Disproportional distribution of radioactive elements in the marine ecosystems surrounding the accident site of the Fukushima Daiichi Nuclear Power Plant

Shigeharu Moriya[1, 3, *], Hideaki Otsu[2], Kumiko Kihara[1, 4], Yukari Kato[1], Misao Itouga[1], Kenji Sakata[1], Jun Kikuchi[1, 3], Hiroshi Yamakawa[5, 6] and Jiro Suga[6]

1) RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama city, Kanagawa, 230-0045, JAPAN

Shigeharu Moriya, Kumiko Kihara, Yukari Kato, Misao Itouga, Kenji Sakata and Jun Kikuchi

2) RIKEN Nishina Center for Accelerator-Based Science, 2-1 Hirosamwa, Wako city, Saitama, 351-0198, JAPAN

Hideaki Otsu

3) Graduate School of Medical Life Science, Yokohama-city University, 1-7-29 Suehiro-cho, Tsurumi-ku, Yokohama city, Kanagawa, 230-0045, JAPAN

Shigeharu Moriya and Jun Kikuchi

4) Department of Biological and Chemical Systems Engineering, National Institute of Technology, Kumamoto College, 2627 Hirayama-Shinmachi, Yatsushiro-shi, Kumamoto 866-8501, JAPAN

Kumiko Kihara

5) Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo, 108-8477, JAPAN

Hiroshi Yamakawa

6) Japan Academy of Underwater Sciences, 3-9-1-301 Botan, Koto-ku, Tokyo, 135-0046, JAPAN

Hiroshi Yamakawa and Jiro Suga

*) Corresponding author: Shigeharu Moriya

RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama city, Kanagawa, 230-0045, JAPAN

Tel +81-45-508-7221, Fax +81-45-508-7363

smoriya@riken.jp

Abstract

Background

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

Results

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

Conclusions

These findings suggest that several different entry paths are available for radioactive elements to access the biological components of the ecosystem, and these paths may explain the disproportional and patchy distribution of radioactive elements among marine ecosystems.

Keywords

Radio isotope, Marine eco-system, Biofilm

Background

Following the Great East Japan Earthquake and catastrophic tsunami in March of 2011, the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident led to the release of large amounts of radioactive materials into the atmosphere and marine environment.

This environmental input of radioactive elements from the FDNPP introduced into the marine waters a variety of radioisotopes, especially radiocesium isotopes. Public monitoring

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

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

Materials and Methods

Sampling

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

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

Sediment core samples were collected at Area 2 using a piston corer prototype. The "core sampler" was composed of a 4.5 cm diameter/28 cm height cylinder and inscribed piston. Core samples were collected during a separate dive in weeks 6 (core sample 1) and 7 (core samples 2 – 4).

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

Sample preparation

The seawater samples were immediately filtered with two 24-mm-wide 0.22 μm Durapore filters (Millipore, MA, USA) sample until they clogged. All samples were frozen at -20°C in a field freezer prior to transportation back to laboratory.

In the laboratory, the samples were dried at 105°C overnight except for the filters, water 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 μm pore size membrane filters and stored at -20°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°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

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

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

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

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

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

The radioactivity of the marine animals, macroalgae and biofilm samples were normalized by weight, and the radioactivity of the water samples and sediment core samples were normalized by volume.

Element analysis

To quantitatively analyze the content of 37 elements (^{7}Li, ^{9}Be, ^{23}Na, ^{24}Mg, ^{27}Al, ^{39}K, ^{43}Ca, ^{45}Sc, ^{47}Ti, ^{51}V, ^{52}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{98}Mo, ^{111}Cd, ^{133}Cs, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{152}Sm, ^{153}Eu, ^{158}Gd, ^{159}Tb, ^{164}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{172}Yb, ^{175}Lu, and ^{202}Hg) in the samples (i.e., surface salts, surface microbes, biofilms), the samples were predigested with 5 mL of concentrated HNO_3 for 1 h at room temperature. Next, the organic components were completely decomposed by wet-ashing using a microwave sample preparation system (Multi-Wave-3000, Perkin Elmer, MA USA) [8]. The digested samples were brought up to a volume of 50 mL with MilliQ water (MQW) and filtered through 5B filter paper (Advantec, Tokyo, Japan). The concentrations of the mineral elements were determined by Inductively 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-1 HCl

178 [8]. The mineral nutrient concentration in the samples was calculated as a unit (mg g^{-1} 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 (^{134}Cs and ^{137}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}Cs and ^{137}Cs in addition to natural radioisotope signals, such as ^{40}K (data not shown), for

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

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

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

Table 1. Peak detection in the samples

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

Macroalgae	++	++	+
Biofilms on bottom rocks	++	++	–

++: significant peak detected from all samples

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

-: significant peak not detected from any sample

*: Particles were filtered from water using a 0.22 μm filter, and the radioactivity was measured

Sediment core samples were obtained from the sampling point "Area 2" by the core sampler. The analysis of these sediment core samples indicated the presence of both radiocesium and radiosilver. The measured radioactivities are shown in Additional file 1: Supplementary Table 1. To better understand the radioactivity in the same volume of space, we employ the unit Bq/liter to express the radioactivity of the core samples because the core samples contain different materials along their depth profile.

In Figure 1, the distribution of radioactivity along the sediment column is shown. Both radiocesium and radiosilver are distributed similarly in the surficial part of the core

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

Figure 1

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

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

296 However, the ratio between ^{110m}Ag and ^{137}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}Ag and

299 ^{137}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}Ag/^{137}Cs of

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

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

303

304 Figure 2

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

306 average ^{110m}Ag/^{137}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}Cs, open bars show ^{137}Cs and gray bars show ^{110m}Ag. Radioactivity is

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

310

311 Figure 3

312 Radioactivity of ^{134}Cs, ^{137}Cs and ^{110m}Ag in the macrobenthos samples. Panel a shows the

313 average $^{110m}Ag/^{137}Cs$ 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}Cs, open bars represent ^{137}Cs and gray bars represent ^{110m}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 ^{110m}Ag and ^{137}Cs.

322 Our results indicated that sea snails (Buccinidae) and oysters (*Crassostrea gigas*)

323 consistently presented a high ratio of $^{110m}Ag/^{134}Cs$, which was also observed for the sponge

324 *Cliona chilensis*. Samples of the Ascidian *Halocynthia roretzi* showed a relatively high ratio

325 of $^{110m}Ag/^{134}Cs$ (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 $^{110m}Ag/^{137}Cs$ in the spider, *Atypus karschi*

328 indicated that the reaction center Cu in hemocyanin can be replaced by Ag, or ^{110m}Ag in this

329 case [3]. Similarly, the sampled mollusks may have actively incorporated ^{110m}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}Cs = 21.7923, ^{137}Cs = 37.3885, ^{110m}Ag = 134.6388 (Bq/kg

333 dry biomass)) than in the shell (^{134}Cs = 12.6245, ^{137}Cs = 18.8071, ^{110m}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}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}Cs = 645,0563,

348 927.5638, 944.2312; ^{137}Cs = 997.5926, 1437.1948, 1588.1469; ^{110m}Ag = 295.6794, 255.9421,

349 213.5846 (Bq/kg dry biomass) and the shell measurement results are ^{134}Cs = 18.6579,

350 51.1889, 28.2958; ^{137}Cs = 30.3881, 80.5451, 43.8699; ^{110m}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}Ag from the surrounding water and retain the

366 element even after moving to clean water that does not contain ^{110m}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

		^{134}Cs Bq/kg dry	^{137}Cs Bq/kg dry	^{110m}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

nd: not detected

"Cesium-philic" biofilm

Microbial communities that grow on surfaces of solid materials in the presence of water are known as biofilms. Our observations suggest that the biofilm community specifically accumulates radiocesium. Therefore, we analyzed the relationship between the microbial consortia within the biofilms and their elemental composition.

Quantified element data were combined with radioactivity data for radiocesium and utilized for the statistical analysis. Ribosomal RNA genes were sequenced and used to construct a dataset for the statistical analysis.

A HCA from the dataset of elements and radiocesium (Fig. 4) in which an accumulation of radioactive cesium, Cr, Ni, Sc, Rb, Li and cesium (Cs cluster) was performed for the week 1 sample. Another remarkable profile from the elemental analysis is observed in week 3, 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 contributes 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 (Additoinal 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}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}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}Co and ^{137}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 ^{110m}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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Competing interests

The authors declare no competing financial interests.

Availability of data and materials

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

Ribosomal RNA sataset are available in the DDBJ sequence read archive with accession number DRA004367.

Electronic supplementary material

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

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

Additional file 3: Supplementary Table 3: Radio activity of macro benthos samples. (XLSX 46 KB)

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

Additional file 5: Supplementary Table 5: oading plot results of PC1 minus - PC2 minus. (XLSX 51 KB)

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

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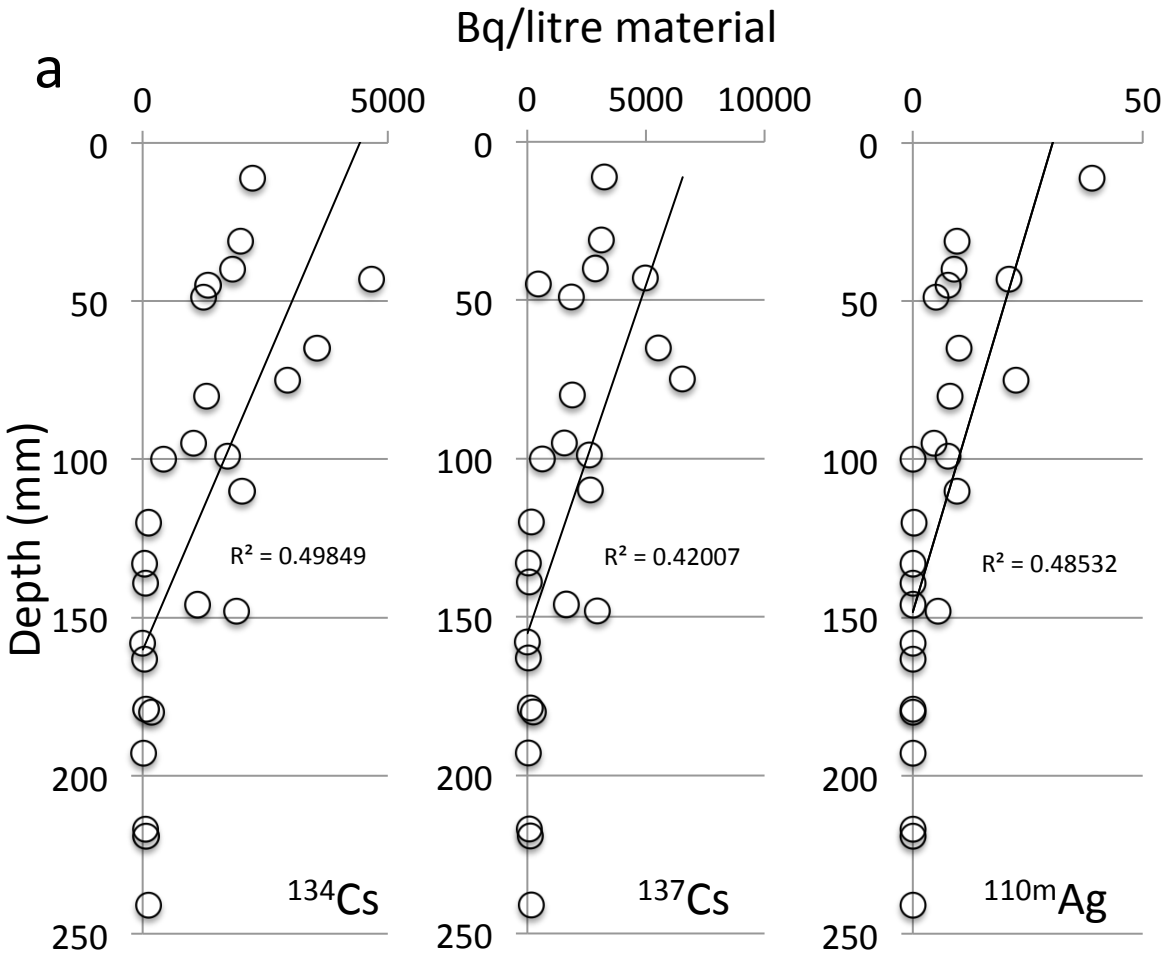
597

Fig.1


a
Bq/litre material
0
5000
0
5000
10000
0
50
Depth (mm)
0
50
100
150
200
250
R² = 0.49849
R² = 0.42007
R² = 0.48532
134Cs
137Cs
110mAg

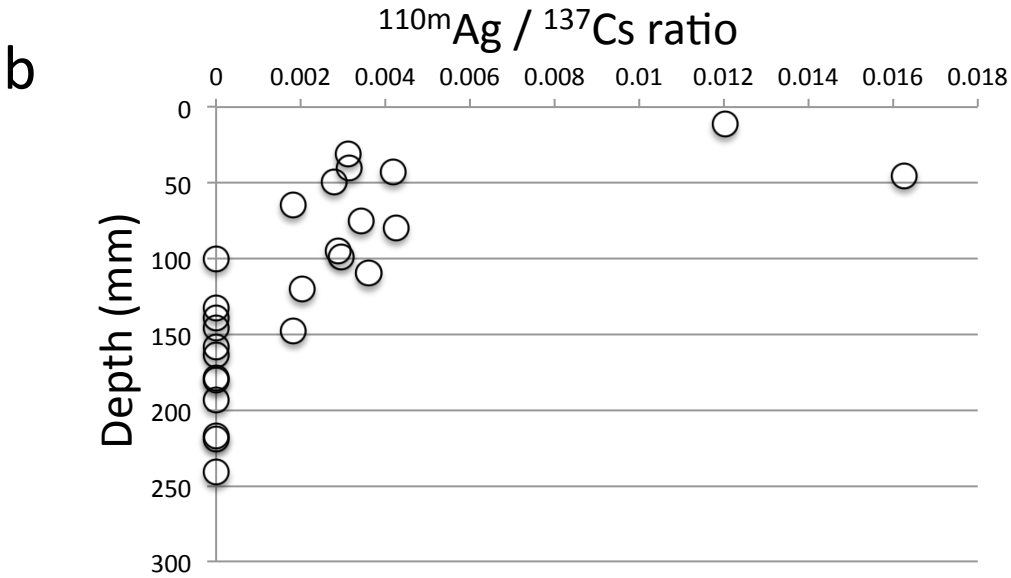
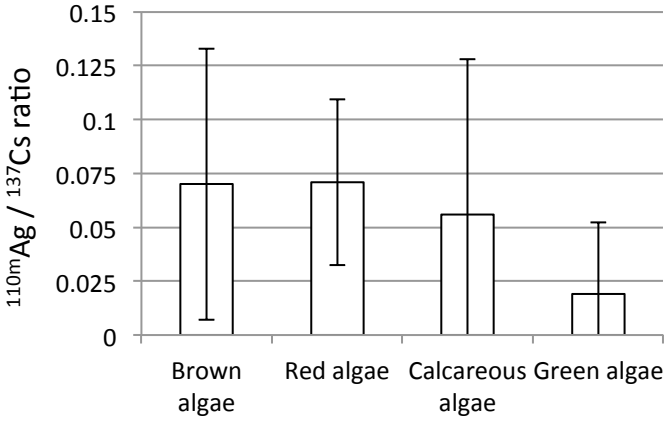

b
110mAg / 137Cs ratio
0
0.002
0.004
0.006
0.008
0.01
0.012
0.014
0.016
0.018
Depth (mm)
0
50
100
150
200
250
300

Fig.2

a


0.15
0.125
0.1
0.075
0.05
0.025
0
110mAg / 137Cs ratio
Brown algae
Red algae
Calcareous algae
Green algae

b

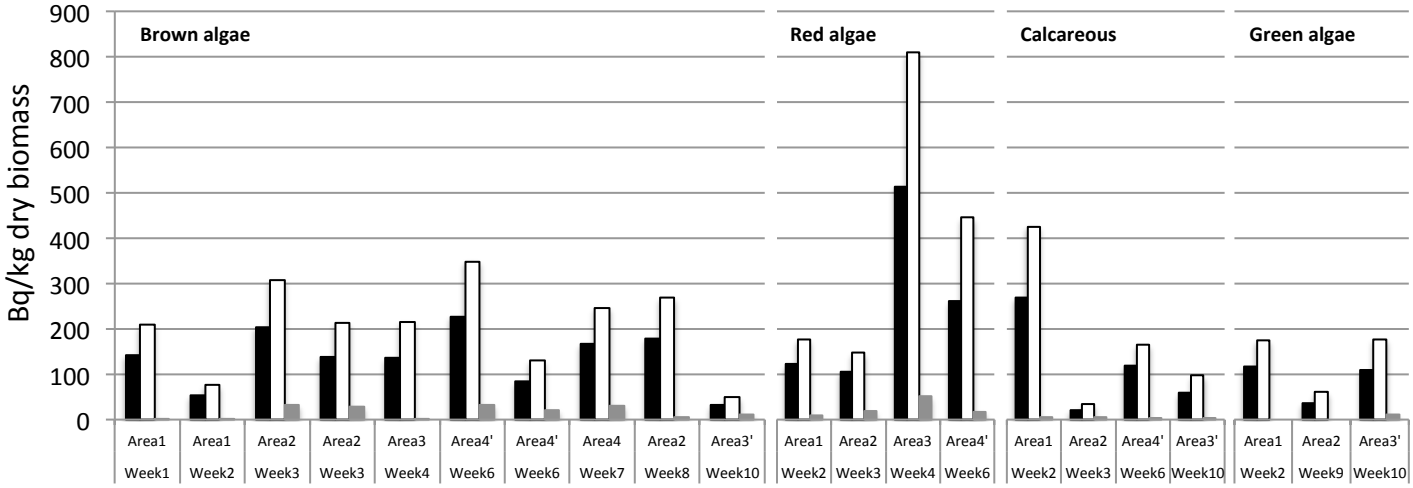
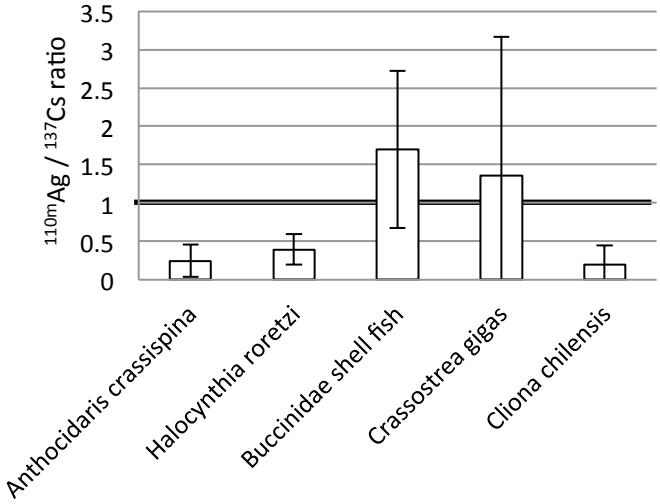

900
800
700
600
500
400
300
200
100
0
Bq/kg dry biomass
Brown algae
Red algae
Calcareous
Green algae
Area1 Week1
Area1 Week2
Area2 Week3
Area2 Week3
Area3 Week4
Area4' Week6
Area4' Week6
Area4 Week7
Area2 Week8
Area3' Week10
Area1 Week2
Area2 Week3
Area3 Week4
Area4' Week6
Area1 Week2
Area2 Week3
Area4' Week6
Area3' Week10
Area1 Week2
Area2 Week9
Area3' Week10

Fig.3

a


^{110m}Ag / ^{137}Cs ratio
3.5
3
2.5
2
1.5
1
0.5
0
Anthocidaris crassispina
Halocynthia roretzi
Buccinidae shell fish
Crassostrea gigas
Cliona chilensis

b

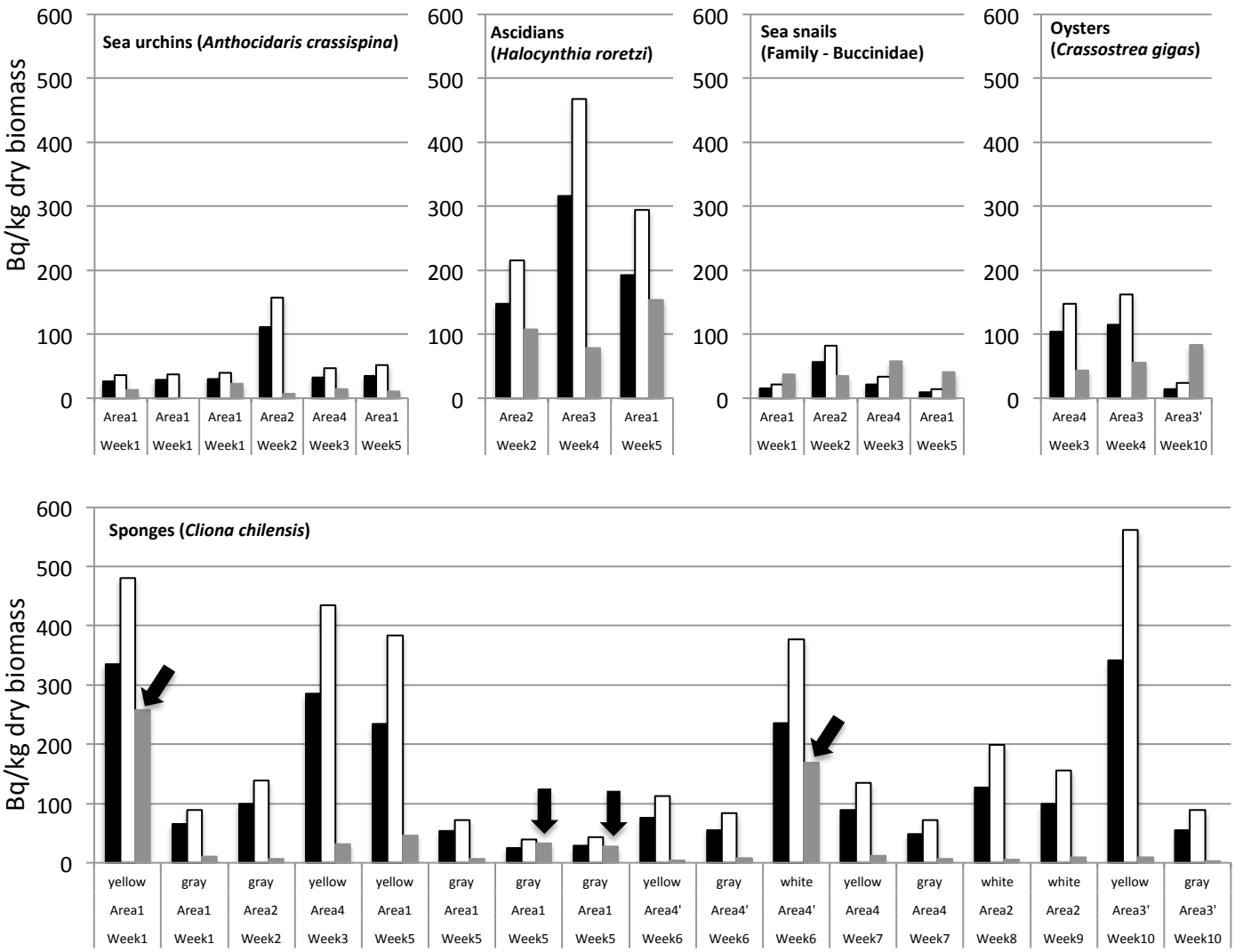
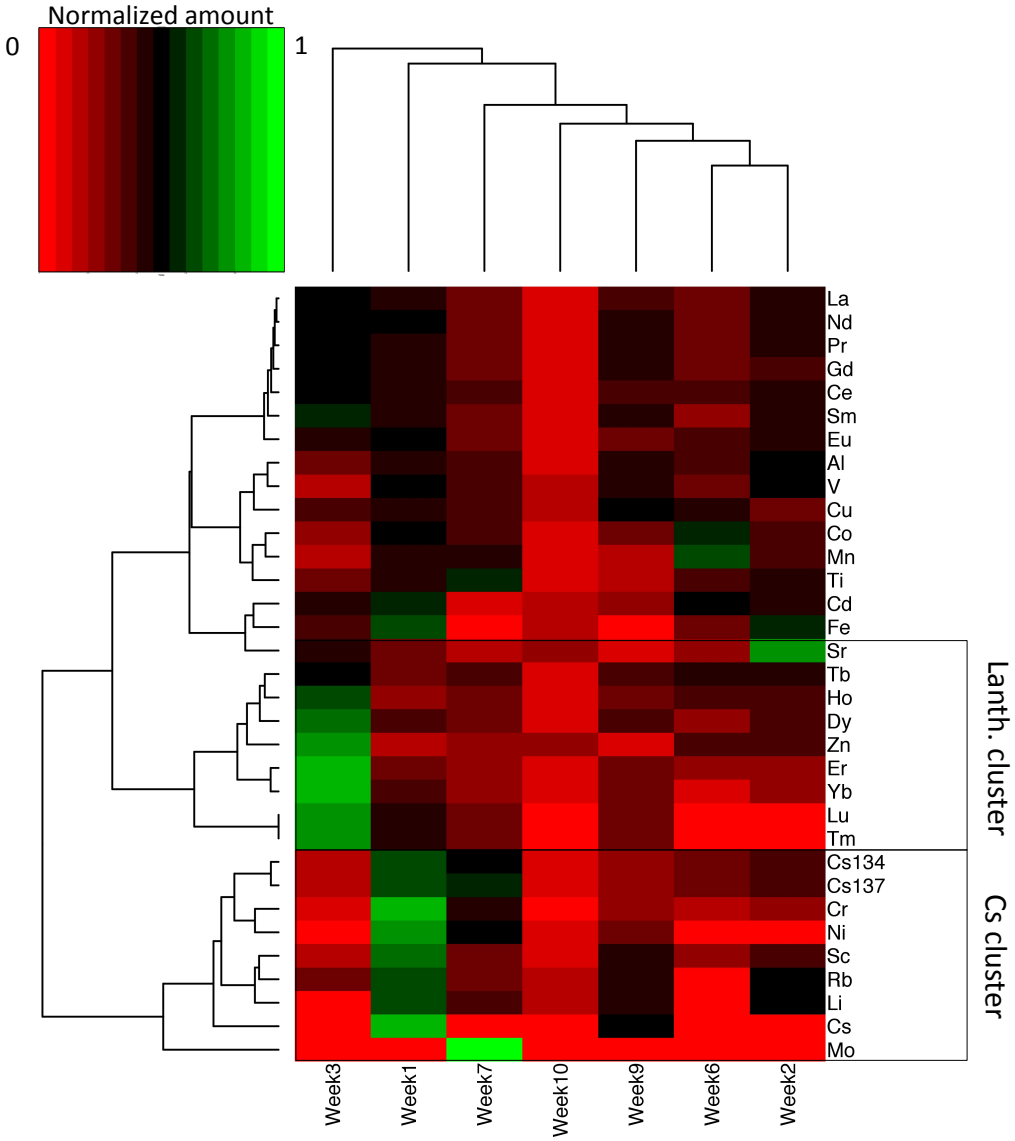

Bq/kg dry biomass
600
500
400
300
200
100
0
Sea urchins (*Anthocidaris crassispina*)
Area1 Week1
Area1 Week1
Area1 Week1
Area2 Week2
Area4 Week3
Area1 Week5
Ascidians (*Halocynthia roretzi*)
Area2 Week2
Area3 Week4
Area1 Week5
Sea snails (Family - Buccinidae)
Area1 Week1
Area2 Week2
Area4 Week3
Area1 Week5
Oysters (*Crassostrea gigas*)
Area4 Week3
Area3 Week4
Area3' Week10
Sponges (*Cliona chilensis*)
yellow Area1 Week1
gray Area1 Week1
gray Area2 Week2
yellow Area4 Week3
yellow Area1 Week5
gray Area1 Week5
gray Area1 Week5
gray Area1 Week5
yellow Area4' Week6
gray Area4' Week6
white Area4' Week6
yellow Area4 Week7
gray Area4 Week7
white Area2 Week8
white Area2 Week9
yellow Area3' Week10
gray Area3' Week10

Fig.4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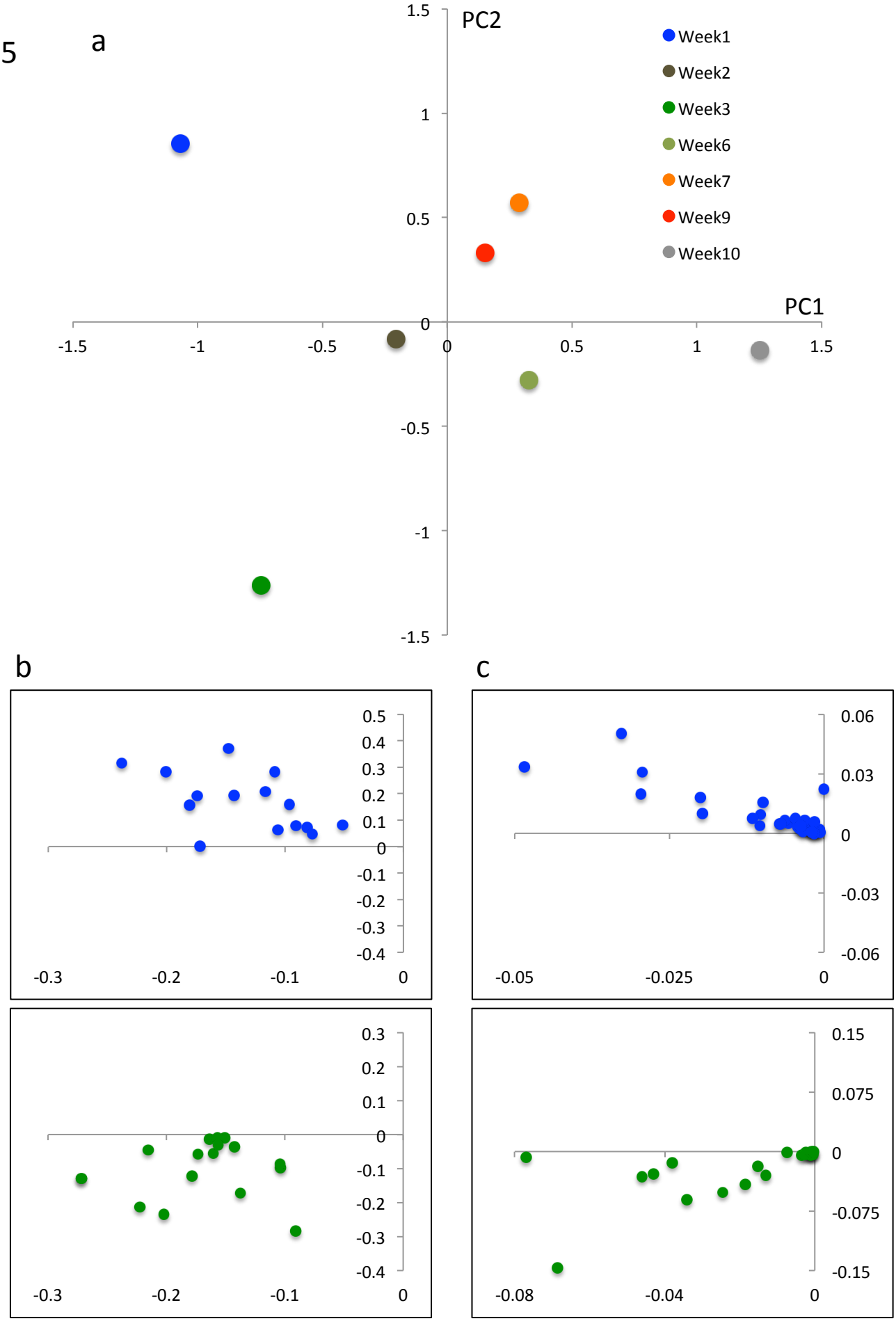
Nd
Pr
Gd
Ce
Sm
Eu
Al
V
Cu
Co
Mn
Ti
Cd
Fe
Sr
Tb
Ho
Dy
Zn
Er
Yb
Lu
Tm
Cs134
Cs137
Cr
Ni
Sc
Rb
Li
Cs
Mo
Lanth. cluster
Cs cluster
Week3
Week1
Week7
Week10
Week9
Week6
Week2

Fig.5


a
PC2
PC1
Week1
Week2
Week3
Week6
Week7
Week9
Week10
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1
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0
-0.5
-1
-1.5
-1.5
-1
-0.5
0
0.5
1
1.5
b
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.3
-0.2
-0.1
0
c
0.06
0.03
0
-0.03
-0.06
-0.05
-0.025
0
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.3
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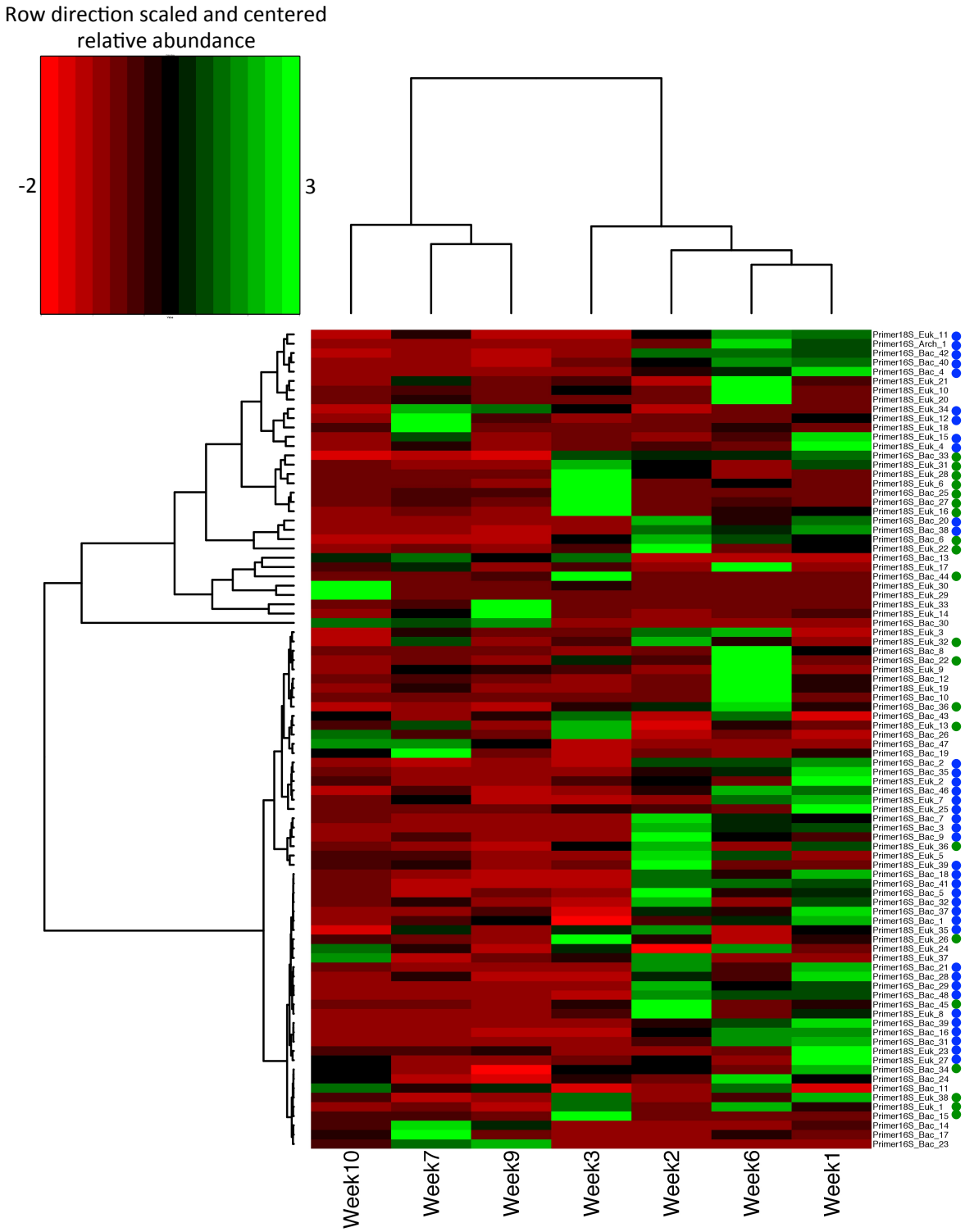
Fig.6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Primer18S_Euk_11
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Primer18S_Euk_20
Primer18S_Euk_34
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Primer18S_Euk_15
Primer18S_Euk_4
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Primer18S_Euk_28
Primer18S_Euk_6
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Primer18S_Euk_16
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Primer16S_Bac_6
Primer18S_Euk_22
Primer16S_Bac_13
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Primer16S_Bac_44
Primer18S_Euk_30
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Primer18S_Euk_14
Primer16S_Bac_30
Primer18S_Euk_3
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Primer16S_Bac_22
Primer18S_Euk_9
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Primer18S_Euk_19
Primer16S_Bac_10
Primer16S_Bac_36
Primer16S_Bac_43
Primer18S_Euk_13
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Primer16S_Bac_19
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Primer16S_Bac_35
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Primer16S_Bac_7
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Primer18S_Euk_36
Primer18S_Euk_5
Primer18S_Euk_39
Primer16S_Bac_18
Primer16S_Bac_41
Primer16S_Bac_5
Primer16S_Bac_32
Primer16S_Bac_37
Primer16S_Bac_1
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Primer16S_Bac_16
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Primer18S_Euk_27
Primer16S_Bac_34
Primer16S_Bac_24
Primer16S_Bac_11
Primer18S_Euk_38
Primer18S_Euk_1
Primer16S_Bac_15
Primer16S_Bac_14
Primer16S_Bac_17
Primer16S_Bac_23
Week10
Week7
Week9
Week3
Week2
Week6
Week1