exposed to ^{137}Cs in a 5-l plastic container for 6 days, following the same activities as those used for the seawater exposure experiment. At the end of the exposure period, *Artemia sp.* was filtered and resuspended in clean seawater. Two different-sized groups of *C. chanos* (six samples in each group) were prepared for food exposure experiment and one group ($n = 6$) for dissection at the end of food exposure. Six *C. chanos* were placed in a 10-l closed-circuit constantly aerated glass aquarium (T: 27 ± 0.5 °C; salinity: 35‰) and fed radiolabeled Artemia for 2 h. Control fish were placed in the aquarium as control for any tracer recycling via seawater. The control fish were regularly counted and no activity was detected in them, which indicates the absence of detectable recycling of dietary tracer. After the feeding period (2 h), all *C. chanos* were counted and flowing seawater conditions were restored for 24 h (flux: 50 l h^{-1}; T: 27 ± 0.5 °C; salinity: 35 ‰). Individuals were counted at 2-h intervals to follow the depuration kinetics of radiotracers from whole-body fish. Six individuals were collected at the end of the 24-h depuration period and dissected to determine the radiotracer distribution between organs (e.g., muscle and skeletal bones, viscera, head, skin and fin, and gills).

2.5. Data analysis

The uptake of radiotracers from water was expressed as a change in concentration factors (CFs). The CF of ^{137}Cs is defined as the ratio of activity in the fish (Bq g^{-1}) to that in water (Bq mL^{-1}), which is calculated as the mean before and after exposure for each time point. The uptake rate was calculated as the slope of the linear regression obtained by plotting CF against time of exposure multiplied by the concentrations of dissolved Cs. The uptake kinetics was described using a single-component first-order kinetic model (Metian et al., 2007):

$$CF_t = CF_{ss}\left(1-e^{-kt}\right), \quad (1)$$

where CF_t and CF_{ss} are CFs at time t (days) and steady state, respectively, and k is the constant rate (d^{-1}). Radiotracer elimination was expressed in terms of percentage of remaining activity, that is, activity at time t divided by initial activity measured in the organisms at the beginning of the depuration period. The plot of radiotracer loss against time displayed an exponential curve, whose kinetics was described by the following single-component exponential model:

$$A_t = A_0 x\ e^{-k_e t}, \quad (2)$$

where A_t and A_0 are remaining activities (%) at time t (days) and 0, respectively, and k is the depuration rate constant (d^{-1}), which allows calculation of the radiotracer biological half-life ($t_{1/2b}$):

$$t_{1/2\,b} = \frac{\ln 2}{k_e}. \quad (3)$$

2.6. Modeling exposure and food chain transfer

Under steady-state conditions, ^{137}Cs accumulation in fish can be calculated by the following equation (Wang and Wong 2003, Reinfelder et al., 1998a, b):

$$BAF = \frac{k_u}{k_e} + \frac{AE.IR.BCF}{k_{e.f}} \quad (4)$$

where *BAF* is the ^{137}Cs bioaccumulation factor in the fish, k_u is the ^{137}Cs net uptake constant rate from the aqueous phase (d^{-1}), k_u is the elimination constant rate following the uptake from the dissolved phase (d^{-1}), *AE* is the metal assimilation efficiency, *IR* is the fish feeding rate (in fraction of body weight d^{-1}), and k_{ef} is the elimination constant rate following the uptake from food (d^{-1}). *AE* was calculated as the percentage of metal retained in the fish after 24 h. In this experiment, we used an *IR* value of 0.073/d dry weight basis. *BCF* was calculated as the ratio of metal concentration in ingested prey (Bq g^{-1}) to that in the dissolved phase (Bq l^{-1}).

The constants of the models and their statistics were estimated by iterative adjustment of the model and using the nonlinear curve-fitting routines in the Origin 8 software. Statistical significance was always set at $\alpha = 0.05$.

3. Result and discussion

No fish mortality was recorded during the acclimation period nor uptake or depuration experiments. In general, the accumulation of a radioactive substance by an organism occurs when the uptake rate exceeds the excretion rate (Metian et al., 2007). As stated previously, fish in the marine environment may accumulate ^{137}Cs directly from seawater or ingested food. Absorption through both pathways may occur either simultaneously or at different times, depending on migratory patterns or food habits of the fish concerned (Smith et al., 2002a, b). This experiment has established the uptake and elimination rates of

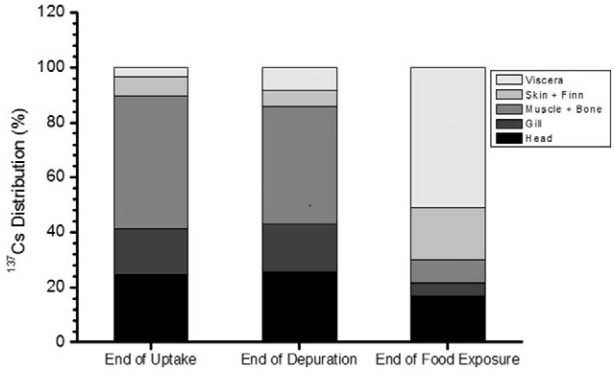

Fig. 2. Body distribution (mean % ± SD) of ^{137}CS in *Chanos chanos* during seawater (after 10 days of exposure; end of uptake and after 6 days of depuration; end of depuration and feeding experiment (24-h after feeding with *Artemia* sp.; end of food exposure).

Table 2
Organ distribution of cesium in *Chanos chanos* (mean ^{137}Cs Distribution ± SD, n = 6) at the end of the uptake and depuration of ^{137}Cs accumulated via seawater and feeding with ^{137}Cs-contaminated *Artemia sp*. Results are expressed as percentages of total ^{137}Cs activity.

Organs	Seawater		Food
	End of uptake (%)	End of depuration (%)	End of feeding (%)
Head	24.74 ± 1.24	25.71 ± 1.28	17.02 ± 0.85
Gills	16.58 ± 0.83	17.2 ± 0.86	4.57 ± 0.23
Muscle & bone	48.16 ± 2.41	43.08 ± 2.15	8.27 ± 0.41
Skin & fins	7.13 ± 0.36	5.64 ± 0.3	19.15 ± 0.96
Viscera	3.39 ± 0.17	8.37 ± 0.42	50.99 ± 2.55

cesium in the milkfish *C. chanos* using a nondestructive radiotracer technique. As absorption followed two independent pathways, comparisons could be made between them.

The uptake of ^{137}Cs displayed a steady-state exponential kinetics (Fig. 1 and Table 1). The estimated values of the kinetic parameters and their associated statistics are shown in Table 1. The CFs of ^{137}Cs for body sizes 2.46, 5.29, 8.32, and 9.86 g at steady-state period were 10.66, 9.93, 5.78, and 3.98 mL g^{-1} in whole-body fish, respectively. At the end of the uptake experiment, the highest ^{137}Cs load was observed in the muscle and skeletal bones (>48% of the total body load). ^{137}Cs was mainly accumulated in the head, gills, skin and fins, and viscera (24.74%, 16.58%, 7.13%, and 3.39% of the total body load, respectively; Fig. 2 and Table 2).

Na^+, Cl^+, and K^+ are actively absorbed by the intestine of marine teleost (i.e, *C. chanos*) from the seawater ingested by osmoregulatory water absorption (Smith et al., 2000). Small amount of cesium (Cs^+) ions, which are generally considered as biochemical analogs of K^+ (Relman, 1956), can be detected in both fish (i.e., bones) and seawater. These findings strongly suggest that the metabolism of Cs is closely associated with that of K^+ in teleost. Using log-linear regression, Rowan and Rasmussen (1994) found that the bioaccumulation of ^{137}Cs by fish was a negative function of potassium, and it occurs not only in the environment but also in fish body. At the end of depuration, 43.08% of ^{137}Cs still remained. This is lower than that at the end of uptake (48.16%). Only a small amount of Cs taking change with K (50.8%), and K is removed from the bones and muscle and excreted to the environment via the K-transporting pathway in the gill ionocytes. As the proportion of ^{137}Cs in gills at the end of depuration was 0.62% higher than that at the end of uptake, turnover of ^{137}Cs with K occurs in the gills during the depuration period. Although the elimination route of Cs^+ remains unclear, the K^+-excreting pathway in the branchial epithelium on gill ionocytes is a strong factor determining the elimination mechanism of cesium (Smith et al., 2000).

When noncontaminating conditions were restored, the whole-body depuration kinetics of ^{137}Cs was best described by a single-component exponential model (Fig. 3 and Table 1). The maximum bioaccumulation of ^{137}Cs were 49.14%, 22.80%, 12.70%, and 39.97% in *C. chanos* with body sizes of 2.46, 5.29, 8.32, and 9.86 g, respectively. The estimated depuration constant rates of *C. chanos* with body sizes 2.46, 5.29, 8.32, and 9.86 g were 0.71, 0.55, 0.49, and 0.46 d^{-1}, respectively, and consequently, their derived biological half-lives range from 0.97 to 1.51 days (Table 1).

The depuration kinetics of the radionuclides ingested with food from the whole-body *C. chanos* was best fitted by a single exponential model (Fig. 3 and Table 1). Different-sized *C. chanos* (2.46 and 8.32 g) exhibited similar assimilation efficiencies of 36.98% and 35.24% close to depuration constant rates (k_e) of 3.26 and 3.26, respectively (Fig. 4 and Table 1). At the end of the depuration period, a major proportion of ^{137}Cs was found to be essentially accumulated in the viscera (50.99% of the total body load) (Fig. 2 and Table 2). However, ^{137}Cs

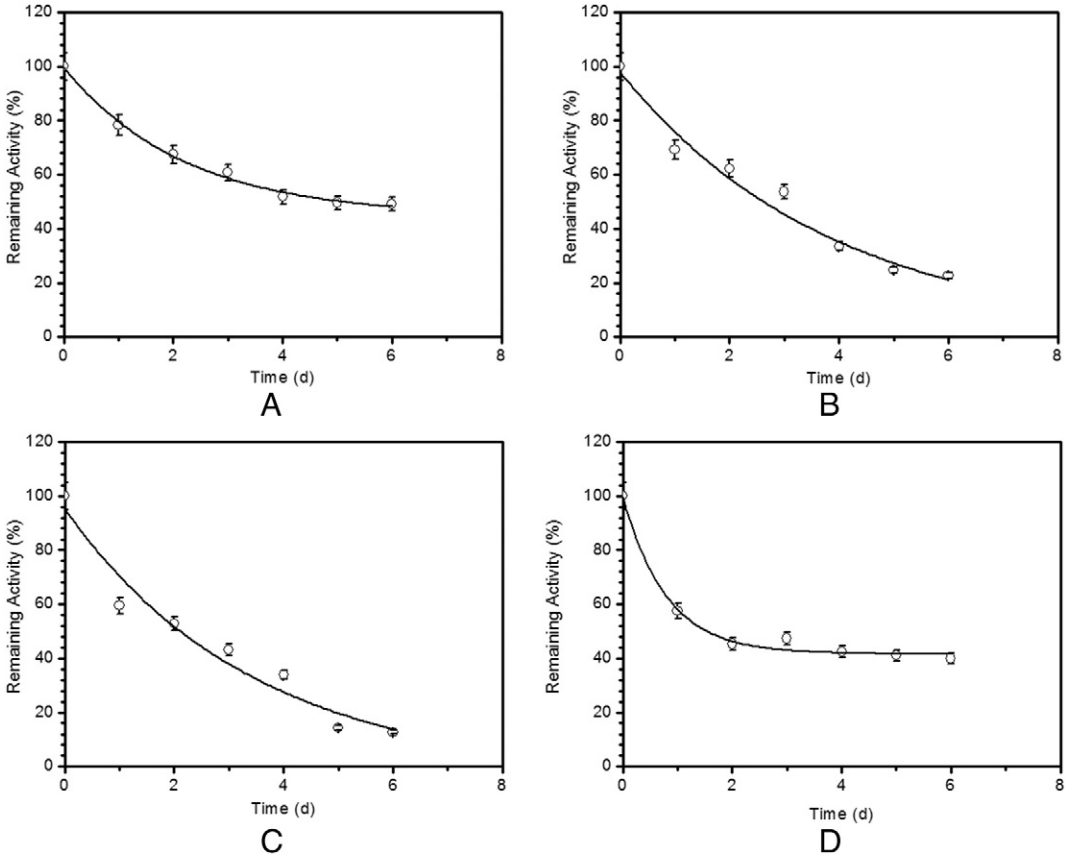

Fig. 3. Depuration kinetics of ^{137}Cs in the *Chanos chanos* (A. 2.46 g, 5.29 g, C. 8.32 g, D. 9.86 g) maintain for 6 days in noncontaminated seawater (remaining activity, %; mean ± SD; n = 6 per body size).

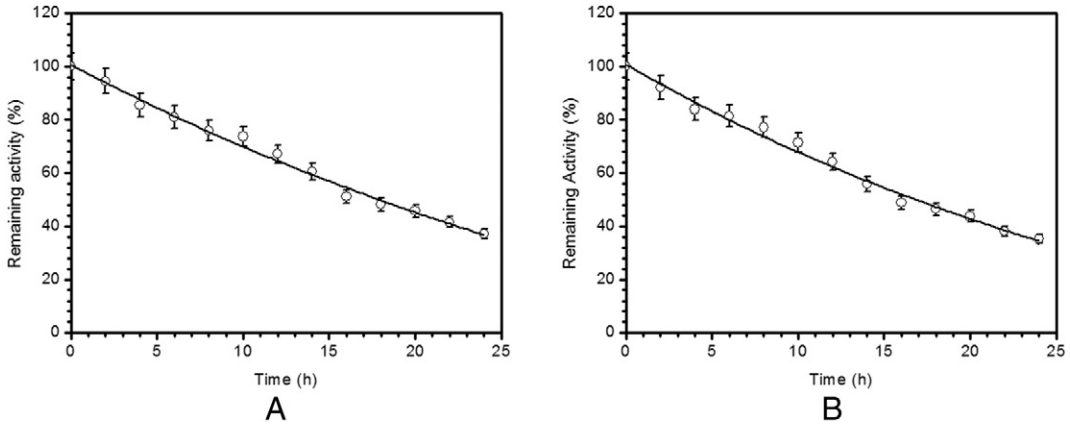

Fig. 4. Depuration kinetics of ^{137}Cs in the *Chanos chanos* (A. 2.46 g and B. 8.32 g) after a 2-h feeding on radiolabeled *Artemia* sp. for ^{137}Cs followed by 24-h of depuration (n = 6).

was mainly distributed in the head, gills, muscle and skeletal bones, skin and fins, and viscera, among which the gills presented the lower average load (8.27%) (Fig. 2 and Table 2).

Species within a taxonomic group but with different environmental habitat can exhibit widely varying Cs accumulation and depuration rates. An important ecological factor is the trophic level of the fish (piscivores bioaccumulate more than planktivores and benthivores) (Rowan and Rasmussen, 1994). In this experiment, *C. chanos* are generally fed algae and invertebrates and have a CF between 3.98 and 10.66, which is similar to other fish species (Table 3). In addition to the trophic level, different experimental conditions (e.g., food composition, labeling techniques, depuration conditions, and duration of radiolabeling for the fish) may partly explain the discrepancies observed among studies.

The body size of the fish is also found to have an influence on the accumulated contaminants. It has been reported that the accumulation of trace element is size-dependent for both freshwater and seawater fish (Smith et al., 2002a, b, Strong and Luoma, 1981). Smaller fish generally have a faster rate of uptake and accumulate higher concentration of contaminants than larger fish (Smith et al., 2002a, b). Physiological differences in their metabolic demand are the primary cause for the different contaminant uptakes among fish, although size-dependent surface-to-volume ratio, feeding behavior, and concentrations of enzymes that influence accumulation may also play some roles in these differences (Bruner et al. 1994). Because of the possibility of body size influencing the accumulation of Cs, the effects of body size on milkfish CF were examined (Fig. 5). The value of CF in *C. chanos* increased with a decrease in body size (Fig. 5). A comparison of biokinetic parameters between the four different fish body size groups indicated that the bioaccumulation of ^{137}Cs was the highest in the smallest fish (2.46 g), because of its significantly higher uptake rate than others. The uptake and depuration rates also indicated a similar pattern for CFss.

In general, cesium occurs in very low concentrations in open ocean waters. In addition, ^{137}Cs concentration in the surface seawater of the Pacific Ocean was 1–2 Bq m^{-3} before the Fukushima nuclear power plant (FNPP1) accident, which is incorporated with the Fukushima-derived ^{137}Cs (Bailly du Bois et al., 2011; Kumamoto et al., 2013). The fate and cycling of radioactive isotopes in the ecosystem have long been a subject of vital interest to ecologists and health physicists (Davis and Foster, 1958). Although the issue of radiocesium bioaccumulation is of major ecological and radiological importance, its analysis is impeded by the fact that most of the monitoring data have been collected only for calculating site-specific doses used by local regulatory agencies (Baptist and Price, 1962). Suseno and Prihatiningsih (2014) have described statues monitoring ^{137}Cs at marine coasts in Indonesia between 2011 and 2013. These monitoring activities were conducted not only in seawater and sediment, but also in marine biota. Although Cs itself is not biologically essential, the pathways that cycle this element within the ecosystem and the processes that lead to its bioconcentration are the fundamental physiological processes that dominate ecosystem functions (Baptist and Price, 1962).

Environmental conditions, such as salinity and temperature, have been widely demonstrated of their possibility of influencing the accumulation and depuration rates in fish (e.g., Zumholz et al., 2007; Topcuoglu, 2001; Nakahara et al., 1977). It also appears that concentration and composition of food may have an impact on the accumulation, assimilation, tissue distribution, and retention of radionuclides in mussels (Wang and Fisher, 1999). This study helps determine the uptake, retention, and assimilation efficiency of *C. chanos*, which were placed, on purpose, under specific temperature, salinity, and feeding regime conditions. These parameters have been chosen to be the most representative ones encountered on the field. Barman et al. (2012) showed that cesium accumulation rates of *C. chanos* decreased with the increase of salinity. As Tanjung Pasir (the collection site of *C. chanos* in this study) is located in estuarine waters, where salinity varies very much, cesium uptake rates in *C. chanos* may also change accordingly. The discrepancies observed between *C. chanos* parameters and the ones of other fish species might be strengthened by these changing conditions. Further studies are required to test the effects of seasonal variations and salinity changes on the accumulation of cesium in this Indonesian species.

4. Conclusion

At present, Indonesia has a research power reactor, and is planning to establish another reactor at Jakarta Bay. There is a possible risk of *C. chanos* from the aquaculture land in Jakarta Bay accumulating ^{137}Cs released from such a power reactor. Moreover, demand for the milkfish in Indonesia has been increasing every year. Therefore, bioaccumulation research of ^{137}Cs is required to assess the uptake and depuration kinetics of *C. chanos* from Jakarta Bay that were exposed to ^{137}Cs through seawater and food. In addition, investigations on the body distribution of ^{137}Cs in the milkfish at the end of the bioaccumulation experiment are necessary. The uptake of ^{137}Cs by *C. chanos* via seawater displayed a simple exponential kinetic model suggesting that the CFs of ^{137}Cs in the milkfish (weight 2.46–9.86 g) at a steady-state period were between 10.66 and 3.98 mL g^{-1} after 10 days of exposure. The depuration rate was observed to be low, with only 22.80–49.14% of ^{137}Cs absorbed by

Table 3
Cesium concentration factor (CF) in fish.

Biota	CFss (seawater exposure)	Ref
Psetta maxima	2.5	Jeffree et al. (2006)
Micropogon undulatus	11	Baptist and Price (1962)
Lutjanus argentimaculatus	10	Zhao et al. (2001)
Epinephelus sp.	3	Guimaraes (1992)
Scyliorhinus canicula	0.5	Jeffree et al. (2006)
Chanos chanos	3.98–10.66	Present study

the whole body of *C. chanos*, which was absent 6 days after exposure. By contrast, depuration occurred quickly for radiolabeled food uptake, reaching 20% of retention within 10 days after exposure. Muscles and viscera of the milkfish exhibited the highest degree of end uptake and end depuration of ^{137}Cs from seawater and feeding. ^{137}Cs ingested with food (*Artemia sp.*) was efficiently assimilated (35–37%) and weakly retained in milkfish tissues (Tb½ = 0.97–1.51 days).

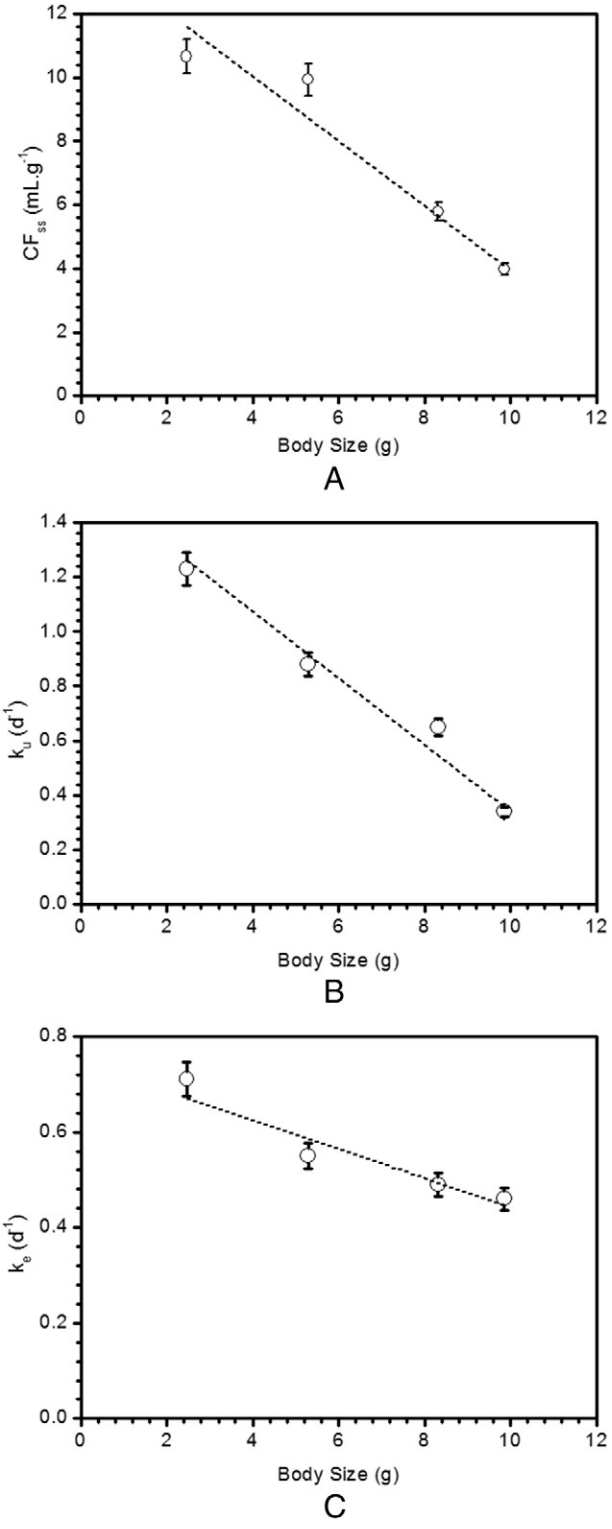

Fig. 5. Measured internal concentration factor in steady state period; CFSS(A), uptake rate; ku(B) and depuration rate; (C) of ^{137}Cs as function of time for different body sizes in *Chanos chanos* determined after 10 days of exposure.

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