

1 Disproportional distribution of radioactive elements in the marine ecosystems surrounding  
2 the accident site of the Fukushima Daiichi Nuclear Power Plant  
3  
4 Shigeharu Moriya<sup>1, 3, \*</sup>, Hideaki Otsu<sup>2</sup>, Kumiko Kihara<sup>1, 4</sup>, Yukari Kato<sup>1</sup>, Misao Itouga<sup>1</sup>, Kenji  
5 Sakata<sup>1</sup>, Jun Kikuchi<sup>1, 3</sup>, Hiroshi Yamakawa<sup>5, 6</sup> and Jiro Suga<sup>6</sup>  
6  
7 1) RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-cho, Tsurumi-ku,  
8 Yokohama city, Kanagawa, 230-0045, JAPAN  
9 Shigeharu Moriya, Kumiko Kihara, Yukari Kato, Misao Itouga, Kenji Sakata and Jun  
10 Kikuchi  
11 2) RIKEN Nishina Center for Accelerator-Based Science, 2-1 Hirosamwa, Wako city,  
12 Saitama, 351-0198, JAPAN  
13 Hideaki Otsu  
14 3) Graduate School of Medical Life Science, Yokohama-city University, 1-7-29 Suehiro-cho,  
15 Tsurumi-ku, Yokohama city, Kanagawa, 230-0045, JAPAN  
16 Shigeharu Moriya and Jun Kikuchi

- 17 4) Department of Biological and Chemical Systems Engineering, National Institute of  
18 Technology, Kumamoto College, 2627 Hirayama-Shinmachi, Yatsushiro-shi, Kumamoto  
19 866-8501, JAPAN
- 20 Kumiko Kihara
- 21 5) Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo,  
22 108-8477, JAPAN
- 23 Hiroshi Yamakawa
- 24 6) Japan Academy of Underwater Sciences, 3-9-1-301 Botan, Koto-ku, Tokyo, 135-0046,  
25 JAPAN
- 26 Hiroshi Yamakawa and Jiro Suga
- 27
- 28 \*) Corresponding author: Shigeharu Moriya
- 29 RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-cho, Tsurumi-ku,  
30 Yokohama city, Kanagawa, 230-0045, JAPAN
- 31 Tel +81-45-508-7221, Fax +81-45-508-7363
- 32 [smoriya@riken.jp](mailto:smoriya@riken.jp)

33 Abstract

34 Background

35 After the Fukushima Daiichi Nuclear Power Plant accident, various surveys were performed  
36 to measure the extent of radioactive material contamination in the marine sediments,  
37 surface waters, plankton and fish. Six months after the event, relatively high radiocesium  
38 contamination could still be detected in the fish and sediment of the coastal demersal  
39 environment. To determine the distribution of radioactive material in the demersal  
40 ecosystem adjacent to the event, we sampled and analyzed the dominant macroorganisms  
41 attached to the seafloor as well as environmental material, including biofilms, from the  
42 coastal demersal environment around Hisanohama Port, which is less than 30 km south of  
43 the accident site.

44

45 Results

46 Our results showed that variable ratios of radiosilver/radiocesium occurred in the  
47 macrobenthos, macroalgae and sediment samples. However, the biofilm samples displayed  
48 high radiocesium contamination but did not show radiosilver contamination.

49

50 Conclusions

51 These findings suggest that several different entry paths are available for radioactive

52 elements to access the biological components of the ecosystem, and these paths may explain

53 the disproportional and patchy distribution of radioactive elements among marine

54 ecosystems.

55

56 Keywords

57 Radio isotope, Marine eco-system, Biofilm

58

59 Background

60 Following the Great East Japan Earthquake and catastrophic tsunami in March of 2011,

61 the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident led to the release of large

62 amounts of radioactive materials into the atmosphere and marine environment.

63 This environmental input of radioactive elements from the FDNPP introduced into the

64 marine waters a variety of radioisotopes, especially radiocesium isotopes. Public monitoring

65 showed that during the several months after the first explosion at the FDNPP, a rapid  
66 decrease in radioisotopes was observed in the nearby seawater. A joint official survey team  
67 from the Tokyo Power Electric Company (TEPCO), Fukushima prefectural government and  
68 the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT)  
69 reported that the  $^{137}\text{Cs}$  contamination level in the seawater quickly decreased from over 10 k  
70 Bq/liter to less than 100 Bq/liter within a 2-month period, whereas the  $^{137}\text{Cs}$  found in the  
71 ocean sediments was continuously observed over this period at levels between 100 Bq/dry kg  
72 and 1000 Bq/dry kg until at least September 2012 [1]. This disproportional distribution of  
73 radioisotopes was also observed in marine fish by Wada et al. Wada reported that the  
74 retention time of radiocesium appears to be remarkably longer in the larvae of demersal fish,  
75 e.g., *Sebastes cheni*, than in surface fish, e.g., *Eugraulis japonica* [2]. Similar disproportional  
76 distributions among radionuclides was also reported in a terrestrial ecosystem. Nakanishi  
77 et al. reported  $^{110\text{m}}\text{Ag}$  accumulation in the orb-web spider (*Nephila clavata*) and other  
78 arthropods [3]. Decay-corrected data from Nakanishi's work showed that the radioactivity  
79 ratio between  $^{110\text{m}}\text{Ag}$  and  $^{137}\text{Cs}$  ( $^{110\text{m}}\text{Ag}/^{137}\text{Cs}$ ) in specific samples of arthropods was over 1.0,  
80 whereas the same ratio ( $^{110\text{m}}\text{Ag}/^{137}\text{Cs}$ ) in the soil was 0.0014-0.0023, which indicates that

81 these organisms may actively accumulate radiosilver more than radiocesium. This finding is  
82 inconsistent with the majority of the organisms from land ecosystems, which accumulate  
83 more radiocesium than radiosilver.

84 These disproportional distributions may occur via partitioning in complex ecosystems and  
85 food webs through processes that include biological concentration. To better understand the  
86 fate of radioisotopes in nearshore marine environments, the present study sampled water,  
87 sediment, biofilms, macrobenthos and macroalgae as basic environmental materials and  
88 entry points of organic/inorganic materials into the ecosystem. In the present study,  
89 samples were collected using a self-contained underwater breathing apparatus (SCUBA) at  
90 a depth of 5-10 m along the nearshore area around Hisanohama Port. Sampling was  
91 essentially performed weekly from the end of November 2011 to the beginning of February  
92 2012 at 6 different points. Our results showed that macroalgae and macrobenthos  
93 accumulate and retain both radiocesium and radiosilver. The observed ratios of  
94 radiosilver/radiocesium are variable among the organisms, and they may be dependent on  
95 the specific usage and behavior of the organisms with respect to metals, such as specific  
96 cofactors, oxygen acceptors, metal uptake and other metabolic features. Although sediment

97 also retains both radiocesium and radiosilver, biofilms contain only radiocesium. The  
98 microbial community structure is likely related to the radiocesium accumulation and  
99 retention in microbes.

100

101 Materials and Methods

102 Sampling

103 Sampling was performed from 11 November 2011 to 4 February 2012. The sampling site  
104 was the ocean along the coastline of Hisanohama, Iwaki City, Fukushima, Japan. We  
105 collected samples 10 times within the established time period at 11 Nov (week 1), 20 Nov  
106 (week 2), 26 Nov (week 3), 3 Dec (week 4), 10 Dec (week 5), 17 Dec (week 6), 14 Jan (week 7),  
107 21 Jan (week 8), 29 Jan (week 9) and 4 Feb (week 10). Sampling was performed at 6  
108 different areas near Hisanohama Port: Area 1 (37.153762N 141.006167E, weeks 1 and 5),  
109 Area 2 (approximately 37.149863N 141.001017E, weeks 2, 8 and 9), Area 3 (approximately  
110 37.146579N 141.002390E, week 4), Area 3' (approximately 37.147503N 141.008570E, week  
111 10), Area 4 (approximately 37.133887N 141.002390E, weeks 3 and 7) and Area 4'  
112 (approximately 37.142953N 141.003592E, week 6). These sampling sites are located 30 km

113 south of the Fukushima Nuclear Power Plant #1. Sampling was performed by diving with a  
114 SCUBA unit. Divers sampled the bottom water and surface water (5 L) and collected bottom  
115 stones to obtain biofilms and locally observed macrobenthos and macroalgae.

116 Sediment core samples were collected at Area 2 using a piston corer prototype. The “core  
117 sampler” was composed of a 4.5 cm diameter/28 cm height cylinder and inscribed piston.

118 Core samples were collected during a separate dive in weeks 6 (core sample 1) and 7 (core  
119 samples 2 – 4).

120 Samplings were performed with permission from Fukushima Prefecture and the fishery  
121 association of Iwaki City.

122

123 Sample preparation

124 The seawater samples were immediately filtered with two 24-mm-wide 0.22  $\mu\text{m}$  Durapore  
125 filters (Millipore, MA, USA) sample until they clogged. All samples were frozen at  $-20^{\circ}\text{C}$  in a  
126 field freezer prior to transportation back to laboratory.

127 In the laboratory, the samples were dried at  $105^{\circ}\text{C}$  overnight except for the filters, water  
128 and sediment core samples. The dried samples were crushed by a food mill and settled into a



129 U8 container, and the weight and height were measured in the container. In the case of  
130 biofilms, half of the samples was dried as mentioned above and the other half was captured  
131 on 0.2  $\mu\text{m}$  pore size membrane filters and stored at  $-20^{\circ}\text{C}$  for ribosomal RNA gene analysis.

132 The water samples were dried by boiling. The remaining salts were settled into a U8  
133 container, and the weight and height were measured in the container.

134 Sediment core samples for radioactivity measurements were stored at  $-80^{\circ}\text{C}$ . The cores were  
135 sliced by a clean saw at 5 cm intervals from the surface side of the sediment to the bottom  
136 side. Each piece of the core sample was freeze dried and then settled into a U8 container,  
137 and the weight and height measured in the container.

138

139 Measurement of radioactivity

140 The gamma ray activity of the samples in the containers was measured by a Germanium  
141 (Ge) detector. The detector setup is identical to those described in [4]. The energy resolution  
142 of 2 keV full width at half maximum (FWHM) was achieved at 1332.5 keV gamma rays. The  
143 sample location and the detector were surrounded with 15 cm lead blocks to reduce the  
144 background radiation in the gamma ray spectroscopy. The detection efficiency of the gamma

145 rays was calibrated with an accuracy of 2 - 10% using a multiple gamma ray standard  
146 source, which ranged from 88 keV to 1836 keV. A calibrated  $^{134}\text{gCs}$  source was also used to  
147 correct for the coincidence summing for radioactivity determinations of  $^{134}\text{gCs}$ .

148 Every sample was contained in a U8 plastic container. The U8 container is a standard  
149 container in Japan that is used for measurements of absolute radioactivity with high  
150 accuracy by a Ge detector. The geometry of the container is cylindrical, with a diameter of 47  
151 mm and height of 60 mm. The volume is approximately 100 mL. The efficiency calibration  
152 source is available within the container with a uniform density in the container.

153 Every sample was measured by the Ge detector for at least 8 hours to obtain sufficient  
154 statistics for each gamma ray peak. The relative geometry between the sample and the Ge  
155 detector was carefully reproduced by a guide apparatus at the sample location.

156 Gamma rays from  $^{134,137}\text{Cs}$  and  $^{110\text{m}}\text{Ag}$  were observed for the majority of the samples. The  
157 radioactivity levels for every isotope were determined from the yield of the gamma rays  
158 corrected by the detection efficiency of the Ge detector and a geometrical acceptance  
159 between the sample and the detector. The geometry difference in the height of the sample  
160 from the calibration source was simulated by several electromagnetic simulation codes,

161 including Geant4 [5,6] and EGS5 [7].

162 The radioactivity of the marine animals, macroalgae and biofilm samples were normalized

163 by weight, and the radioactivity of the water samples and sediment core samples were

164 normalized by volume.

165

166 Element analysis

167 To quantitatively analyze the content of 37 elements ( $^7\text{Li}$ ,  $^9\text{Be}$ ,  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{27}\text{Al}$ ,  $^{39}\text{K}$ ,  $^{43}\text{Ca}$ ,

168  $^{45}\text{Sc}$ ,  $^{47}\text{Ti}$ ,  $^{51}\text{V}$ ,  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{57}\text{Fe}$ ,  $^{59}\text{Co}$ ,  $^{60}\text{Ni}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{85}\text{Rb}$ ,  $^{88}\text{Sr}$ ,  $^{98}\text{Mo}$ ,  $^{111}\text{Cd}$ ,  $^{133}\text{Cs}$ ,  $^{139}\text{La}$ ,  $^{140}\text{Ce}$ ,

169  $^{141}\text{Pr}$ ,  $^{146}\text{Nd}$ ,  $^{152}\text{Sm}$ ,  $^{153}\text{Eu}$ ,  $^{158}\text{Gd}$ ,  $^{159}\text{Tb}$ ,  $^{164}\text{Dy}$ ,  $^{165}\text{Ho}$ ,  $^{166}\text{Er}$ ,  $^{169}\text{Tm}$ ,  $^{172}\text{Yb}$ ,  $^{175}\text{Lu}$ , and  $^{202}\text{Hg}$ ) in

170 the samples (i.e., surface salts, surface microbes, biofilms), the samples were predigested

171 with 5 mL of concentrated  $\text{HNO}_3$  for 1 h at room temperature. Next, the organic components

172 were completely decomposed by wet-ashing using a microwave sample preparation system

173 (Multi-Wave-3000, Perkin Elmer, MA USA) [8]. The digested samples were brought up to a

174 volume of 50 mL with MilliQ water (MQW) and filtered through 5B filter paper (Advantec,

175 Tokyo, Japan). The concentrations of the mineral elements were determined by Inductively

176 Coupled Plasma Mass Spectrometry (ICP-MS, NexION300, Perkin Elmer). For the ICP-MS

177 analysis, a portion of the filtrated samples was diluted appropriately with 0.01 mol L<sup>-1</sup> HCl  
178 [8]. The mineral nutrient concentration in the samples was calculated as a unit (mg g<sup>-1</sup> in  
179 dry weight).

180 The obtained ICP-MS data were normalized by the root-sum-of-squares (RSS) levels among  
181 each element. Count data of radioactive cesium (<sup>134</sup>Cs and <sup>137</sup>Cs) were treated with the same  
182 calculation for normalization and then merged with the normalized ICP-MS dataset  
183 (elements table).

184

185 *ssrDNA* analysis

186 Genomic DNA was prepared from stored biofilm samples by bead-beating with a  
187 phenol:chloroform:isoamyl alcohol (PCI) extraction. Bead-beating was performed at 3000  
188 rpm for 5 min (TOMY, Tokyo Japan), and then nucleic acids were recovered by ethanol  
189 precipitation.

190 PCR amplification was performed with the extracted genomic DNA with two primer sets for  
191 16S *ssrDNA*, 515F/806R [9,10], and 18S *ssrDNA*, TAReuk454FWD1/TAReukREV3 [11]. The  
192 primers were modified for Illumina sequencing with a multi-index kit for the Nextera XT

193 sequencing library construction kit (Illumina, CA, USA). We added a “read1” sequence (5’-  
194 TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG -3’) to the forward primers and a  
195 “read1” sequence (5’- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G -3’) to the  
196 reverse primers.

197 PCR was performed with a standard reaction of ExTaq DNA polymerase according to the  
198 manufacturer’s instructions (Takara Bio, Kyoto Japan) with 25 pmol of each primer/50 µl  
199 reaction. The reactions were performed as a two-step PCR (10 cycles of 94°C for 30 s, 55°C  
200 for 45 s and 72°C for 60 s followed by 20 cycles of 94°C for 30 s and 72°C for 60 s) for 16S and  
201 3-step PCR (30 cycles of 94°C for 30 s, 55°C for 45 s and 72°C for 60 s) for 18S. The amplified  
202 products were purified by 1% agarose gel electrophoresis. The obtained amplified products  
203 were applied to the NexteraXT (Illumina CA USA) sequencing library construction process  
204 without the “tagmentation” process.

205 A multi-index sequencing library was applied to the MiSeq sequencing kit v1 and read by  
206 Illumina MiSeq according to the instructions in the paired-end sequencing mode. Data were  
207 treated by MiSeq reporter software, and the reads were obtained from individual samples.  
208 After the extraction of reads, reads from weeks 4, 5 and 8 for the 18S ssrDNA were

209 insufficient for analysis. Therefore, the following analysis was performed using reads from  
210 weeks 1, 2, 3, 6, 7, 9 and 10.

211 The obtained read data were mapped onto the ribosomal RNA sequence library SILVA  
212 release 108 by the program package QIIME [12] using “Prefix-suffix OTU picking”, and the  
213 absolute abundance OTU table at the family level was constructed. Count zero data were  
214 manually removed from the table, and the relative abundance values were calculated. 16S  
215 data and 18S data were treated independently and then merged into a single OTU table  
216 with the relative abundance values.

217 Sequence data have been deposited in the DDBJ sequence read archive with accession  
218 number DRA004367.

219

220 Statistical analysis

221 For the statistical analysis, we used the program package R [13]. First, we drew a heatmap  
222 chart with the hierarchical cluster analysis (HCA). The heatmap.3 function in the GMD  
223 package, which is a package for non-parametric distance measurements between two  
224 discrete frequency distributions [14], was used to draw the heatmap with the HCA.

225 Independent heatmaps were drawn from each element table and the OTU table with a  
226 two-axis HCA. A scaling option was used for the OTU table treatment according to the row  
227 direction but was not used for the elements table treatment.

228 A principal components analysis (PCA) was performed with the merged dataset, including  
229 both the out table and elements table, using the prcomp function in R. Score plots were  
230 drawn with principal component 1 (PC1) (x-axis) and PC2 (y-axis). The loading plots for  
231 quadrants II and III were manually drawn.

232 Spearman's rank correlation coefficient between the elements dataset and the OTUs dataset  
233 was calculated with the cor function in R. A correlation matrix was used to draw the  
234 heatmap chart with the HCA using the heatmap.3 function of R.

235

236 Results and Discussion

237 Heterogeneous distribution of artificial radioisotopes in the coastal marine environment  
238 around Hisanohama

239 According to our gamma ray energy spectra, we found significant peaks corresponding to  
240  $^{134}\text{Cs}$  and  $^{137}\text{Cs}$  in addition to natural radioisotope signals, such as  $^{40}\text{K}$  (data not shown), for

241 all samples with the exception of the seawater samples. We also detected significant  $^{110m}\text{Ag}$   
242 signals from the macrobenthos, macroalgae and sediment samples. Significant signals of the  
243 radioisotopes  $^{134}\text{Cs}$ ,  $^{137}\text{Cs}$  and  $^{110m}\text{Ag}$  were not detected from the control samples taken from  
244 Nishi-kawana inside Tokyo Bay, which is approximately 280 kilometers from the FDNPP.  
245 Although  $^{134}\text{Cs}$  (half-life of 2.0652 years) and  $^{137}\text{Cs}$  (half-life of 30.1 years) from prior nuclear  
246 weapons tests in the Pacific Ocean and the Chernobyl disaster might have persisted in the  
247 seabed around Japan, our analysis of samples collected near Nishi-kawana did not detect  
248 these signals. Therefore, we concluded that the radioisotopes detected in the samples in this  
249 study originated from the FDNPP disaster rather than the environmental background or  
250 past nuclear weapon tests.

251

252 As presented in Table 1, among the results from the radioactivity measurements of a variety  
253 of samples, we noticed a heterogeneous distribution between radiocesium ( $^{134}\text{Cs}$  and  $^{137}\text{Cs}$ )  
254 and radiosilver ( $^{110m}\text{Ag}$ ). The retention of radiocesium was observed in all sediments,  
255 macrobenthos, macroalgae and biofilm samples, whereas non-detectable signals of these  
256 isotopes were observed in the surficial and bottom water. This result is surprising because



257 the  $^{134}\text{Cs}$  radioactivity detected in the Pacific Ocean near the accident site was  $3900 \text{ Bq/m}^3$   
258 (3.9 Bq/liter) in June 2011, which is one of the highest levels ever recorded [15]. Although  
259 our results do not directly indicate that marine organisms and sediments are able to  
260 accumulate radioisotopes at the concentrations found in this environment, we must consider  
261 that they are able to retain radioisotopes for at least up to 9 months, which represents the  
262 time lapse between the disaster and sample collection.

263

264 Table 1. Peak detection in the samples

Material	Peak detection		
	$^{134}\text{Cs}$	$^{137}\text{Cs}$	$^{110\text{m}}\text{Ag}$
Surface water	–	–	–
Bottom water	–	–	–
Particles in surface water *	–	–	–
Particles in bottom water *	–	–	–
Sediments	++	++	++
Macrobenthos	++	++	+

Macroalgae	++	++	+
Biofilms on bottom rocks	++	++	-

265 ++: significant peak detected from all samples

266 +: significant peak detected from certain samples (not all samples)

267 -: significant peak not detected from any sample

268 \*: Particles were filtered from water using a 0.22  $\mu\text{m}$  filter, and the radioactivity was  
269 measured

270

271 Sediment core samples were obtained from the sampling point "Area 2" by the core sampler.

272 The analysis of these sediment core samples indicated the presence of both radiocesium and

273 radiosilver. The measured radioactivities are shown in Additional file 1: Supplementary

274 Table 1. To better understand the radioactivity in the same volume of space, we employ the

275 unit Bq/liter to express the radioactivity of the core samples because the core samples

276 contain different materials along their depth profile.

277 In Figure 1, the distribution of radioactivity along the sediment column is shown. Both

278 radiocesium and radiosilver are distributed similarly in the surficial part of the core

279 samples, which contains a large amount of radioactivity. The calculated regression line for  
280 the data showed that both radiocesium and radiosilver occur at depths above approximately  
281 155 mm. The similar distribution of radiocesium and radiosilver suggest that both  
282 radioisotopes start to precipitate at almost the same time and they share a similar  
283 precipitation pattern. The ratio of radioactivity between  $^{110m}\text{Ag}$  and  $^{137}\text{Cs}$  is 0.0046 on  
284 average, which is based on the detected radiosilver of each 5 cm slice of the core samples  
285 (Fig. 1b).

286

287 Figure 1

288 Radioactivity of the sliced sediment core. Panel a shows the results of  $^{134}\text{Cs}$ ,  $^{137}\text{Cs}$  and  $^{110m}\text{Ag}$   
289 measurements. Open circles indicate each sliced core sample. All 4 core samples were  
290 collected inside Hisanohama Port (Area 2). Radioactivity is expressed as Bq/liter material.  
291 The regression lines are calculated as linear approximation lines, and the  $R^2$  value is  
292 indicated beside each line. Panel b shows the  $^{110m}\text{Ag}/^{137}\text{Cs}$  ratios. Panel a shows the  
293 individual  $^{110m}\text{Ag}/^{137}\text{Cs}$  ratio of each core slice.

294

295 In the macrobenthos and macroalgae samples, we also detected radiosilver and radiocesium.

296 However, the ratio between  $^{110m}\text{Ag}$  and  $^{137}\text{Cs}$  varied among the samples.

297 As shown in Figure 2a and Additional file 2: Supplementary Table 2, macroalgae contained

298 a relatively low ratio of radiosilver relative to radiocesium. The ratio between  $^{110m}\text{Ag}$  and

299  $^{137}\text{Cs}$  averaged 0.06 to 0.07 among brown, red and calcareous algae (Fig. 2a); nevertheless, it

300 varied among individuals (Fig. 2b). Except for green algae, the ratio of  $^{110m}\text{Ag}/^{137}\text{Cs}$  of

301 macroalgae was approximately 10-fold larger than that of the surrounding sediments. Green

302 algae contained almost no  $^{110m}\text{Ag}$ .

303

304 Figure 2

305 Radioactivity of  $^{134}\text{Cs}$ ,  $^{137}\text{Cs}$  and  $^{110m}\text{Ag}$  in the macroalgae samples. Panel a shows the

306 average  $^{110m}\text{Ag}/^{137}\text{Cs}$  ratio in the macroalgae sample, and panel b shows the individual

307 results for the macroalgae. The measured radioactivity is expressed by the bar graph.

308 Closed bars show  $^{134}\text{Cs}$ , open bars show  $^{137}\text{Cs}$  and gray bars show  $^{110m}\text{Ag}$ . Radioactivity is

309 expressed as Bq/kg of dried sample (Bq/kg dry biomass).

310

311 Figure 3

312 Radioactivity of  $^{134}\text{Cs}$ ,  $^{137}\text{Cs}$  and  $^{110\text{m}}\text{Ag}$  in the macrobenthos samples. Panel a shows the  
313 average  $^{110\text{m}}\text{Ag}/^{137}\text{Cs}$  ratio in the macrobenthos sample, and panel b shows the individual  
314 results for the macrobenthos. The measured radioactivity is expressed by the bar graph.  
315 Closed bars represent  $^{134}\text{Cs}$ , open bars represent  $^{137}\text{Cs}$  and gray bars represent  $^{110\text{m}}\text{Ag}$ .  
316 Radioactivity is expressed as Bq/kg of the dry weight of the samples (Bq/kg dry biomass).  
317 The arrowhead indicates the relatively high concentration of radiosilver found in the sponge  
318 samples (i.e., the main body).

319

320 As shown in Figures 3a and 3b and Additional file 3: Supplementary Table 3, the  
321 macrobenthos samples showed a variable ratio between  $^{110\text{m}}\text{Ag}$  and  $^{137}\text{Cs}$ .

322 Our results indicated that sea snails (Buccinidae) and oysters (*Crassostrea gigas*)  
323 consistently presented a high ratio of  $^{110\text{m}}\text{Ag}/^{134}\text{Cs}$ , which was also observed for the sponge  
324 *Cliona chilensis*. Samples of the Ascidian *Halocynthia roretzi* showed a relatively high ratio  
325 of  $^{110\text{m}}\text{Ag}/^{134}\text{Cs}$  (Fig. 3a).

326 Certain mollusks, such as sea snails and oysters, are known to use hemocyanin as an oxygen

327 carrier [16]. A study on the relatively high ratio of  $^{110m}\text{Ag}/^{137}\text{Cs}$  in the spider, *Atypus karschi*  
328 indicated that the reaction center Cu in hemocyanin can be replaced by Ag, or  $^{110m}\text{Ag}$  in this  
329 case [3]. Similarly, the sampled mollusks may have actively incorporated  $^{110m}\text{Ag}$  from  
330 environmental water and taken up the radioisotope into the blood system. To corroborate  
331 this hypothesis, our results showed a higher ratio of radiosilver compared with that of  
332 radiocesium detected in the body ( $^{134}\text{Cs} = 21.7923$ ,  $^{137}\text{Cs} = 37.3885$ ,  $^{110m}\text{Ag} = 134.6388$  (Bq/kg  
333 dry biomass)) than in the shell ( $^{134}\text{Cs} = 12.6245$ ,  $^{137}\text{Cs} = 18.8071$ ,  $^{110m}\text{Ag} = 17.7947$  (Bq/kg dry  
334 biomass)).

335 A similar mechanism for accumulation and retention of the relatively high observed amount  
336 of radiosilver in *Halocynthia roretzi* is expected. Ascidians accumulate vanadium into their  
337 blood cells [17]. Although the function of vanadium accumulation is not yet fully understood  
338 and the mechanism of metal uptake in ascidians is still under investigation, these  
339 organisms may accumulate other metals using vanadium binding protein(s). Our samples of  
340 ascidians are from *Halocynthia roretzi*, and a high specificity for vanadium has not been  
341 reported in this species [18]. Our hypothesis is that a metal binding factor within the  
342 *Halocynthia roretzi* blood system with a low specificity for vanadium is able to promote

343  $^{110m}\text{Ag}$  accumulation. Although our hypothesis is based on indirect evidence, it is partially  
344 supported by the observation that the *Halocynthia roretzi* body (including the blood system)  
345 showed a larger amount of radiosilver than was found in the shell, which can contain  
346 radiosilver because of attached organisms on the surface of the shell. We investigated three  
347 individuals of *Halocynthia roretzi*, and the body measurement results are  $^{134}\text{Cs} = 645,0563,$   
348  $927.5638, 944.2312;$   $^{137}\text{Cs} = 997.5926, 1437.1948, 1588.1469;$   $^{110m}\text{Ag} = 295.6794, 255.9421,$   
349  $213.5846$  (Bq/kg dry biomass) and the shell measurement results are  $^{134}\text{Cs} = 18.6579,$   
350  $51.1889, 28.2958;$   $^{137}\text{Cs} = 30.3881, 80.5451, 43.8699;$   $^{110m}\text{Ag} =$  not detected,  $3.3797,$  not  
351 detected (Bq/kg dry biomass).

352 The sea urchin (*Anthocidaris crassispina*) samples and the majority of the sponge samples  
353 (*Cliona chilensis*) presented ratios of radiosilver/radiocesium that were lower relative to that  
354 of the other macrobenthos (Fig. 3a, b). Nevertheless, the feeding pattern of sea urchins and  
355 sea snails are similar because they are both deposit feeders, and the feeding patterns of  
356 sponges, oysters and Ascidians are also similar because they are all filter feeders.  
357 Interestingly, 4 of the 16 specimens of sponge presented remarkably high  
358 radiosilver/radiocesium ratios of approximately 0.5 (Fig. 3b, arrowheads). Variation were

359 not observed in the radiosilver/radiocesium ratio corresponding to the three color variations  
360 of the samples of *Cliona chilensis*. A previous report showed that certain sponge species  
361 exhibit a strong ability to incorporate a variety of metal elements, including silver [19].  
362 Indeed, sponges are known as "a sentinel organism" that can be used as a marker to monitor  
363 heavy metal pollution in water because they present a high capacity for specific metal  
364 accumulation [20]. According to Genta-Jouve et al., *Acanthella acuta*, which is a species of  
365 commonly found sponge, can incorporate  $^{110m}\text{Ag}$  from the surrounding water and retain the  
366 element even after moving to clean water that does not contain  $^{110m}\text{Ag}$  [19]; however, among  
367 the six sponge species analyzed in that study, this feature was only observed in *Acanthella*  
368 *acuta*. In our study, four specimens of sponge incorporated relatively high ratios of  
369 radiosilver relative to radiocesium (Fig. 3b arrowhead); therefore, we regarded these  
370 sponges as a "silver accumulator" species.

371 Thus, macroalgae and macrobenthic species present varying capacities to incorporate and  
372 retain radioisotopes based on their biological features. In addition, the  
373 radiosilver/radiocesium ratios of these organisms are higher than those in the sediment.

374 Although we detected both radiocesium and radiosilver from almost all biological samples in



375 this research, an interesting exception was observed because radiosilver was not detected in  
376 any of the biofilm samples, including biofilms collected from Area 2 (weeks 2, 8 and 9) (Table  
377 2). This result suggested that microbes that compose biofilms specifically accumulate and  
378 retain radiocesium but not radiosilver, even when the surface of nearby sediments contains  
379 both radioisotopes.

380

381 Table 2. Radioactivity of the biofilm samples

		<sup>134</sup> Cs Bq/kg dry	<sup>137</sup> Cs Bq/kg dry	<sup>110m</sup> Ag Bq/kg dry
Week1	Area1	1141.77	1655.17	nd
Week2	Area2	671.00	932.18	nd
Week3	Area4	306.90	429.21	nd
Week4	Area3	429.21	2353.58	nd
Week5	Area1	926.40	1436.73	nd
Week6	Area4'	538.56	829.08	nd
Week7	Area4	844.49	1543.05	nd
Week8	Area2	818.66	1607.65	nd

Week9	Area2	359.77	591.46	nd
Week10	Area3'	190.29	294.20	nd

382 nd: not detected

383

384 “Cesium-philic” biofilm

385 Microbial communities that grow on surfaces of solid materials in the presence of water are

386 known as biofilms. Our observations suggest that the biofilm community specifically

387 accumulates radiocesium. Therefore, we analyzed the relationship between the microbial

388 consortia within the biofilms and their elemental composition.

389 Quantified element data were combined with radioactivity data for radiocesium and utilized

390 for the statistical analysis. Ribosomal RNA genes were sequenced and used to construct a

391 dataset for the statistical analysis.

392 A HCA from the dataset of elements and radiocesium (Fig. 4) in which an accumulation of

393 radioactive cesium, Cr, Ni, Sc, Rb, Li and cesium (Cs cluster) was performed for the week 1

394 sample. Another remarkable profile from the elemental analysis is observed in week 3,

395 where high relative amounts of lanthanide elements were measured (Lanth cluster).

396

397 Figure 4

398 Heatmap chart of the element and radioactivity data from the biofilm samples based on the  
399 hierarchical clustering analysis (HCA). The heatmap chart was sorted by the HCA for both  
400 axes (elements axis and sample axis). Boxes indicate clusters of radiocesium (Cs cluster) or  
401 lanthanides (Lanth cluster).

402

403 A PCA score plot (Fig. 5a) of the merged dataset between the elements/radiocesium and the  
404 microbial consortia showed that the week 1 sample was placed in the PC1 negative – PC2  
405 positive direction and the week 3 sample was placed in the PC1 negative – PC2 negative  
406 direction. As mentioned above, the week 1 sample contained high relative amounts of the Cs  
407 cluster signals, and the week 3 sample contained high relative amounts of the Lanth cluster  
408 signals. Consistent with those findings, the PCA loading plot showed that the Cs cluster was  
409 placed in the PC1 negative – PC2 positive direction (Fig. 5b, Additional file 4:  
410 Supplementary Table 4), and the lanthanide cluster was placed in the PC1 negative – PC2  
411 negative direction (Fig. 5b, Additional file 5: Supplementary Table 5). The results of the

412 HCA and PCA analyses suggested that a “cesium-philic” biofilm occurred at the sampling  
413 point in week 1 and a “lanthanide-philic” biofilm occurred at the sampling point in week 3.

414

415 Figure 5

416 PCA results among the sampling sites with microbial consortia data and element data with  
417 radiocesium. Panel a is the score plot of the PCA among sampling sites. Each colored dot  
418 indicates each sampling site. The color code is superimposed on the PCA score plot. Panel b  
419 indicates the loading plot of the elements data. The upper panel shows the plot of elements  
420 that contribute to the PC1 minus and PC2 plus direction, indicating that they contribute to  
421 separate week 1 samples in the PC1 minus and PC2 plus direction. The lower panel shows  
422 the plot of elements that contribute to the PC1 minus and PC2 minus direction, indicating  
423 that they contribute to separate week 3 samples in the PC1 minus and PC2 minus direction  
424 in the PCA score plot. Panel c indicates the loading plot of the microbial consortia data. The  
425 upper panel displays the plot of OTUs that contribute to the PC1 minus and PC2 plus  
426 direction, indicating that they contribute to separate week 1 samples in the PCA score plot.  
427 The lower panel shows the plot of the OTUs that contribute to the PC1 minus and PC2

428 minus direction, indicating that they contribute to separate week 3 samples in the PCA  
429 score plot.

430

431 The PCA results from both datasets of elements and microbes showed candidate microbes in  
432 the “cesium-philic” and “lanthanide-philic” biofilms on the loading plots because those plots  
433 were placed in same loading direction, e.g., PC1 negative – PC2 positive (Cs cluster) or PC1  
434 negative – PC2 negative (Lanth cluster) (Fig. 5c, Additional file 4 and 5: Supplementary  
435 Table 4 and 5). Indeed, the HCA heat map of the microbial consortia showed that the PC1  
436 negative – PC2 negative-directed microbes mainly appeared in the week 1 column (Fig. 6  
437 blue dots). The same tendency was observed with the Lanth cluster microbes (Fig. 6 green  
438 dots). These results suggest that these two OTU sets are positively correlated with the  
439 Lanth cluster (week 3 sample) and Cs cluster (week 1 sample). The correlation heat map  
440 chart between the element dataset and the OTU dataset supports this result

441

442 Figure 6

443 Heatmap chart of microbial consortia data for biofilms with a hierarchical clustering

444 analysis (HCA).

445 The heatmap chart has been sorted by the HCA for both axes (OTU axis and sample axis).

446 Green and blue dots indicate contributing OTUs for the PC1 minus and PC2 plus direction

447 and the PC1/PC2 minus direction, respectively. The relative intensity value has been scaled

448 and centered in the row direction.

449

450 The radiocesium and Cs cluster elements are positively correlated to other multicellular

451 eukaryotes, such as *Hexacorallia* and *Oligochaete*. For bacteria, high relative intensities of

452 *Bacteroidetes*, *Rhodobacteraceae* and Gamma-proteobacteria were observed (Additional file

453 6: Supplementary Table 6 and Fig. 6). Although a primer set that did not target Archaea

454 was used, Archeal sequences were obtained in this work by sequencing amplicons generated

455 by the 16S ribosomal RNA primers set. An uncultured Crenarcheota in marine group I was

456 observed to be one of the dominant OTUs from the biofilm sample of week 1 (Additional file

457 6: Supplementary Table 6).

458 The function of these microorganisms is poorly understood. However, Alpha- and

459 Gamma-proteobacteria have been obtained from biofilms on the surface of nuclear fuel pools

460 [21]. According to Sarro et al. [21], biofilms obtained from nuclear fuel pools accumulate  $^{60}\text{Co}$ .  
461 Cobalt accumulation in the week 1 sample was suggested by the PCA (Fig. 5b and  
462 Additional file 4: Supplementary Table 4) despite a lack of  $^{60}\text{Co}$  signal indicated by  
463 gamma-ray spectrography. The microbial community retrieved from the nuclear fuel pool  
464 was described as capable of accumulating both  $^{60}\text{Co}$  and  $^{137}\text{Cs}$  as reported by Tisakova et al.  
465 [22]. Our results suggest that the biofilm sampled in week 1 presents similar characteristics  
466 to the microbes described by Sarro et al. and Tisakova et al.

467

## 468 Conclusions

469 In this study, we found heterogeneous distributions of radiocesium and radiosilver among a  
470 variety of sample types from the coastal environment near the FDNPP disaster. Specific  
471 organisms that use hemocyanin, such as shell fish and Ascidians, accumulate and retain  
472  $^{110\text{m}}\text{Ag}$ , and similar observations have been reported in terrestrial spiders. We also found  
473 radiosilver in the sediment samples, suggesting that particular materials can be sources of  
474 radiosilver for these organisms, even if particles filtered from the water have low levels of  
475 radioactivity in our samples.

476 However, the biofilm samples did not display detectable signals of radiosilver, although the  
477 amount of radiocesium in these samples was higher than that found in other marine  
478 organisms. Biofilms may still act as an entry point for radiocesium to enter into the  
479 ecosystem. In this paper, we could not assess the functional mechanisms by which biofilms  
480 accumulate cesium specifically; thus, future research that includes cultivation experiments  
481 and meta-transcriptome analyses should be performed to further investigate the microbial  
482 communities described in this work and provide insights into this complex topic.

483

484 Declarations

485 Author's contributions

486 SM, HO and JS designed research. SM, HO, KK, HY and JS performed sampling. YK and  
487 MI performed ICP-MS analysis and KS and JK partially performed sample preparation for  
488 ICP-MS and advice for statistical analysis. Sample preparation and molecular biological  
489 experiment was performed by SM and KK. Radioactivity measurement has been done by  
490 HO. SCUBA diving safety control and sampling arrangement has been done by HY and JS.

491



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498

499 Competing interests

500 The authors declare no competing financial interests.

501

502 Availability of data and materials

503 The datasets used and/or analysed during the current study except for ribosomal RNA  
504 sequences data and datasets that showed in manuscript or supplementary tables are  
505 available from the corresponding author on reasonable request.

506 Ribosomal RNA dataset are available in the DDBJ sequence read archive with accession

507 number DRA004367.

508 Electronic supplementary material

509

510 Additional file 1: Supplementary Table 1: Radio activity of core samples. (XLSX 44 KB)

511

512 Additional file 2: Supplementary Table 2: Radio activity of macro algae samples. (XLSX 47

513 KB)

514

515 Additional file 3: Supplementary Table 3: Radio activity of macro benthos samples. (XLSX

516 46 KB)

517

518 Additional file 4: Supplementary Table 4: Loading plot on PC1 minus - PC2 plus. (XLSX 52

519 KB)

520

521 Additional file 5: Supplementary Table 5: oading plot results of PC1 minus - PC2 minus.

522 (XLSX 51 KB)

523

524 Additional file 6: Supplementary Table 6: Cs cluster taxon in Week1 sample. (XLSX 30 KB)

525

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531

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Fig.1

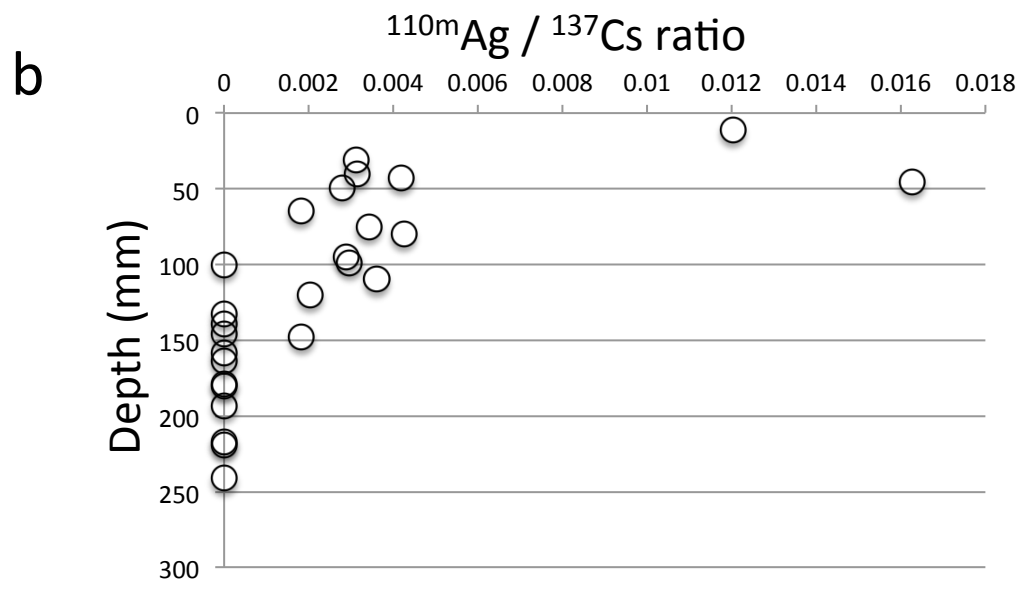
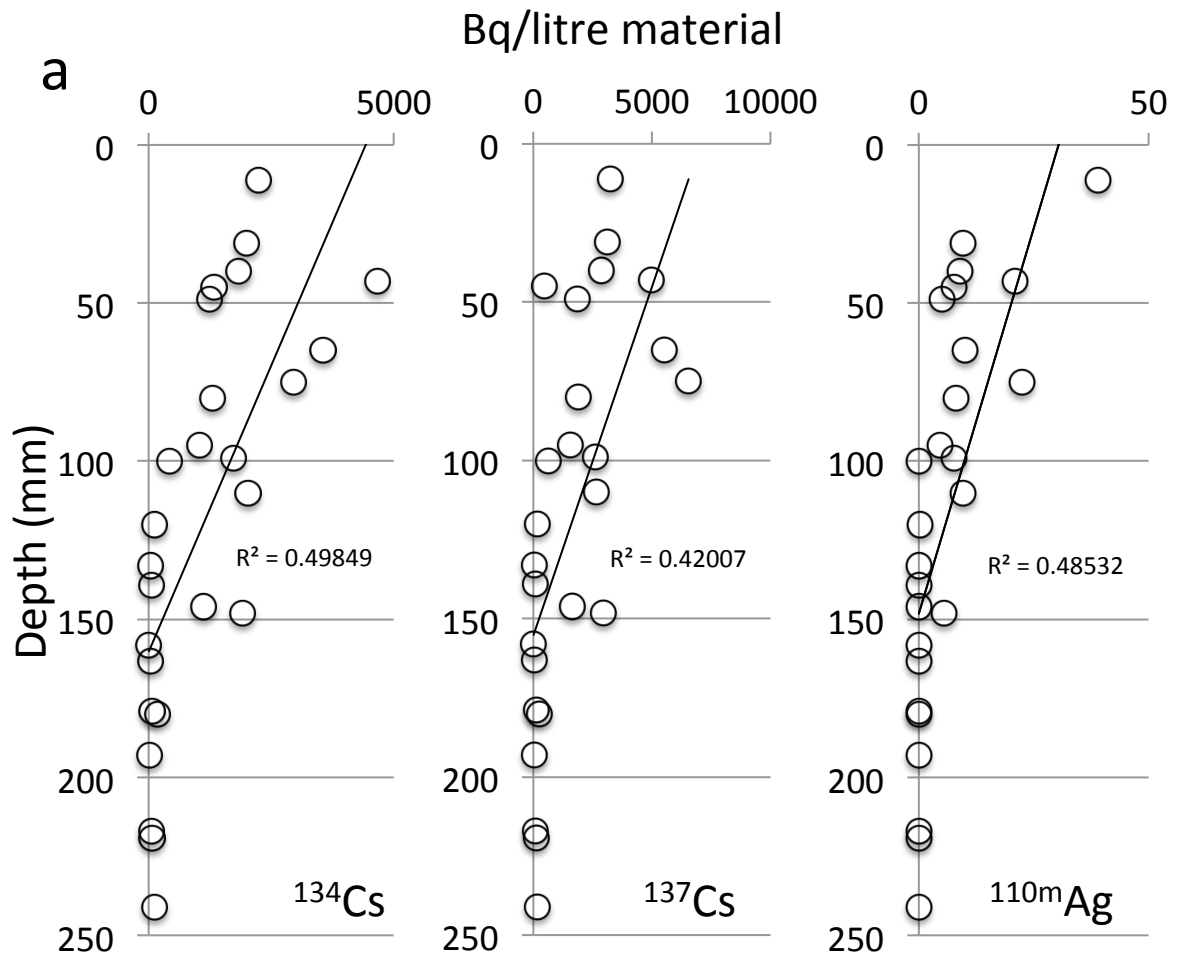




Fig.2

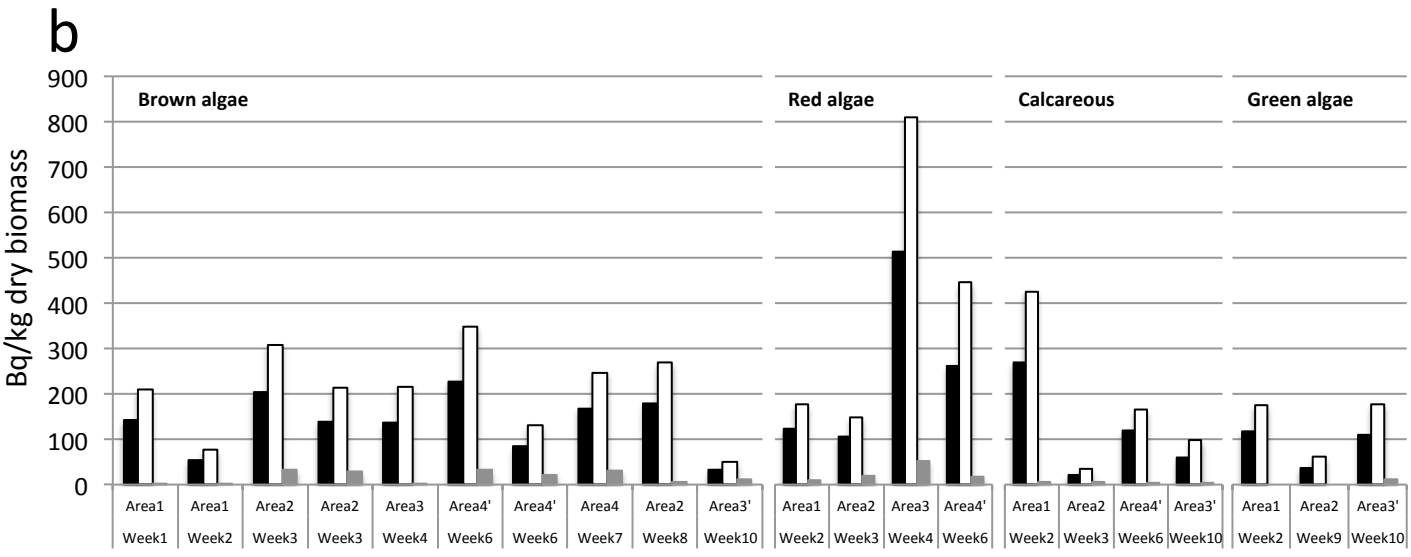
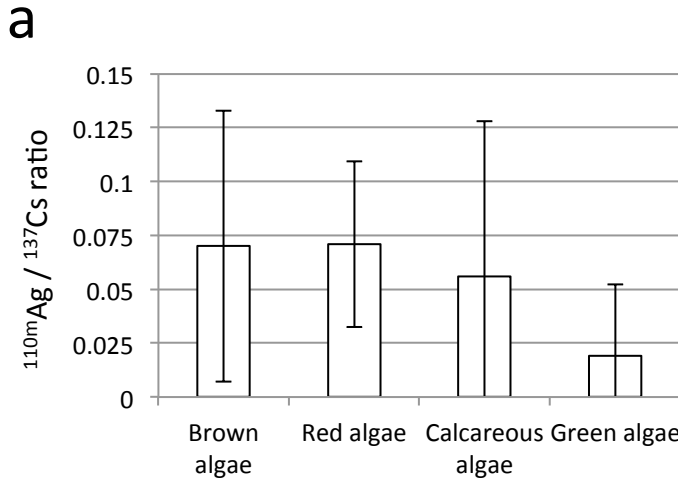


Fig.3

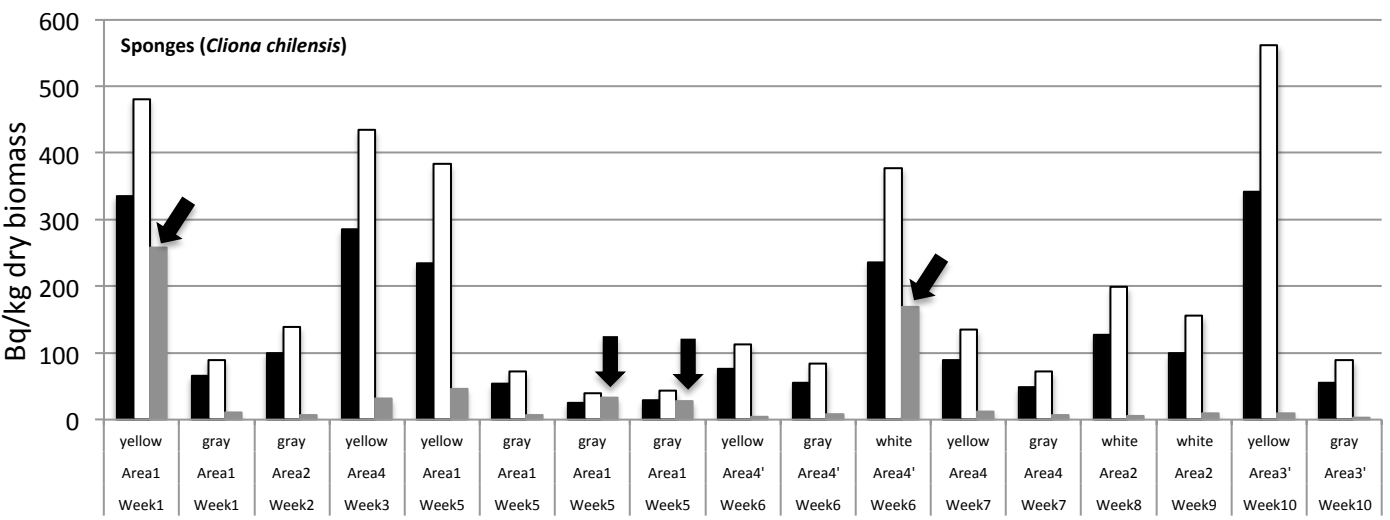
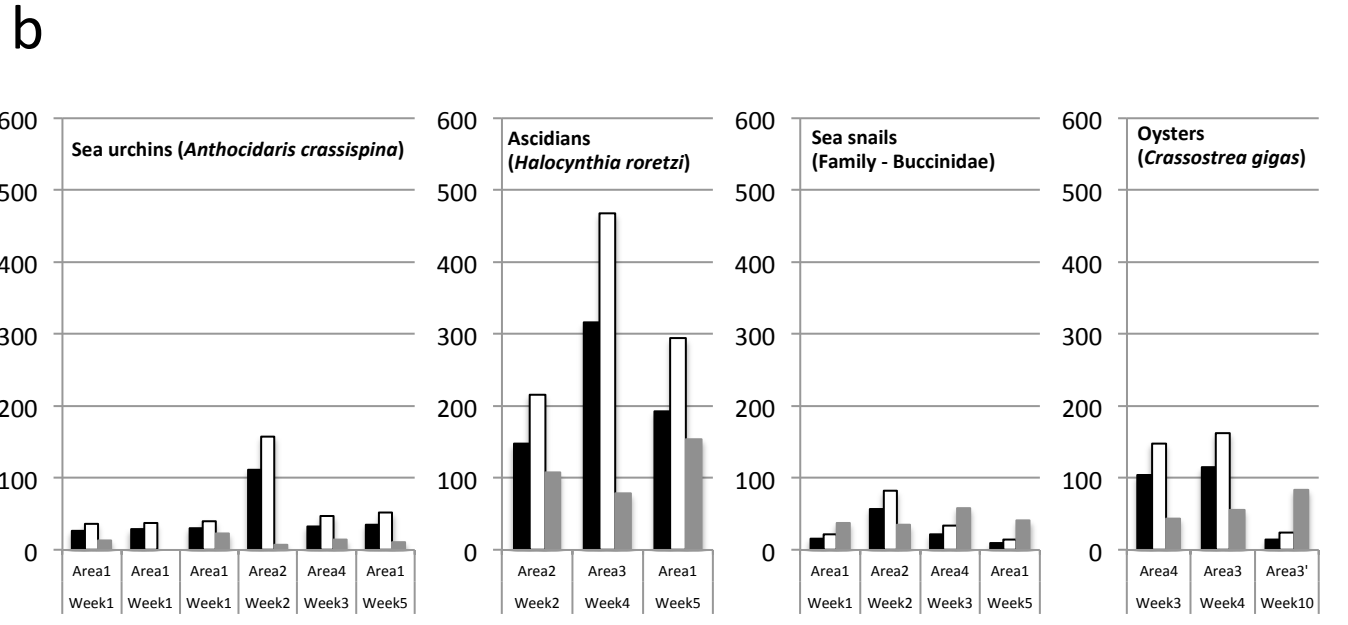
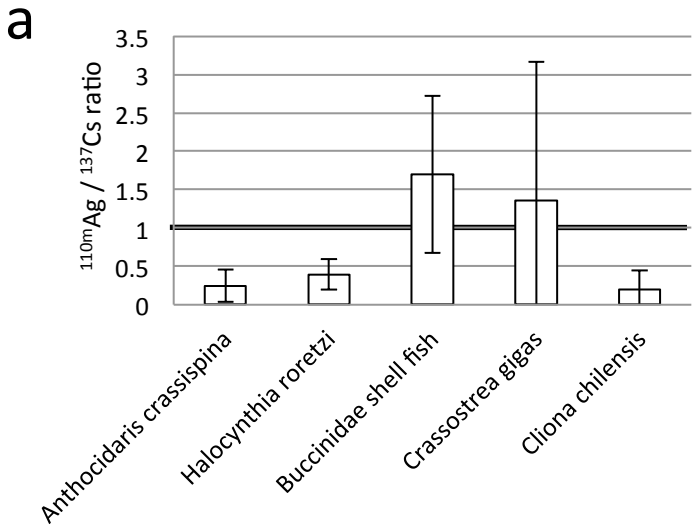


Fig.4

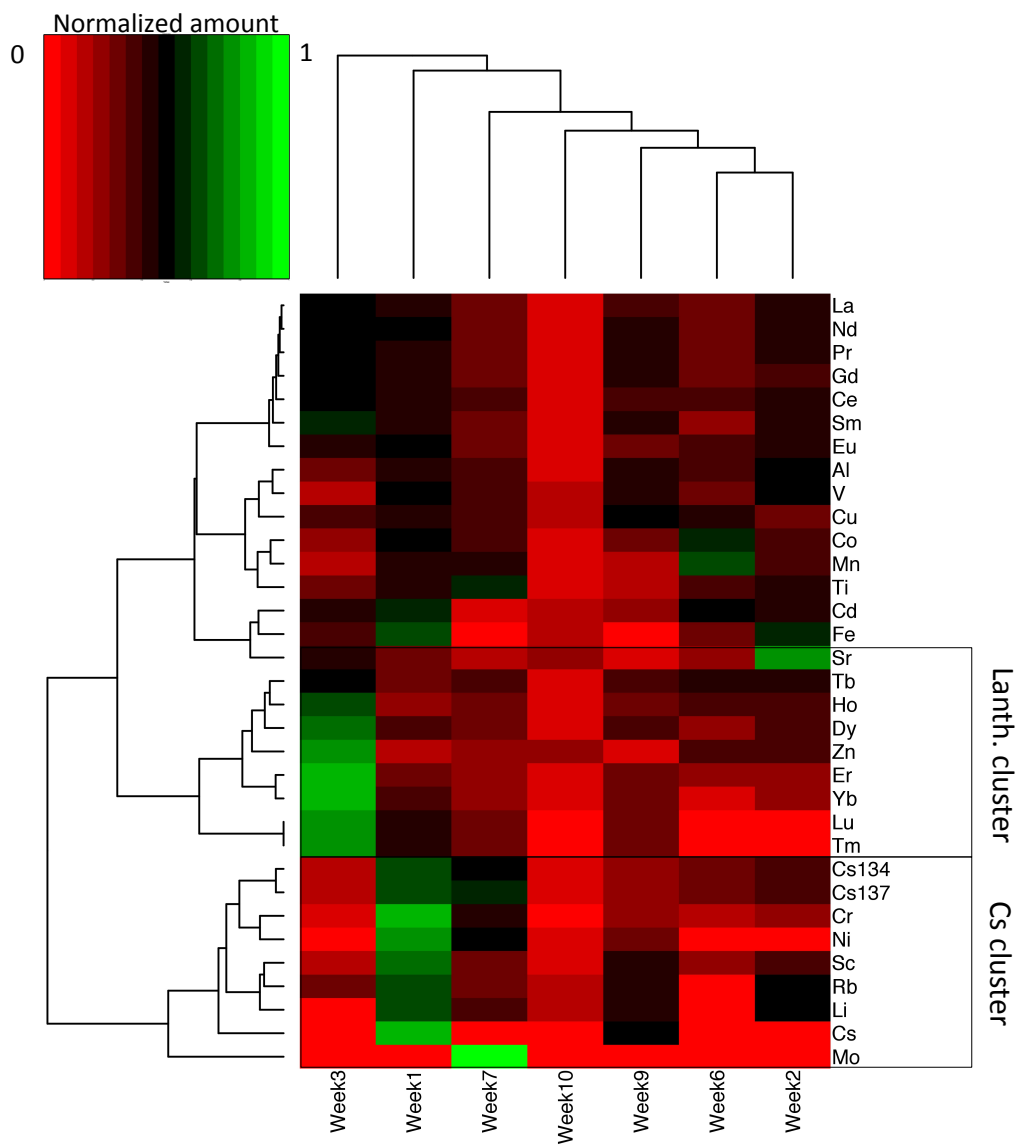
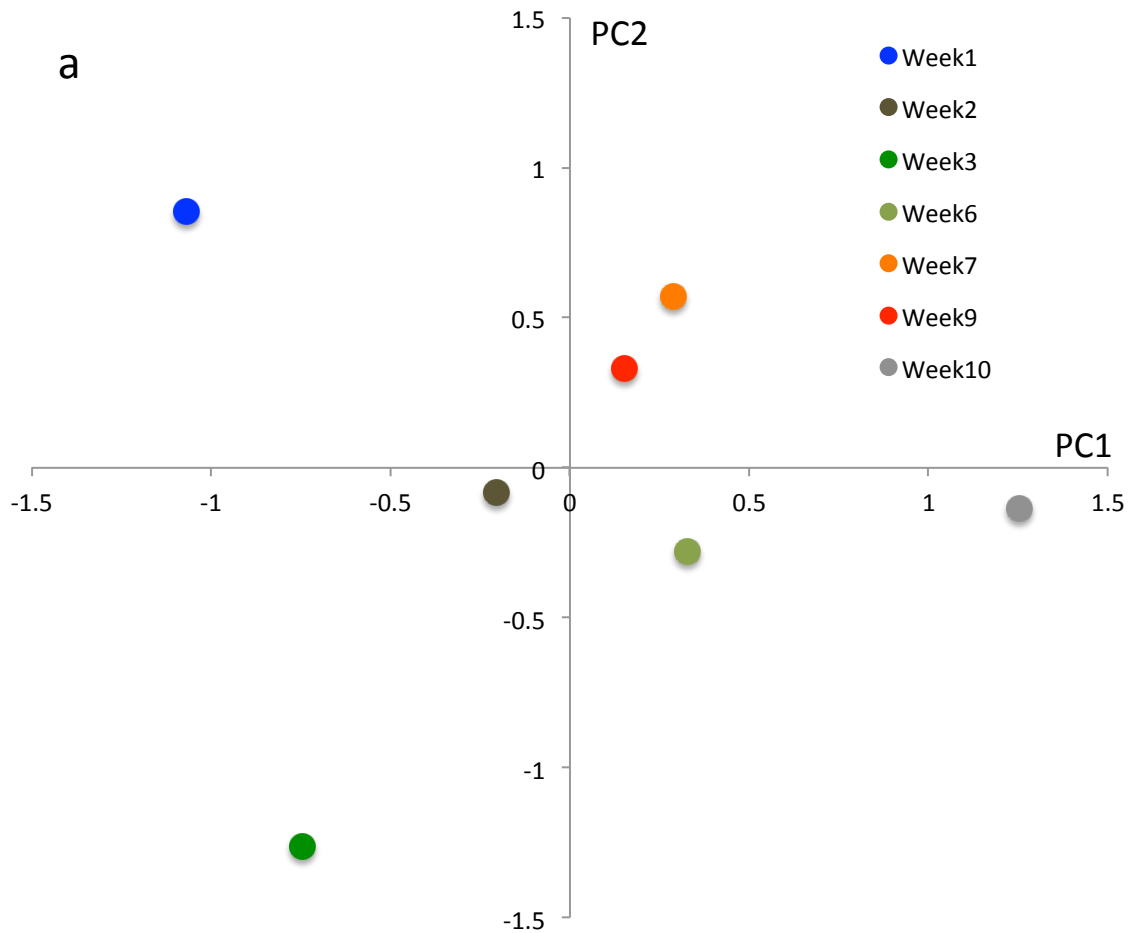
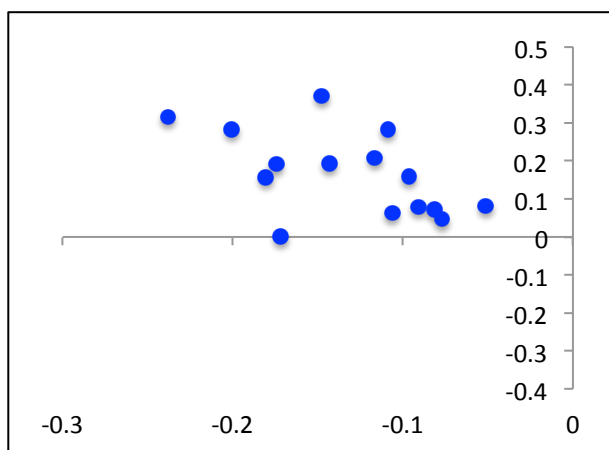


Fig.5

a



b



c

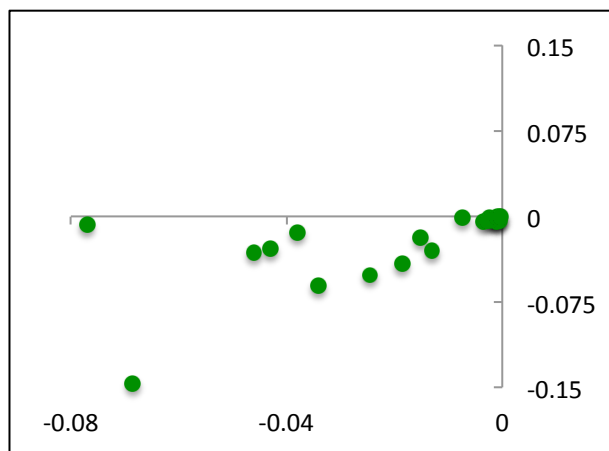
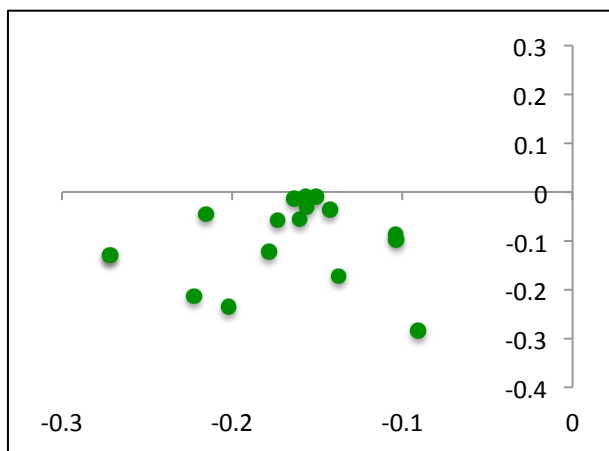
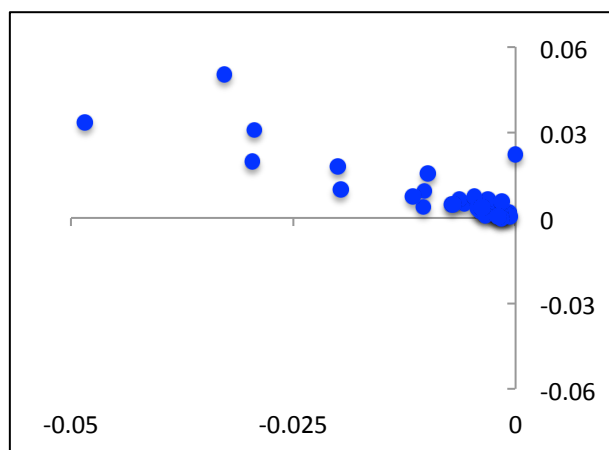


Fig.6

Row direction scaled and centered

