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Reference organisms for assessing the impact of ionizing radiation on the environment of the southern Baltic Sea

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Abstract

Assessing the impact of ionizing radiation on the marine environment requires a well–defined methodology, which includes, among other elements, the analysis of exposure and effects. One of the most important components of the assessment system is the choice of reference organisms specific for the assessed area that fulfill requirements such as radioecological sensitivity, widespread distribution, and amenability to research and monitoring. The following species specific to the southern Baltic Sea that represent diversified ecological niches were proposed as reference organisms. *Polysiphonia fucoides* was proposed as a representative of macroalgae. Pelagic and benthic fauna were represented by *Crangon crangon* (crustacean), *Saduria entomon* (crustacean), *Hediste diversicolor* (polychaete), and *Mytilus trossulus* (mollusc). Fish were represented by *Clupea harengus* (pelagic planctotrophic fish), *Gadus morhua* (pelagic carnivorous fish), and *Platichthys flesus* (benthic fish). Activity concentrations of ^{137}Cs were determined in reference biota as well as in seawater, as required for the total dose-rate evaluation, and relevant concentration factors were calculated.

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INTRODUCTION

Development of a system methodology for assessing the impact of ionizing radiation on marine organisms is one of the key issues in the assessment of the state of the environment of the Baltic Sea. Until now, preventive measures addressed assessment of the effects of ionizing radiation on humans in order to provide effective forms of protection. However, the assumption that by protecting humans from the effects of ionizing radiation, the environment is automatically protected (ICRP 1991; Brown et al. 2006) is no longer valid. Legal documents, regarding the improvement of the state of the marine environment, that are coming into force at present, focus on selecting appropriate parameters and setting target values for the assessment of the status of the environment. One example of these acts is the Marine Strategy Framework Directive, which states that concentrations of contaminants, including radionuclides, in the marine environment should be at levels that do not give rise to pollution effects. Additionally, the requirements set for the environmental status are more restrictive than in the case of e.g. seafood.

The system of environmental impact assessment in the case of radioactive substances is comprised of a number of elements: planning, problem formulation, assessment, and risk characterization (Jonas, Gilek 2004; Brown et al. 2006). Assessment is the most important module because the assessment results determine what corrective measures are taken. The assessment includes exposure analysis as well as effects analysis (Jonas, Gilek 2004). Exposure analysis refers to the process of estimating the

intensity, frequency, and duration of exposure to radionuclides currently present in the environment or of estimating hypothetical exposure that might arise from accidental releases (Brown et al. 2004).

Many methodologies and models have been proposed for exposure assessment (Kumblad et al. 2006, Brown et al. 2006, Jones et al. 2003, Lepicard et al. 2004).

The most critical step in conducting an assessment using any model is the choice of reference organisms (Burger et al. 2006). Radionuclide concentrations in the selected organisms allow one to determine the internal dose, while the concentrations measured in the abiotic environment allow one to determine the external dose. The sum of those two values provides information on the total dose rate of the given radionuclide affecting the organism and the final biological effect is related to the magnitude of the adsorbed dose.

According to Brown et al. (2006), the reference organisms have to fulfill certain requirements:

- be typical or representative for the assessed area,
- represent all ecological niches or all trophic levels in the ecosystem as far as possible,
- possess considerable radioecological sensitivity: bioaccumulation efficiency, time response to pollution, reflect trends in the environment (long-term or seasonal),
- be common and widely distributed,
- be amenable to research and monitoring.

The main purpose of this paper was to collect information on ^{137}Cs activity concentrations in fauna and flora specimens representing species specific to the southern Baltic, which could serve as reference organisms for assessing the impact of ionizing radiation on the marine environment.

The radioactive isotope of cesium - ^{137}Cs - was selected as the radionuclide of interest because it is a nuclide of solely anthropogenic origin with the relatively long half-life of 30.05 years (Bé et al. 2008). The Baltic Sea is still considered the marine area most polluted with ^{137}Cs (IAEA 2005) and cesium activities in marine matrices in the Baltic Sea are much higher than those of other artificial isotopes (HELCOM 1995, 2003, 2009; Zalewska, Lipska 2006, Ikaheimonen et al. 2009). The major part of ^{137}Cs in the Baltic Sea were deposited as a result of the accident at the Chernobyl nuclear power plant in 1986. It is estimated that the overall load of ^{137}Cs that reached the sea from this source was 4.7 TBq, comprising 82% of the total amount of ^{137}Cs in Baltic seawater (Nielsen et al. 1999; HELCOM 2003).

Materials And Methods

Sampling and sample preparation

Detailed information on sampling site locations, sampling dates, and the characteristics of individual samples is presented in Table 1 and Fig. 1.

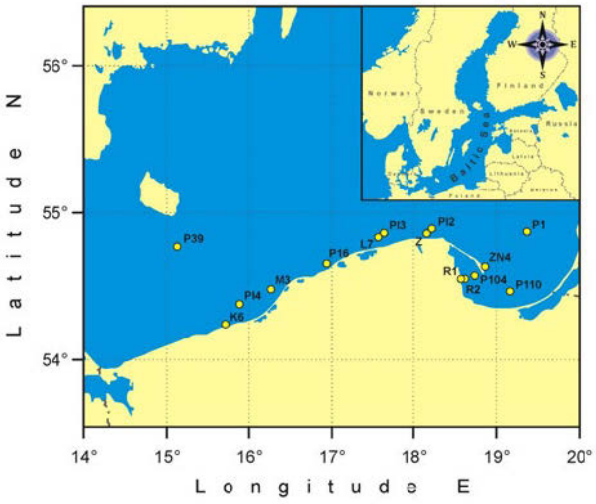

Fig. 1. Sampling locations.

Macrophytes

Macrophytobenthos sampling was carried out in the coastal zone of the Gulf of Gdańsk, in the vicinity of Redłowski Cliff (R1) (Table 1, Fig. 1) in 2009. The plants were collected at a depth of 2 m by a scuba diver, from a sea floor area enclosed in a metal frame of 0.5 × 0.5 m dimensions. *Polysiphonia fucoides* was separated out and its wet weight was determined. In order to determine the amount of radionuclide activity, the plant sample was dried, its dry weight was determined, and then it was ashed at 450°C and homogenized. The activity of ^{137}Cs in benthic plants was measured using the gamma spectrometry method (see section 2.2 Gamma spectrometry analysis).

Benthic macroinvertebrates

The benthic organisms *Mytilus trossulus*, *Saduria entomon*, and *Crangon crangon* were collected using a bottom dredge at different locations (Table 1, Fig. 1). Collected samples were immediately frozen onboard

Table 1

Dates, sampling stations, and characteristics of faunal and floral organisms sampled.

Date	Sampling station	Latitude	Longitude		Number of individuals	Mean length (cm)	Wet Weight (g)
Fish							
13.02.2009	Pl4	54,3333	15,5000	*Platichthys flesus*	5	28	510
				Gadus morhua	4	37	670
				Clupea harengus	15	24	510
14.02.2009	Pl4	54,3333	15,5000	*Platichthys flesus*	5	26	500
				Gadus morhua	4	37	595
				Clupea harengus	16	20	500
19.02.2009	Pl3	54,8333	17,5000	*Clupea harengus*	20	22	500
20.02.2009	Pl2	54,9167	18,1667	*Platichthys flesus*	5	29	535
				Gadus morhua	4	34	550
20.02.2009	Pl3	54,8333	17,5000	*Platichthys flesus*	3	24	230
				Gadus morhua	4	37	570
21.02.2009	Pl4	54,3333	15,5000	*Platichthys flesus*	5	28	600
21.02.2009	Pl2	54,9167	18,1667	*Gadus morhua*	4	36	820
				Clupea harengus	20	20	520
22.02.2009	Pl2	54,9167	18,1667	*Clupea harengus*	16	23	510
Mollusc							
09.09.2010	R2	54,4683	18,6433	*Mytilus trssulus*	182	3.95	121.5
Crustacean							
03.04.2001	Pl4	54,3333	15,5000	*Saduria entomon*	47	-	115.0
11.06.2001	ZN4	54,6667	18,8333		20	-	65.2
10.06.2001	M3	54,4500	15,9833		118	-	52.2
08.06.2001	P39	54,7333	15,1333		53	-	39.0
10.06.2001	M3	54,4500	15,9833	*Crangon crangon*	49	-	106.0
Polychaets							
9.06.2010	K6	54,1540	15,3200	*Hediste diversicolor*	-	-	11.3
10.06.2010	L7	54,5000	17,3210				
10.06.2010	Z	54,8750	18,0833				
10.06.2010	P16	54,3800	16,4800				
14.06.2010	P104	54,5817	18,7900				
Macroalgaea							
8.05.2009	R1	54,4849	18,5721	*Polysiphonia fucoides*	-	-	34.3

the ship without any additional treatment and were transported to the laboratory. The soft tissue of the mussels was separated, freeze-dried, ashed at 450°C, and homogenized. In the cases of *Saduria entomon* and *Crangon crangon*, the whole body of the animal was used for the analysis. Frozen samples were freeze-dried, ashed at 450°C, and homogenized. Ashed samples were immersed into the counting boxes of appropriate shape and size for gamma spectrometry analysis.

Hediste diversicolor was sampled using a 0.1 m² van Veen grab. Collected organisms were transported to the laboratory in formalin solution. Wet biomass was determined gravimetrically. Then the sample was dried, dry weight was determined, the sample was ashed at 450°C, homogenized, and immersed into the counting boxes of appropriate shape and size for gamma spectrometry analysis.

Fish

Fish samples were collected in 2009 at three locations: Pl 2, Pl 3, and Pl 4 (Table 1, Fig. 1). Three species were chosen for analysis: *Gadus morhua*, *Clupenga harengus*, and *Platichthys flesus*. Fish muscle tissue was separated, dried, ashed at 450°C, and ^{137}Cs was determined using gamma spectrometry analysis.

Seawater

^{137}Cs concentration in seawater is routinely analyzed within the HELCOM MORS program. Seawater samples were taken in the southern Baltic Sea region at stations K6, ZN4, M3, and P39 in 2001, at K6, Ł7, and P110 in 2009, and at P1, K6, B13, Ł7, and P16 in 2010 (Fig. 1). ^{137}Cs activity concentration in seawater samples was measured using two methods. In 2001, the radiochemical method was applied and preparation of samples followed the procedure described in Zalewska & Lipska (2006). In 2009 and 2010, ^{137}Cs activity was determined by gamma spectrometry. In this method, as an initial step, 20 mg Cs^+ was added to acidified seawater samples as a carrier. Cesium was absorbed on 10 g of ammonium phosphomolybdate (AMP) during 20 min. steering. Then the AMP was separated and dried and the radionuclide activity was measured using gamma spectrometry.

Gamma-spectrometric analysis

Two gamma spectrometry instruments were used in ^{137}Cs analysis. Determinations in *P. fucoides*, *M. trossulus*, *H. diversicolor*, and in seawater samples were carried out using an HPGe detector with a relative

efficiency of 18% and a resolution of 1.8 keV for a peak of 1332 keV of ^{60}Co in the laboratory of the Institute of Meteorology and Water Management in Gdynia. The detector was coupled with an 8192-channel computer analyser. Concentrations of ^{137}Cs in *S. entomon*, *C. crangon*, and in fish samples were determined in the Central Laboratory for Radiological Protection in Warsaw using gamma spectrometry with an HPGe detector with an energy resolution of 1.8 keV for ^{60}Co (1332 keV) and a relative efficiency of 30% coupled with the multichannel analyser Canberra MULTIPORT II MCA with GENIE-2000.

The reliability and accuracy of the measurements were verified by the participation of both laboratories in the HELCOM–MORS Proficiency Test Determination of Radionuclides in Fish Flesh Samples organized by IAEA-MEL Monaco (IAEA-414, Irish and North Sea Fish). Fish flesh is recognized as a substitute for ashed biological samples, since they are of nearly the same density as the prepared samples. The results of ^{137}Cs and ^{40}K determination, as well as information on accuracy and precision, are presented in Table 2 (IAEA, 2010).

RESULTS AND DISCUSSION

The key element in assessing the impact of exposure of marine flora and fauna to ionizing radiation is the selection of specific species that fulfill the requirements of reference organisms. The reference organisms - representatives of the marine flora and fauna - were selected based on long-term studies in the southern Baltic Sea carried out within monitoring and scientific investigations. All species recommended in this paper are typical and representative of the southern Baltic Sea region. A red alga, *P. fucoides,* was selected as the representative of benthic macrophytes (Zalewska, Saniewski 2011a, b; Zalewska 2011). The mussel *M. trossulus*, crustacean *S. entomon*, polychaete *H. diversicolor*, and fish *P. flesus* were selected as representatives of benthic fauna. Pelagic fauna representatives included the planktonic crustacean *C. crangon*, the pelagic planktotrophic fish *C. harengus*, and the planktonic carnivorous fish *G. morhua* (Table 3). The selected species represent all the ecological niches and, as far as possible, all trophic levels (Table 3). The choice of reference organisms excluded e.g. benthic bacteria and phytoplankton, since Brown et al. (2006) pointed out that these have minor importance in the assessment of ionizing radiation impact on the marine environment.

The second step of the assessment methodology involved determining activity concentrations of the radionuclide of interest in the reference organisms and in the surrounding environment, since it is the source of the radioisotopes. These results can then be used to calculate concentration factors, which are defined as the ratio of the radionuclide concentration in the organism to the concentration of this radionuclide in seawater, the direct source of the radionuclide:

$$CF = \frac{C_{RO}}{C_{SW}}$$

where CF is the concentration factor for the reference organism expressed in $dm^3\ kg^{-1}$; C_{RO} is the activity concentration of radionuclide in the whole body of the reference biota (Bq kg^{-1} wet weight); and C_{SW} is the activity concentration of the radionuclide in seawater (Bq dm^{-3}).

If the topical data on radionuclide concentrations is unavailable, the concentration factors and the respective radionuclide concentrations in seawater could be used to calculate the needed concentrations in reference organisms. The content of the

Table 2

Results of the HELCOM-MORS Proficiency Test Determination of Radionuclides in Fish Flesh (IAEA 414 – certified reference material) – Laboratory N°4 - Institute of Meteorology and Water Management, Maritime Branch, Gdynia, Poland, Laboratory N°5, Central Laboratory for Radiological Protection, Warsaw, Poland, Reference Date: 01 January 1997, after IAEA (2010).

Laboratory	Analyte	IAEA Value (Bq kg^{-1} d.w.)	IAEA Unc. (Bq kg^{-1} d.w.)	Lab. Value (Bq kg^{-1} d.w.)	Lab. Unc. (Bq kg^{-1} d.w.)	Lab. Unc. (%)	Rel. Bias (%)	Trueness	Precision (%)	Precision	Final Score
N°4	^{40}K	481	16	474.5	19.3	4.1	1.35	Passed	5.2	Passed	Acceptable
	^{137}Cs	5.18	0.10	5.06	0.64	12.6	2.32	Passed	12.8	Passed	Acceptable
N°5	^{40}K	481	16	517	14	2.8	-7.59	Passed	18.14	Passed	Acceptable
	^{137}Cs	5.18	0.10	5.15	0.20	3.9	0.58	Passed	8.17	Passed	Acceptable

Table 3

^{137}Cs concentrations in faunal and floral organisms and in seawater, as well as calculated concentration factors.

Ecological niche	Reference organisms	Representative species	Date	^{137}Cs in biota (Bq kg^{-1} w.w.)	^{137}Cs in seawater (Bq m^{-3})	Concentration factor (dm^3 kg^{-1} w.w.)
Benthic flora	Benthic macrophytes	*Polysiphonia fucoides*	8.05.2009	2.19 ±0.27	48,1 ±0.9	46 ±6
Benthic fauna	Benthic mollusc	*Mytilus trossulus*	9.09.2010	0.75 ±0.08	39,8 ±1.0	19 ±2
	Benthic crustacean	*Saduria entomon*	3.04.2001	2.55 ±0.09	63,4 ±1.4	40 ±2
			11.06.2001	3.22 ±0.14	57,7 ±2.4	56 ±3
			10.06.2001	3.05 ±0.15	63,4 ±1.4	48 ±3
			8.06.2001	3.69 ±0.20	64,5 ±3.5	57 ±4
			Mean value	3.13 ±0.15	62,3 ±2.2	50 ±3
	Benthic fish	*Platichtys flesus*	13.02.2009	5.02 ±0.08	37,7 ±0.9	133 ±4
			14.02.2009	5.87 ±0.07	37,7 ±0.9	156 ±4
			20.02.2009	4.80 ±0.09	39,2 ±0.9	122 ±4
			20.02.2009	5.53 ±0.10	39,2 ±0.9	141 ±4
			21.02.2009	6.38 ±0.08	37,7 ±0.9	169 ±5
			Mean value	5.52 ±0.08	38,3 ±0.9	144 ±4
	Polychaetes	*Hediste diversicolor*	9-14.06.2010	2.00 ±0.68	37,7 ±1.4	53 ±18
Pelagic fauna	Pelagic crustacean	*Crangon crangon*	10.06.2001	4.63 ±0.20	63,4 ±1.4	73 ±4
	Pelagic planctotrophic fish	*Clupea harengus*	13.02.2009	3.87 ±0.07	37,7 ±0.9	103 ±3
			14.02.2009	5.27 ±0.08	37,7 ±0.9	140 ±4
			19.02.2009	5.02 ±0.07	39,2 ±0.9	128 ±3
			21.02.2009	5.13 ±0.06	39,2 ±0.9	131 ±3
			22.02.2009	5.67 ±0.10	39,2 ±0.9	145 ±4
			Mean value	4.99 ±0.08	38,6 ±0.9	129 ±4
	Pelagic carnivorous fish	*Gadus morhua*	13.02.2009	5.92 ±0.11	37,7 ±0.9	157 ±5
			14.02.2009	4.98 ±0.08	37,7 ±0.9	132 ±4
			20.02.2009	4.97 ±0.09	39,2 ±0.9	127 ±4
			20.02.2009	6.73 ±0.11	39,2 ±0.9	172 ±5
			21.02.2009	6.52 ±0.07	39,2 ±0.9	166 ±4
			Mean value	5.82 ±0.09	38,6 ±0.9	151 ±4

radionuclide in organisms is the information necessary to assess the status of the marine environment and the impact of ionizing radiation on marine organisms, as well as to assess the situation in the case of accidental or episodic events.

The selected organisms show considerable differences in radioecological sensitivity, resulting mainly from their morphological and physiological differences and from their life histories. Specific organisms are chosen as reference organisms primarily because of their ability to accumulate radionuclides, which is expressed as bioaccumulation efficiency. Concentration factors can be a measure of bioaccumulation efficiency.

The results of ^{137}Cs concentrations measured (on a wet weight basis) in the reference organisms are presented in Table 3 and Fig. 2A. The corresponding concentrations of ^{137}Cs in seawater, collected simultaneously and in close physical proximity to the flora and fauna sampling sites, are given in Table 3. Calculated values of concentration factors are also presented in Table 3 and Fig. 2B.

The maximal concentration factor, 151 ±4 dm^3 kg^{-1}, was determined for cod (Fig. 2B). Slightly lower values were found in herring, 144 ±4 dm^3 kg^{-1}, and flounder, 129 ±4 dm^3 kg^{-1}. The following organisms are listed in decreasing order of *CF*: *C. crangon*, *H. diversicolor*, and *S. entomon*. The minimal *CF* value was determined for *M. trossulus*, the organism commonly considered as the standard bioindicator species of all

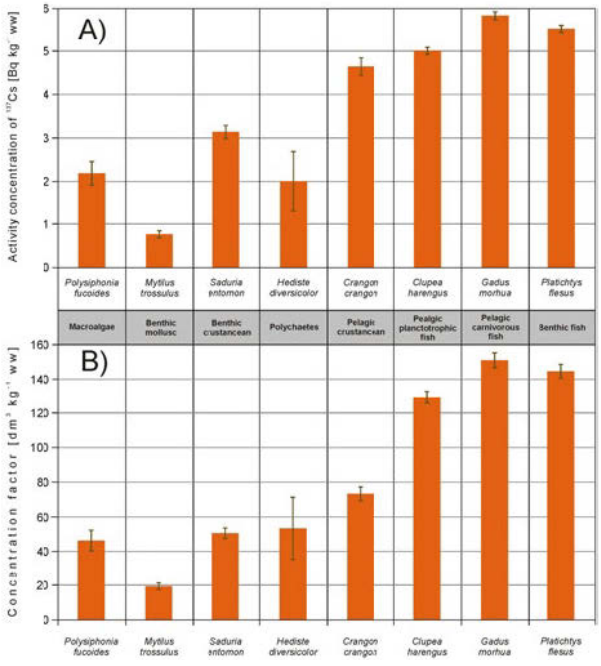

Fig. 2. Activity concentrations of ^{137}Cs (A) and concentration factors (B) in reference organisms.

types of pollutants in the marine environment (Fig. 2B).

The differences in ^{137}Cs concentrations observed in the analyzed species (Fig. 2A) are largely consistent with the distribution of *CF* values. The crustaceans *C. crangon* and *S. entomon* present an outstanding

exception, since the concentrations of ^{137}Cs in these animals were higher than the corresponding CFs. This is probably indicative of a lower bioaccumulation efficiency.

As already mentioned, the bioaccumulation ability is closely related to the morphology and physiology of an organism, hence also to its means of sustenance. The fact that *G. morhua* was characterized by the highest *CF* results mainly from its final position in the food web, which results in the biomagnification of radionuclides. Besides, the higher concentration factors determined for fish can be directly related to the prolonged bioaccumulation process, which extends over their entire lifespan, i.e. at least several years, as is common for other marine fauna species.

Benthic plants are entirely different, because the bioaccumulation period in benthic plants comprises only the particular seasons of the year when the vegetation is actively growing. Taking into account this short bioaccumulation period, the *CF* obtained for *P. fucoides* is unexpectedly high. Analyses of seasonal changes in ^{137}Cs concentrations in selected macrophytes, conducted in our laboratory, showed that seasonality significantly influenced bioaccumulation efficiency, especially during the vegetation period (Zalewska 2012a, 2012b).

The bioaccumulation process is directly related to an organism's response time to pollution. A quick reponse time can be helpful in reference organisms. In the case of most benthic plants, the response time is very short. That is why these organisms should be considered the best bioindicators of radioisotope concentration changes on a short time scale. Fish, on the other hand, seem to be the perfect organisms to monitor long-term changes in the environment (Fig. 3). The mean concentrations of ^{137}Cs in fish from the Polish sector of the Baltic Sea increased significantly after the Chernobyl accident (1986). Since 1990, a continuous decreasing trend in the isotope content has been observed, reflecting its declining concentrations in seawater.

However, it must be stressed that the response time to pollution depends to a considerable extent on the magnitude of the pollution signal. Significant concentrations of ^{137}Cs in fish measured shortly after the Fukushima break down (2011) are a good example of this issue (unpublished results).

All the species examined in this study are specific to the southern Baltic Sea area, common, and widely distributed. Certain organisms also fulfill the requirements of a good bioindicator in that they are

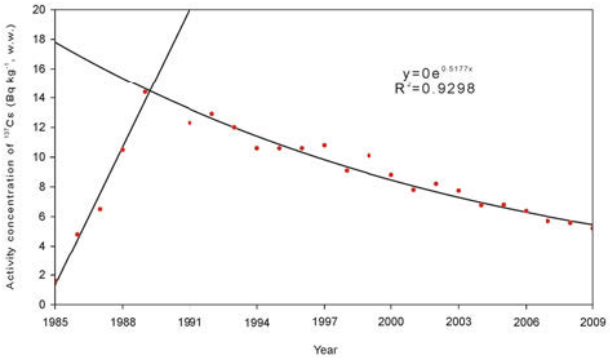

Fig. 3. Changes in average ^{137}Cs activity in fish from 1985 to 2009.

amenable to research and monitoring. They are already being used to monitor radioactive substances in the marine environment as part of the HELCOM MORS PRO programme, which is conducted in the Polish sector of the Baltic Sea as part of the National Environmental Monitoring Programme (IMGW 2004-2006).

CONCLUSIONS

- All the selected species of flora and fauna comply with the requirements characterizing reference organisms and are recommended for assessing the influence of ionizing radiation on marine organisms of the southern Baltic Sea.
- The concentrations of ^{137}Cs determined in the examined organisms and in seawater were used to establish concentration factors, which can be further applied to assess the impact of radiation on marine fauna and flora, especially in the case of an accident.
- Organisms from the higher trophic levels revealed significantly higher concentration factors, because of both the biomagnification of isotopes and the prolonged bioaccumulation period, extending e.g. in the case of fish to a number of years.

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