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LATEST UPDATE ON ENVIRONMENTAL EMERGENCY RESEARCH

The natural environment after the Great East Japan Earthquake

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The natural environment after the Great East Japan Earthquake

About the theme of this issue - the natural environment after the Great East Japan Earthquake

1. Introduction

Our lives have been affected in various ways by the Great East Japan Earthquake (GEJE) and the Fukushima Nuclear Accident that was one of the consequences of the quake. In this issue, we will focus on the impacts of the GEJE on the natural environment, and more particularly on the flora and fauna of affected areas and their habitats and ecosystems.

If ecosystems collapse, ecosystem services such as the supply of agricultural, forestry, and fishery products or recreational use that we benefit from will likely be affected. Once flora and fauna and their habitats are contaminated with radioactive substances, people may also be newly exposed to radiation by entering contaminated areas or ingesting contaminated plants and animals. Furthermore, radioactive substances currently confined to forests and other nature areas may be discharged by further natural disasters or other causes and end up in populated areas. These possibilities greatly affect prospects for rehabilitating farming, forestry, and fisheries industries in affected areas, and for rebuilding communities that are safe for people to live in. In other words, changes on the natural environment caused by the GEJE are highly relevant to our own everyday lives.

2. Impacts of the GEJE on the surrounding natural environment

The impacts of the GEJE on the natural environment can be divided roughly into two types — those caused by the earthquake and accompanying tsunami, and those caused by radioactive substances released as a result of the nuclear accident. We have laid out the impacts of the GEJE on the natural environment in the Figure 1 below.

In addition to damage suffered directly by the coastal environment, impacts of the earthquake and tsunami include the disappearance of tidal flats and other changes resulting from restoration and reconstruction projects such as seawall construction (A in the Figure 1).

Impacts from the nuclear accident include the distribution and inflow of radioactive substances in forests, lakes, rivers and the sea; the accompanying transfer of those substances to flora and fauna; and possible genetic effects. Radioactive substances whose absorption to soil or other substrates impedes their transfer may also be carried to populated areas as a result of floods and other extreme events (B in the Figure 1). In addition, human efforts to deal with radioactive contamination may also impact ecosystems. For example, in areas evacuated because of serious contamination by radioactive substances, the disappearance of the human population will likely lead to changes in the number and behavior of wildlife. Decontamination operations aimed at promptly reducing radiation doses

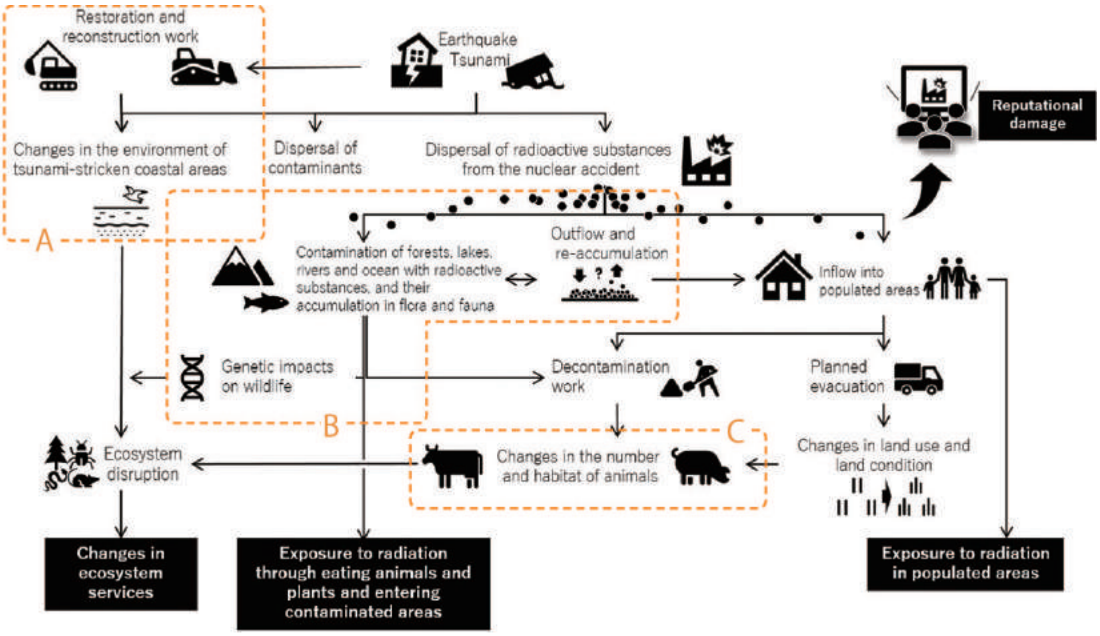

Figure 1. Impacts of the GEJE on the natural environment

could also affect wildlife habitat and behavior (C in the Figure 1). Taking wild boars as an example, their increase in Fukushima Prefecture was a major issue before the GEJE, but various factors including evacuation of the human population mentioned above have made it even more difficult to curb the increase in wild boar numbers.

3. Pursuing issue-driven research: overview of this issue

Addressing these issues requires a sound understanding of the current reality and mechanisms involved. In this issue, we introduce the outcomes of four relevant research projects. The first article describes changes brought about by the GEJE on the coastal ecosystem, and the recovery and conservation of that ecosystem (related to A in the Figure 1). The second article describes the movements of the radioactive substances in a river basin and dam reservoir after recent heavy rainfall events (related to B in the Figure 1). The third article presents methods and results of ecosystem monitoring research conducted in an area in which people no longer live as a result of planned evacuation (related to C in the Figure 1). The fourth article describes a project to assess radiation-induced DNA mutation risks by creating genetically modified plants and cultured cells that enable detection of repairs to DNA damaged by radiation from contaminated soil (related to B in the Figure 1).

4. Conclusions and future works

Various measures are currently being implemented to ensure that radioactive substances released into the natural environment from the nuclear accident do not adversely affect our lives and health. In Fukushima Prefecture, restrictions on the shipment of marine fish and other products have gradually eased, but many items and areas are still subject to restrictions on distribution and consumption. Wildlife conservation and management are also being pursued under the “Fukushima Biodiversity Plan” and “Wildlife Conservation and Management Project Plan”. Tackling the contamination of forests with radioactive substances continues to be a major challenge. The research outcomes described in this publication will hopefully aid consideration of how we should proceed with future efforts to rehabilitate the environment.

Although they have not been covered in this issue, we are also pursuing researches on other themes included in the Figure 1—the impacts of planned evacuation on land use and condition, and evaluation of radiation doses, in populated areas. The treatment and disposal of decontamination wastes is another very complex issue that we are researching. We hope that you will look forward to future issues of this publication.

The changes brought about by the Great East Japan Earthquake on the coastal ecosystem, and the recovery and conservation of that ecosystem

Highlights of this research

1. The Tohoku region's many tidal flats are vital ecosystems that serve as habitats for macrozoobenthos such as bivalves, crabs, and polychaetes; as nursery grounds for commercially important fish species; and as places for water purification.

2. The GEJE brought dramatic changes on the topography, sediment characteristics and vegetation of many of those tidal flats, causing a temporary loss in macrozoobenthic diversity.

3. However, their biota has steadily recovered during the six years since the earthquake, and many endangered species can be found on the tidal flats in Fukushima Prefecture.

4. Tidal flats in the region now face a new threat in the form of restoration projects that alter the environment in ways that may lead to their disappearance. Efforts are, however, made to preserve and restore biodiversity by rehabilitating tidal flats and salt marshes.

Tidal flats and saltmarshes on the Tohoku region's Pacific coastline suffered extensive physical disturbance as a result of ground liquefaction, seismic subsidence of up to 1.2 m, and the tsunami, which inundated some locations to a depth of up to 20 m. In this article, I present the results of research that we have carried out since the GEJE on the environment and biota of tidal flats in the region, and report also on several topics related to the recovery and conservation of those tidal flats six years after the disaster.

1 The functions of tidal flats

Many tidal flats dot the coastline stretching from Fukushima Prefecture to Miyagi Prefecture (Figure 1). Brackish waters and coastal areas where tidal flats form boast a biological productivity on par with that of tropical rainforests. Organic matter produced by photosynthesis is used by many benthic animals, birds and fish through the food chain, thereby constituting the basis for high secondary production (animal biomass production per unit area).

Tidal flats are home to macrozoobenthos such as polychaetes, bivalves, and crabs that sustain themselves by feeding on benthic microalgae and phytoplankton. Organic matter in the mud is actively decomposed (mineralized) by the action of microorganisms, with nitrogen also being removed after mineralization through microbial nitrification and denitrification processes. These processes comprise what are generally recognized as the water purification function of tidal flats. Macrozoobenthos also burrow into sediment, reworking it as they do so (bioturbation) and further promoting microbial activity.

Tidal flats serve as habitat for macrozoobenthos, fish, birds and many other species, thereby helping to maintain the biodiversity of the region. They are also home to many species exploited by we humans as fishery resources. For example, the flatfish that are commercially important species of Sendai Bay

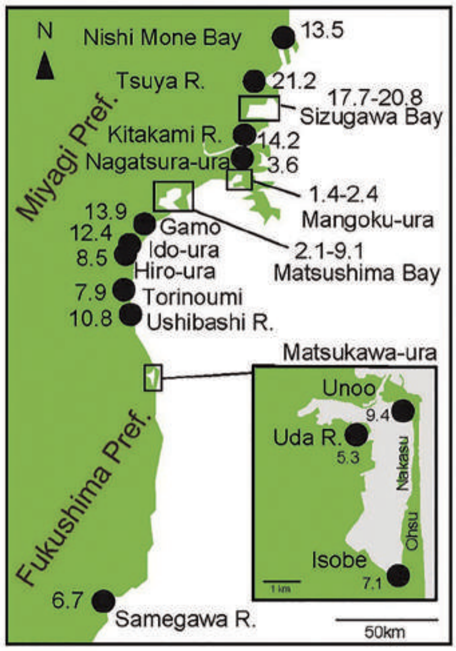

Figure 1. Major tidal flats in Fukushima Prefecture and Miyagi Prefecture. The figures show maximum inundation depth (m) of the 2011 tsunami.

spend their larval and juvenile stages in the coastal tidal flats and shallows. Other species that depend on tidal flats for part of their life cycle include *Portunus trituberculatus* (swimming crab), *Lateolabrax japonicus* (Japanese sea bass), and *Acanthogobius flavimanus* (yellowfin goby). Also commercially harvested in the tidal flats and adjacent waters of Sendai Bay are *Ruditapes philippinarum* (Manila clam), *Monostroma nitidum* (green nori), *Porphyra yezoensis* (nori), and *Crassostrea gigas* (Pacific oyster). We therefore benefit both directly and indirectly in various ways from tidal flats. We refer to these benefits collectively as tidal flat ecosystem services.

2 Changes in the environment and biodiversity of tidal flats caused by the GEJE

How did the GEJE change the tidal flat environment and biota? In summer 2011, we researched the impact of the GEJE tsunami on Gamo Lagoon in Sendai, Miyagi Prefecture. Gamo Lagoon was highly eutrophic before the earthquake, with a thick surface layer of sludge making it unsuitable as habitat for macrozoobenthos. However, when we examined sediment grain size in the lagoon in the summer of 2011, we found that the tsunami had carried away the sludge to leave a sandy bottom that was much better-quality habitat for macrozoobenthos. The tsunami also caused 47 species of bivalves and other macrozoobenthos to temporarily disappear. Its impact however proved to differ markedly depending on species, with the populations of some polychaetes and amphipods rapidly recovering within several months.

In addition to species diversity, the term “biodiversity” encompasses ecosystem diversity (the existence of many different kinds of habitat) and genetic diversity (the presence of individuals with many different genetic constitutions within the same species). Coastal areas include many different kinds of habitat that contribute to the biodiversity of the whole coastal ecosystem: these habitats include tidal flats, reed beds (saltmarshes), sandy shores, sand dune vegetation, seagrass beds, rocky shores, freshwater ponds and swamps (Figure 2). The GEJE had major impacts on this ecosystem diversity. In Gamo Lagoon, for example, 84% of the reed marsh, 99% of the sand dune vegetation, and 52% of coastal forests were washed away by the tsunami. The tidal flats in the Mangoku-ura district of Ishinomaki and Hoso-ura district of Minamisanriku no longer appeared at low tide owing to seismic subsidence, and new tidal flats formed in parts of Ido-ura Lagoon in Sendai that were previously occupied by reed marsh and coastal forest. Research has revealed that this kind of loss, modification, and creation of habitat as a result of the GEJE had severe impacts on the diversity of the species living in affected coastal areas (The Ecological Society of Japan Tohoku Branch 2016).

Figure 2. The diverse habitats of coastal areas. Manila clam fishery at Matsukawa-ura (top left), reed marsh and seagrass bed at Unoo, Matsukawa-ura (top center, bottom left), sandy shore at Torinoumi, Watari, Miyagi Prefecture (top right), rocky shore at Kesennuma Bay (bottom center), Suijin Pond (freshwater pond) in Yamamoto, Miyagi Prefecture (bottom right)

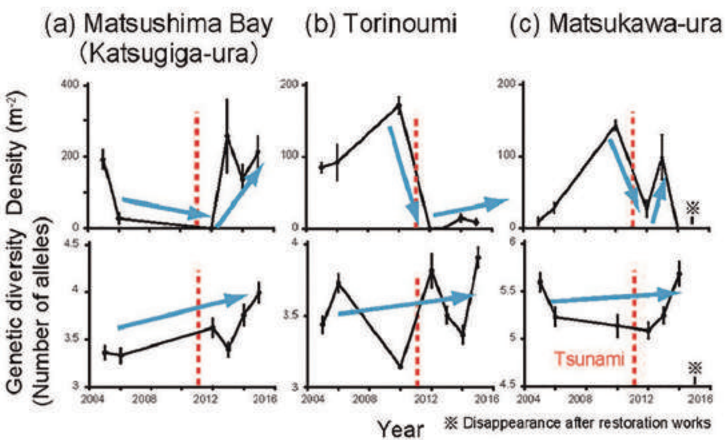

Figure 3. Change in density (top) and genetic diversity (bottom) of *Batillaria attramentaria* (Japanese mud snail) before and after the GEJE

We then conducted research on the impact of the tsunami on genetic diversity using *Batillaria attramentaria* (Japanese mud snail): despite the dramatic decrease in population caused by the tsunami, genetic diversity had not been affected (Figure 3). This indicates that while the impact of the tsunami drastically reduced the number of mud snails, it had not affected the genetic diversity of the mud snail population. Japanese mud snail density remains lower now, six years after the tsunami, than it was before, but from 2013, juvenile snails born after the tsunami have appeared, a sign that populations are beginning to recover. In Matsukawa-ura, on the other hand, post-disaster restoration work has killed off the Japanese mud snails that survived the GEJE (Figure 3c).

3 Recovery of biota

Many macrobenthic species go through a planktonic larval stage, with larvae being transported and dispersed over long distances by ocean currents. As such, recovery of biodiversity in tidal flats depends on the conservation of adult populations as a source of planktonic larvae (source populations) as well as on proper maintenance of tidal flat habitat. We therefore investigated the distribution of macrozoobenthos populations in many different tidal flats along the Pacific coast of Fukushima and Miyagi Prefectures, focusing in particular on endangered species. Our research from 2011 to 2015 found endangered species such as batillariid snails and *Chasmagnathus convexus* (marsh crab) inhabiting the Same River estuary and Matsukawa-ura in Fukushima Prefecture, and Mangoku-ura and the inner reaches of Matsushima Bay in Miyagi Prefecture (Figure 4). The annual survey of tidal flats carried out since before the GEJE by the Ministry of the Environment under its Monitoring Site 1000 program has also found the populations of macrozoobenthos in Matsukawa-ura to be steadily recovering. (Ministry of the Environment 2017). Salt marsh vegetation is also gradually recovering. The recovery of this vegetation will lead to recovery of salt marsh habitat, thereby enabling colonization by new species. These findings indicate that the habitats and biota of coastal ecosystems in GEJE-affected areas are steadily recovering over time and will eventually transition to a steady-state.

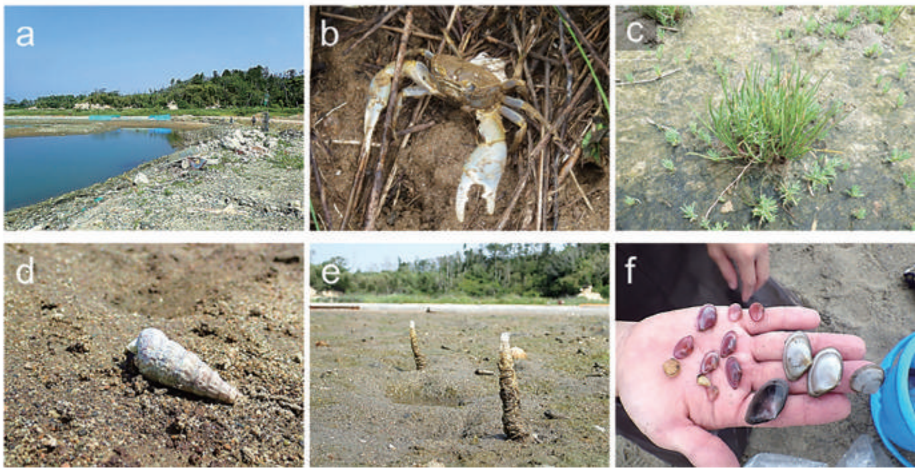

Figure 4. Endangered flora and fauna found after the GEJE. Post-GEJE Unoo tidal flat, Matsukawa-ura (a), marsh crab in Same River, Iwaki (b), marsh plant *Triglochin maritimum* in Unoo (c), mud snail *Cerithidea moerchii* in Uda River estuarine Marsh, Matsukawa-ura (d), polychaete *Chaetopterus cautus* thriving in Unoo (e), and bivalves including *Nitidotellina hokkaidoensis*, *Moerella jedoensis*, and *Macoma praetexta* in the Same River estuary (f).

4 The impact of restoration works and the conservation and restoration of tidal flats

The restoration of seawalls and shore protection structures have led to the fragmentation and loss of habitat in many places in the Tohoku region. Building stronger seawalls requires making them wider as well as taller, and this causes habitat loss as tidal flats, sandy shores and coastal forest in the vicinity get buried under the new seawalls (Figure 5a). Another major issue is the way the seawalls impede the movements of organisms. However, projects have been launched to conserve and restore tidal flats and salt marshes by changing the position of seawalls and constructing channels for the entry of seawater. The brackish environment of the conservation area of Ohsu in Matsukawa-ura has been maintained and marshland inhabited by halophytic plants restored by constructing a tunnel for seawater entry (Fig. 5b & c). Similar initiatives are underway in a number of other locations in Matsukawa-ura (Kanebuchi et al. 2017). At the mouth of the Uda River, which flows into Matsukawa-ura, farmland flooded by the tsunami has become a tidal flat (Udagawa Marsh) now serving as habitat for endangered macrobenthic species such as the mud snail *Cerithidea moerchii* and marsh crab *Chasmagnathus convexus* (Ministry of Environment 2017).

In addition to the impacts of major disasters, we are now also conducting research on the secondary impacts of GEJE-related human disturbance on the environment. Restoration works on the Pacific Coast of the Tohoku region are posing new threats to coastal ecosystems. We feel that it is our responsibility to gather data that can be scientifically evaluated, and to record and communicate what has happened in the region.

Figure 5. Photo a: Disappearance of the tidal flats as a result of seawall construction in the Same River estuary; Photos b & c: Tidal flat restoration in Ohsu conservation area, Matsukawa-ura (Photo b by Dr. Takahide Kurosawa, Photo c by Dr. Takao Suzuki

Future challenges

- The biota of the tidal flats have recovered fairly well over the past six years. However, some endangered species that were never very common have disappeared from the tidal flats since the tsunami.
- We have no previous research findings regarding how much time ecosystems require to recover from a huge tsunami. The data sets from our long-term monitoring since the GEJE should teach us many things about which we had no previous knowledge.
- The Pacific coast of Tohoku continues to be disturbed by restoration works; evaluating the impacts of such works and considering conservation measures are important challenges.

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The effect of dams in storing radiocesium

Highlights of this research

1. During the September 2015 Heavy Rainfall Event in the Kanto and Tohoku Regions, approximately 0.3% of the radiocesium in the Uda River basin in Fukushima Prefecture discharged into the estuary.

2. Matsugabo Dam reservoir stored over 90% of particulate (soil particles, litters etc…) radiocesium flowing into the dam in a year, but this form of radiocesium accumulated on the bottom of the reservoir can play a role in a source of dissolved radiocesium.

3. The amounts of radiocesium transported during heavy rainfall events should be continuously measured. We also need to clarify the origin of dissolved radiocesium in dam reservoirs, and evaluate the necessity of dredging of bottom sediments and other countermeasures.

Radiocesium from the Fukushima Nuclear Accident was deposited in high concentrations in the forests of upper catchments of rivers flowing through the northern part of Fukushima Prefecture's Hamadori region, giving rise to concern over the risk of contamination of downstream urban areas of Hamadori as a result of runoff of radiocesium from the upstream forests. Especially during heavy rainfalls, large amounts of soil particles containing radiocesium flow into rivers and streams to be accumulated in downstream riverbeds, tidal flats and the seashore. Evaluating the amount of radiocesium carried downstream during heavy rains is therefore important for ensuring the safety of people living in the region. In this article, I describe the discharge of radiocesium during the September 2015 Kanto-Tohoku heavy rainfall event that brought the highest precipitation in the northern Hamadori region since the nuclear accident, and the effect of dam reservoirs in storing radiocesium.

1 The Kanto-Tohoku heavy rainfall event of September 2015

The Kanto and Tohoku regions of Japan were hit by torrential rains from September 6 to 11, 2015. Of the areas affected by the nuclear accident, the Uda River basin spanning Fukushima and Miyagi Prefectures suffered particularly heavy precipitation, the nearby weather station at Hippo recording historical highs both for 48 hours (426.5 mm) and 72 hours (483.5 mm). This precipitation caused large-scale soil erosion and mudslides, and owing to the large amounts of soil containing radiocesium that flowed into rivers and streams, considerable amounts of radiocesium are thought to have been carried down from the catchment to the estuary at Matsukawa-ura inlet and the Pacific Ocean.

2 Amount of ^{137}Cs transported by the Uda River in Fukushima Prefecture

We have been continually monitoring the concentration of radiocesium (hereinafter describing as ^{137}Cs because the amount of ^{134}Cs was less than ^{137}Cs due to its short half-life) and suspended solids (soil particles, leaves, etc.) in river water at three points in the Uda River basin (Figure 1) since 2014. In the upper catchment area of a small forest area where we had put monitoring instruments, runoff from the torrential rainfall of September 2015 washed tree branches, soils and rocks into waterways (Figure 2), causing a great increase in the concentration of suspended solids. The runoff from this forest flowed downstream to the Matsugabo Dam, but its transport further downstream was impeded by the dam, whose discharge gates were closed until the morning of September 10. On September 11, however, the dam reservoir reached maximum capacity, requiring discharge of approximately the same amount of water as the inflow (Figure 3). This inevitably resulted in the release downstream of a certain amount of ^{137}Cs.

We accordingly used the data on suspended solid concentration, flow volume, and ^{137}Cs concentration collected at our three monitoring points to calculate the amount of ^{137}Cs transported downstream as sediment as a result of the heavy rainfall event (Table 1). The amounts of ^{137}Cs measured at all three monitoring points for the 10 days of the rainfall event exceeded those for the whole of 2014, indicating the huge amount of sediment runoff. To compare the extent of ^{137}Cs outflow at our three monitoring points, where the amount of ^{137}Cs deposited differs, we calculated ^{137}Cs runoff ratio by dividing the ^{137}Cs outflow volume in suspended sediment measured at each monitoring point by the total amount of ^{137}Cs deposited for each catchment area (i.e., the area upstream from each monitoring point). The ^{137}Cs outflow rate at the three monitoring points was 0.02 to 0.30%, indicating that most of the ^{137}Cs still remained within the upper catchment in spite of such a tremendous discharge.

Results also show the ^{137}Cs runoff ratio at the dam discharge point to be one order of magnitude less than those at the forest and other downstream points. This difference is due to sedimentation of inflow particles on the bottom bed during the process of muddy water flowing downstream in the reservoir: our results indicate that dams can be remarkably effective in stemming the downstream passage of ^{137}Cs. The high runoff ratio at the downstream point compared with the forest catchment area suggests a large impact of runoff from farmland and urban areas distributed mainly in the middle and lower reaches of the catchment. The reason for this high runoff ratio is that decontamination projects on agricultural and residential land ongoing since 2012 and construction of the Soma-Fukushima Road laid the ground bare and vulnerable to runoff of soil. Extensive erosion of agricultural and other land and accumulation of large quantities of sediment on sandbars was actually reported, particularly in the steep middle reaches of the Niida River (Minamisoma city), which flows near the Uda River. However, since no major change in air dose rates in the lower catchment area has been observed since the heavy rainfall event, the impact of contamination in the downstream area is thought to have been limited.

Large volumes of sediment were, however, carried down to the estuary and Matsukawaura inlet. Measurements of the vertical distribution of ^{137}Cs concentration in the sediment near the estuary of the Uda River in Matsukawaura inlet show that whereas in 2013 ^{137}Cs was detected only in the top 15 cm layer of sediment, but some ^{137}Cs was detected in the layer of deeper than 30 cm in December 2015, indicating the deposition of large amounts of sediment carried downstream by the Uda River as a result of the heavy rainfall event. Since ^{137}Cs would continuously be flown out and be deposited in Matsukawaura inlet and the sea beyond, we need to continue to monitor its movement and deposition particularly when the region suffers heavy rainfall.

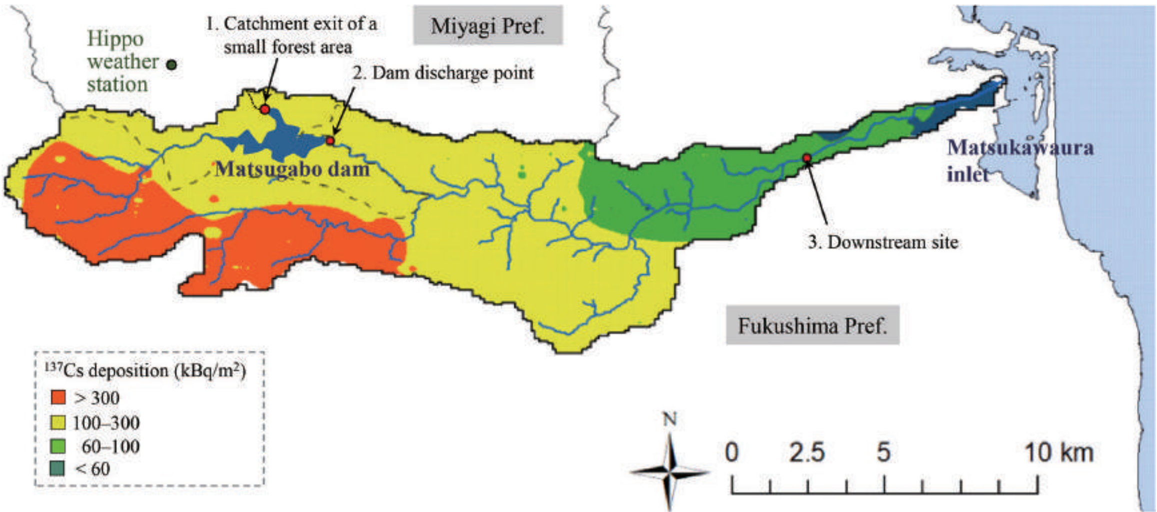

Figure 1. Distribution of ^{137}Cs deposition in the whole Uda River catchment area and our three river water monitoring points (1 to 3)

Figure 2. Water sampling point at the catchment exit of a small forest area (Point 1 in Figure 1).
Left: December 2014, Right: immediately after the heavy rains in September 2015.

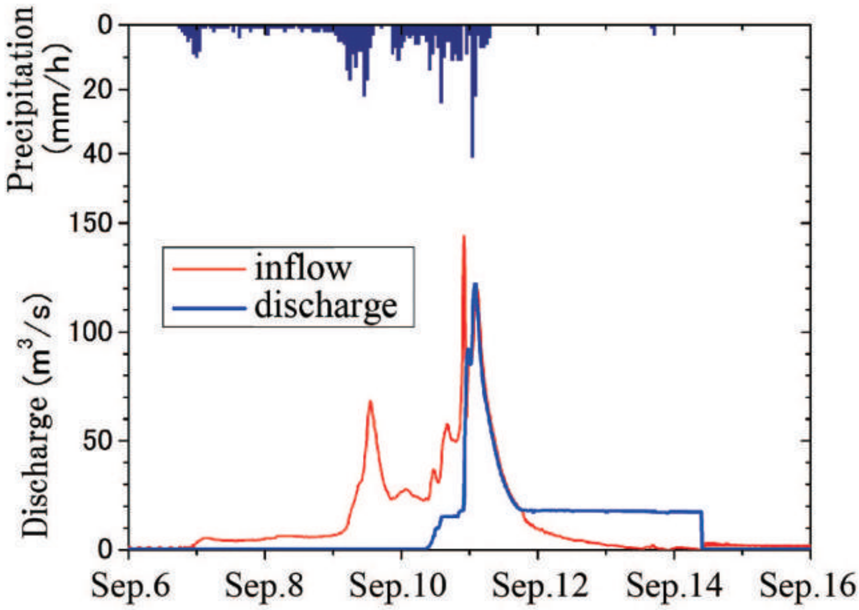

Figure 3. Changes in inflowing and discharged water volume of Matsugabo Dam from September 6 to 16, 2015

Table 1. Amount and outflow rate of particulate ^{137}Cs discharged from catchment areas for the Uda River monitoring points 1 to 3 (Figure 1).

Period	Discharge of ^{137}Cs (×10^8 Bq)			Discharge ratio of ^{137}Cs (%)		
	1. Forest catchment	2. Dam discharge point	3. Downstream site	1. Forest catchment	2. Dam discharge point	3. Downstream site
Sep.6-16, 2015	0.77	3.5	620	0.12	0.02	0.30
Jan.1-Dec.31, 2014	0.44	1.1	120	0.08	0.01	0.06

* Because the discharged amount of dissolved ^{137}Cs during the heavy rainfall event was much smaller than particulate ^{137}Cs, this data was undescribed here.

3 The effect of dams in storing ^{137}Cs

Next, the annual inflow and discharge of ^{137}Cs to and from Matsugabo Dam were calculated separately for particulate (soil particles etc.) and dissolved ^{137}Cs (Fig. 4). In 2014 and 2016, more than 95% of inflowing particulate ^{137}Cs was stored in the dam reservoir, and even 90% was stored in 2015 when the volume of sediment inflow was about twice that of an average year. Although the discharge volume and concentration of dissolved ^{137}Cs has declined year by year, outflow of dissolved ^{137}Cs was 30–60% higher than inflow from 2014 to 2016. One possible reason is that ^{137}Cs contained in the sediment that has accumulated on the bed of the reservoir since the nuclear accident may be eluted into the reservoir water. Since dissolved ^{137}Cs is easily taken into the bodies of fish and other fauna through the food chain after being ingested by microorganisms, data on the concentration of dissolved ^{137}Cs in discharged water will be important to any consideration of the migration of radiocesium to agricultural produce and fishery products.

As described above, while dam reservoirs serve to restrain the discharge of particulate ^{137}Cs, they also appear to behave as a source of dissolved ^{137}Cs. As such, to forecast the migration of ^{137}Cs to agricultural produce in areas downstream from dams, we need to clarify the mechanisms whereby dissolved ^{137}Cs forms in dam reservoirs, and also consider the possibilities for dredging dam sediment and other countermeasures as deemed necessary.

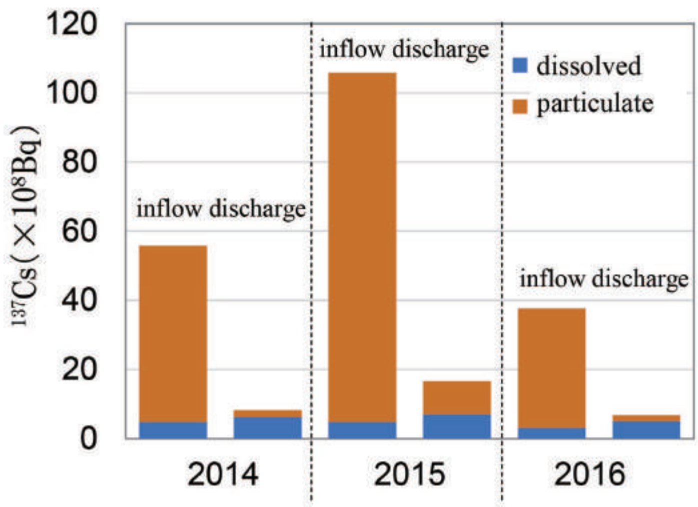

Figure 4. Yearly inflow and discharge of dissolved and particulate ^{137}Cs at Matsugabo Dam for 2014 to 2016.

Acknowledgments: Matsugabo Dam management office provided data on the volume of inflow and discharge at Matsugabo Dam.

Future challenges

- We need to continuously measure river water to monitor the amounts of radiocesium transported by rain and accumulated elsewhere, particularly during heavy rainfall event.

- We also need to clarify the origin of dissolved radiocesium in dam reservoirs, and consider the need for dredging and other countermeasures from the perspectives of cost and risk assessment.

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Monitoring terrestrial ecosystems: methods and results

Highlights of this research

1. Since 2014 we have been monitoring mammals, birds, frogs and insects in the evacuation zone established after the nuclear accident to determine whether familiar species are disappearing and whether mammal pests are increasing and beneficial insects decreasing.

2. We have released and visualized monitoring data as it has become available, and have held events that involve the public in helping to process our data.

3. Statistical analysis of our 2014 monitoring data for bee and flies indicate that there was no significant difference in the number of pollinators (which are beneficial insects) and flies and horseflies (which are pests) between the evacuation zone and surrounding areas.

1 The purpose of monitoring, and methods used

The Fukushima Nuclear Accident forced local residents to evacuate an extensive area of land. Ecosystems in the evacuation zone are expected to change as a result not only of radioactive contamination, but also because of restrictions on farming and other human activities. It is feared that with the disappearance of people, insect pollinators of vegetables and fruit will also disappear, and increasing numbers of wild boar and other wildlife that raid crops will impede the resumption of farming. Fields are gradually becoming overrun with Japanese pampas grass and non-native plant species, and there is concern that in conjunction with these visible changes, the familiar species inhabiting the surrounding satoyama habitats will move elsewhere. Since 2014 the National Institute for Environmental Studies has been monitoring mammals, birds, frogs and insects to determine whether familiar species are disappearing and whether mammal pests are increasing and beneficial insects decreasing as people fear. To collect data over a long period of time from as many locations as possible in the evacuation zone with its restrictions on the entry, we are using low-cost methods that enable automatic data sampling (Yoshioka et al. 2016). We selected species for monitoring on the basis of their relationship to the lives of people in the area, their role in the ecosystem, and the ease with which they could be monitored using automatic sampling methods. We used infrared cameras

Figure 1. Equipment used for ecosystem monitoring: (a) infrared camera trap deployed in national forest etc. to monitor mammals; (b) digital recorder deployed in elementary schools etc. to monitor birds and frogs; (c) malaise trap (left) and flight interception traps (right) for monitoring insects. A digital recorder is attached to the one of the tripods supporting the flight interception traps.

that automatically record passing creatures (camera traps) to monitor mammals, and digital recorders equipped with timer functions to monitor birds and frogs (Figures 1a, 1b).

To investigate bees, flies and other flying insects, we are using traps such as malaise traps and flight interception traps that collect such insects by blocking their flight (Figure 1c). Over the past four years, utilizing these labor-saving survey tools has enabled us to monitor about 50 locations both within and outside the evacuation zone.

2 Sharing and publishing data

We endeavor to share our monitoring data with the general public by publishing it in as transparent a form as possible. We do so to allay fears that have arisen since the nuclear accident that scientists can't be trusted. For mammals, we have published a data paper providing all our data on the instances when camera traps were activated and the frequencies at which different species were detected by them. This data set can be downloaded and analyzed by other researchers as they wish (Fukasawa et al. 2016). A distribution map based on this data set can also be viewed on the BioWM website operated by the NIES Center for Environmental Biology and Ecosystem Studies (Figure 2a). These maps can be used, for example, to obtain information about how many times wild boar have been detected each year at a particular monitoring location in the evacuation zone.

We have similarly published a data paper on birds that presents data derived from recorded birdsong (Fukasawa et al. 2017), together with a distribution map viewable on our KIKI-TORI MAP website (Figure 2b). We also hold public participation Bird Data Challenge events with bird lovers in Fukushima Prefecture to identify and collate species from recorded birdsong. These events are a key part of the system we have built to boost the transparency of our data processing operations and at the same time network with naturalists in Fukushima Prefecture and enable them to deepen their knowledge (Fukazawa et al. 2017).

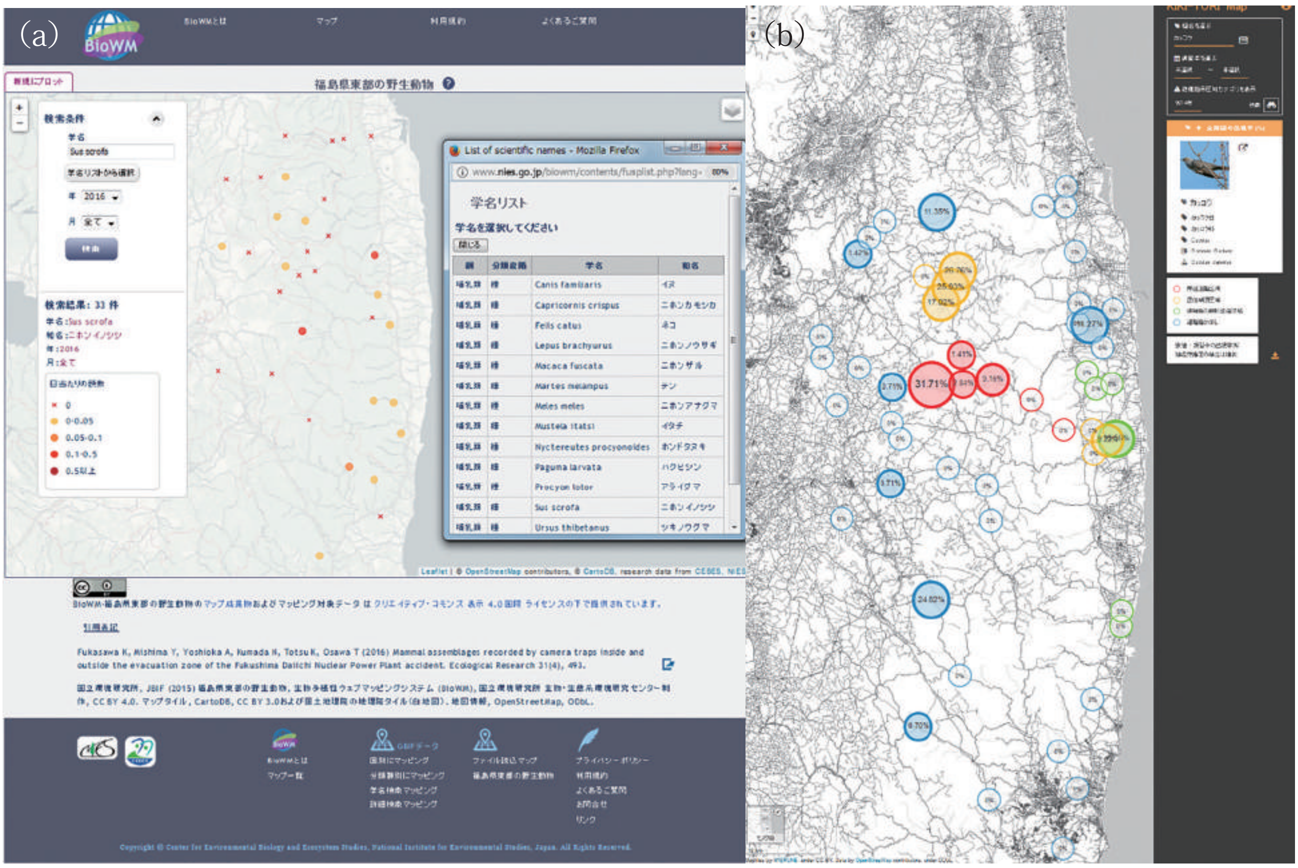

Figure 2. Species distribution data available in map form on the NIES website: (a) BioWM map of mammal distribution in eastern Fukushima (http://www.nies.go.jp/biowm/contents/fukushima.php?lang=en);
(b) KIKI-TORI MAP website providing bird species distribution data in map form (http://www.nies.go.jp/kikitori/contents/map/) (Japanese only)

3 Differences between inside and outside the evacuation zone: flying insects

In addition to publishing our monitoring data, we statistically analyze the same data to compare populations of species inside and outside the evacuation zone. Statistical analysis enables us to infer whether monitoring results showing that a particular species is less or more common inside the evacuation zone than outside are pure coincidence. Since there was a paucity of findings on how the GEJE impacted populations of flying insects, we use the monitoring data for 2014 to statistically analyze and compare data on 46 key taxa and species populations inside and outside the evacuation zone (Yoshioka et al. 2015).

Our analysis reveals Japanese carpenter bees to be the only species that was conspicuously scarce in the evacuation zone, and no particularly noteworthy differences in observed numbers of other taxa inside the evacuation zone (Figure 3). Because the radiation dose at monitoring locations is unlikely to be fatal to insects (Garnier-Laplace et al. 2011, 2013), the decline in Japanese carpenter bee numbers may reflect a decrease in horticultural plants and other usable resources as a result of evacuation of the human population. Some species and taxa were more common inside the evacuation zone than outside. This may be due to the colonization of fields and school playgrounds by weeds that provide both food and habitat to such insects.

Overall, our flying insect monitoring results indicate that at least as of 2014, there had been no major change in the balance of beneficial and pest insects within the evacuation zone. They neither suggest any conspicuous loss of biodiversity among bees and other pollinators, nor do they point to the kind of outbreak in fly and horsefly populations that occurred immediately after the GEJE in areas hit by the tsunami (Hayashi et al. 2012).

However, because this analysis is based on only a single year, caution should be exercised in interpreting our results as firm evidence of an increase or decrease in a particular species since evacuation. We will also likely see new

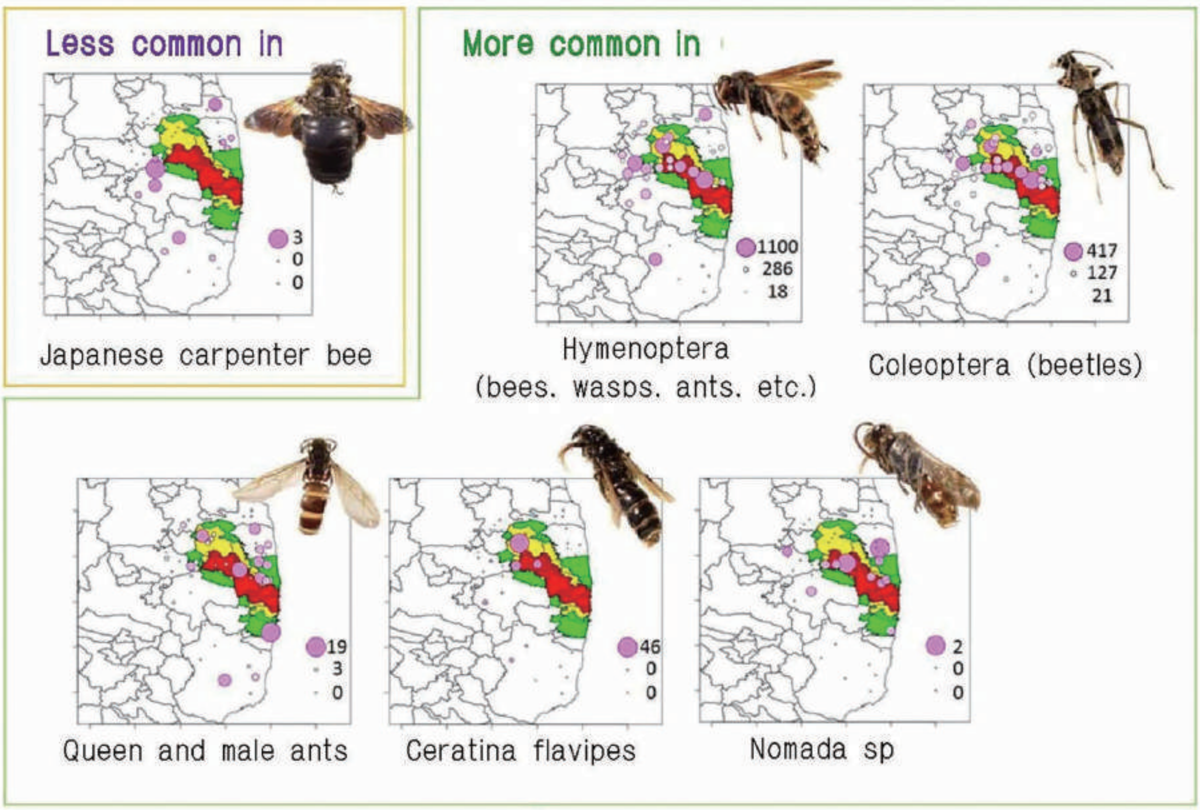

Figure 3. Distribution patterns of species and taxa shown by the statistical analysis conducted on 2014 monitoring data by Yoshioka et al. (2015) to be markedly less or more common in the evacuation zone. The size and intensity of color of the pink circles correspond to the number of individuals collected by malaise traps. At bottom right are the maximum, median, and minimum population values with corresponding circle size and color. The red, yellow, and green areas of the map show the zone deemed difficult for evacuees to return to (red), zones where habitation is restricted (yellow), and zones being prepared for lifting of the evacuation order (green) as designated at that time.

changes in insect communities as a result of natural succession of vegetation in abandoned farmlands and other environmental changes accompanying prolonged evacuation.

Future challenges

- Now that evacuation orders are being lifted over an increasingly wide area, we need to continue monitoring in such locations to investigate the impacts of evacuation. Moreover, since our current monitoring methods have not enabled us to adequately monitor paddy field insects and some other species, we also hope to develop and implement labor-saving methods for monitoring paddy fields to ascertain the impact of resumption of rice cultivation.

- We also plan to publish our monitoring data on frogs and vegetation (land use), and aim to work with researchers in other fields to study the declining utilization of satoyama areas as a result of population decline, and accompanying socioeconomic impacts.

- We will continue to analyze and model the data we have obtained on other organisms as well as insects, and will make every effort to disseminate even more reliable information.

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Development of plants that enable radiation-derived DNA lesions to be spotted with the naked eye

Highlights of this research

1 We created genetically modified plants and cultured cells derived from them that enable visual and quantitative detection of repairs to DNA lesions caused by radiation from contaminated soil.

2 The number of plant DNA lesions increased in proportion to soil radiation dose. Our results indicate that DNA damage is caused mainly by external exposure to radiation.

3 We found that the risk of accumulation of DNA mutations due to soil-derived radiation is no greater than normal background levels at least when the air dose rate remains below 5.6 μ SV/h.

We created genetically modified plants and cultured cells derived from them that enable visual and quantitative detection of repairs to DNA lesions caused by radiation from soil contaminated by the Fukushima Nuclear Accident. We used these materials to investigate whether or not damage to DNA currently being caused by radiation in the soil of Fukushima Prefecture exceeds the repair capacities of plants, resulting in the accumulation of mutations in plant DNA. Based on our results, we also created a DNA mutation accumulation risk map for the zone designated as difficult-to-return zone.

1 Creation of plants that enable detection of DNA repair

The DNA of living organisms is subject to constant damage from environmental stress, but organisms are equipped with mechanisms for quickly repairing lesions by themselves. If, however, this balance is disturbed, and the DNA damage rate exceeds repair rate as a result of, for example, a sudden steep increase in environmental stress, DNA lesions accumulate, resulting in mutations, albeit at a low probability. When mutations occur in certain genes, they may result in the appearance of mutants such as mophological abnormalities and so on (Figure 1). Radiation emitted by radioactive substances deposited in soil after being released into the atmosphere by the Fukushima Nuclear Accident can also damage the DNA of living organisms, and morphological abnormalities have been found in some organisms in areas of Fukushima Prefecture with relatively high radiation levels (Hiyama et al. 2012; Akimoto 2014; Watanabe et al. 2015). The discovery of such morphological abnormalities is giving rise to concern over the accumulation of DNA mutations in organisms inhabiting areas of Fukushima Prefecture with high radiation levels, but DNA mutations that could cause such morphological abnormalities have not yet been identified in specimens showing those abnormalities. This is because, even if decoding the genetic information of organisms showing morphological abnormalities within a wild population turns up DNA mutations, judging with any accuracy whether such mutations have been caused by radiation or by some other factor is very difficult. How, then, can we assess the accumulation of DNA mutations in organisms inhabiting areas of Fukushima Prefecture with high radiation levels?

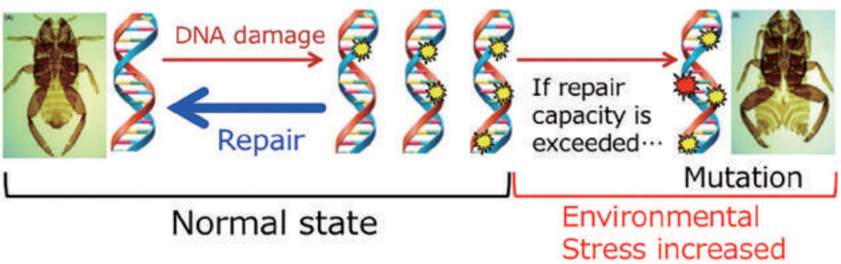

Figure 1. DNA damage and its repair, and expression of mutant traits.

We decided to assess DNA mutation accumulation by examining the relationship between the amount of damage to DNA caused by radiation from the soil of Fukushima Prefecture and the DNA repair capacity of organisms. In other words, if radiation-derived DNA damage remains within the range of repair capacity, there will be a positive correlation between radiation dose and the number of repaired DNA lesions, but if the level of DNA damage exceeds DNA repair capacity, the number of repaired DNA lesions will not increase even if radiation dose increases. This will likely result in accumulation of DNA damage and boost the probability of mutation. To investigate the balance between the generation of DNA damage and repair capacity, we transferred a gene to *Arabidopsis thaliana* (thale cress), a popular model plant, to create genetically modified (GM) plants that enable detection of DNA repair. The gene that we introduced was GU-US, a gene designed to enable detection of repairs effected through homologous recombination, a process used in very similar DNA nucleotide sequences to repair double-strand breaks (DSBs), a type of DNA lesion caused by radiation (Fig. 2A left). The GU-US is derived from the β -glucuronidase (GUS) gene isolated from *E. coli*. If normal GUS protein is supplied with a substrate, cells will be stained blue-green, but because GU-US is designed to cut the GUS gene in half and enable part of the central section to overlap, thus the GU-US could not produce normal GUS protein and cells are not stained. When cells of the transgenic plants are exposed to radiation, DSBs occur with a certain probability within genomic DNA. These DSBs are repaired by homologous recombination in the cells in which they are present, but at the same time, the introduced GU-US gene is also repaired, reverting as a result to the original GUS gene, and thereby causing the cell to be stained blue-green (Figure 2A right). Counting the number of blue-green spots (GUS spots) appearing per individual plant can furnish a quantitative measure of the extent of DNA damage and repair (Figure 2B).

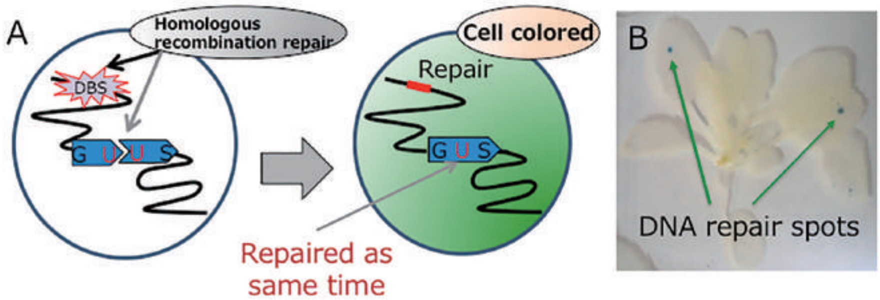

Figure 2 A: Recovery of GU-US gene function through homologous recombination repair.
B: An example of DNA lesion repair detected by using genetically modified plants.

2 Detection of repaired DNA lesions by indoor cultivation in contaminated soil

We cultivated the transgenic plants that we made in contaminated soils taken from three locations in Fukushima Prefecture with different air dose rates and in clean soil to investigate whether these plants would enable us to quantitatively assess DNA repair. We cultivated the plants in treatment lots of 75–100 individuals for 30 days. During this time, the plants received cumulative radiation doses of 57.6 (clean soil), 261, 1340, and 2840 μGy, beginning with lowest dose. On GUS-staining the plants after cultivation, we found the number of DNA lesion repair spots per plant to be 2.5 ± 0.17, 12.99 ± 0.58, 17.62 ± 1.35, and 22.74 ± 1.28 in the order of lowest to highest cumulative radiation dose, respectively, indicating that DNA lesions increase in line with cumulative radiation dose (Figure 3). This result shows that our GU-US transgenic *Arabidopsis thaliana* plants could be used to quantitatively assess DNA damage and repair.

Plants cultivated in soil contaminated with radioactive substances are exposed to both external radiation from the contaminated soil and internal radiation from the absorption of those substances through their roots. To ascertain the respective impacts of external and internal exposure, we cultivated plants for 30 days in clean soil while using contaminated soil as a radiation source to observe the impact of external exposure alone. We also cultivated plants in contaminated soil to observe the impact of radiation on plants exposed to both external and internal radiation. We counted 29.6 ± 3.3 DNA lesion repair spots per plant in plants cultivated in contaminated soil (internal exposure + external exposure), and 32.6± 8.5 spots per plant in plants cultivated in clean soil (external exposure only). The fact that plants cultivated in contaminated soil accumulated 361 Bq/kg of ^{134}Cs and

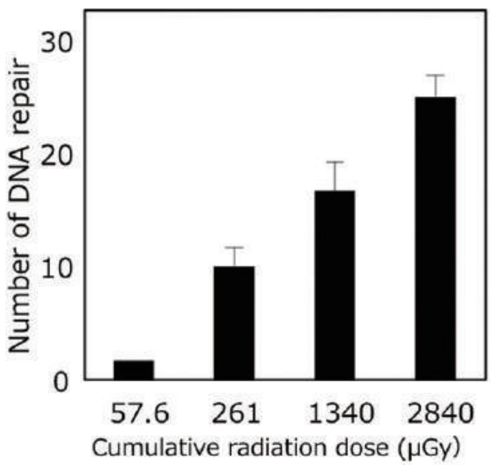

Figure 3. Cumulative radiation dose and number of DNA repair spots.

686 Bq/kg of ^{137}Cs suggest that they were exposed also to internal radiation, but statistical analysis of our results showed no significant difference in the number of DNA lesion repair spots according to presence or absence of internal exposure, indicating that radiation-derived DNA damage in plants is caused mainly by external exposure.

3 Assessing DNA mutation accumulation in contaminated soil in the field using cultured cells

Because indoor cultivation experiments with contaminated soil involve cultivating the plants in soil spread over a limited area, the air dose rate is inevitably lower than in the field. This makes it difficult to verify assessments of DNA mutation accumulation for areas with high cumulative radiation doses by means of indoor experiments. Although it would not be impossible to cultivate the transgenic plants that we created for this study in the field, maintenance of the plants would be difficult owing to the need to cultivate them in the zone designated as difficult-to-return zone; DNA damage caused by ultraviolet radiation present in sunlight would lead to overestimation of DNA lesions caused by radioactivity; and cultivating GM plants in the field may impact the ecosystem in some way. To resolve such concerns, we made cell cultures from our GU-US transgenic plants (Fig. 4A). Since the cells are cultured in Petri dishes with a nutrient source, they can be left for about one month without requiring any maintenance. The Petri dishes can also be buried in the ground to avoid exposure to UV light present in sunlight. To confirm the feasibility of assessing DNA lesion repair in the cultured cells in the same way as for plants, we transplanted cultured cells to Petri dishes and then cultured them for 30 days in contaminated soils with different air dose rates. Our results show a significant positive correlation between the number of DNA lesion repairs and cumulative dose rate (R^2=0.80). This shows that it would indeed be feasible to use cultured cells derived from GU-US transgenic plants to quantitatively assess DNA lesion repair.

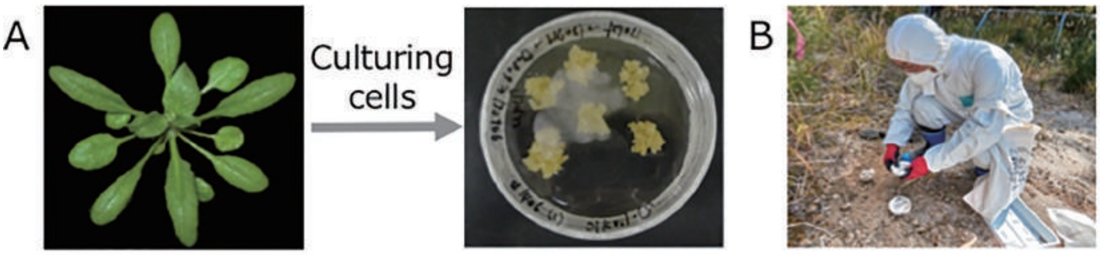

Figure 4. A. Culturing cells from plant containing the GU-US transgene. B. Burying Petri dishes in the ground sites in the field.

We accordingly used these cultured cells to conduct a field experiment that involved burying Petri dishes into which cultured cells had been transplanted 5 cm below the soil surface at the following three locations in Namie, Fukushima Prefecture with various air dose rates: in order of lowest to highest air dose rate, Kakura Community Hall (N37°29′54″ E140°57′48″, air dose rate 0.7 μSv/h), Tsushima Elementary School (N37°33′38″ E140°45′19″, air dose rate 3.3 μSv/h) and Teshichiro Community Hall (N37° 35′ 46″ E140° 45′ 13″, air dose rate 6.6 μSv/h). We transplanted 10 cultured cell blocks to each Petri dish, and buried three Petri dishes together with a cumulative dosimeter at each location (Figure 4B). The Petri dishes were buried in the ground from September 29 to October 25, 2016, making for a total burial time of 620 hours. The cumulative radiation doses during this period were 0.34 mGy at Kakura Community Hall, 1.72 mGy at Tsushima Elementary School, and 3.47 mGy at Teshichiro Community Hall. We retrieved the cultured cells from the Petri dishes, GUS-stained them and recorded the number of DNA lesion repair spots for each Petri dish. We then measured the fresh weight (FW) of the cultured cells, and calculated the number of DNA lesion repair spots per unit FW. Our results were 9.1 ± 9.2 spots/gFW for Kakura Community

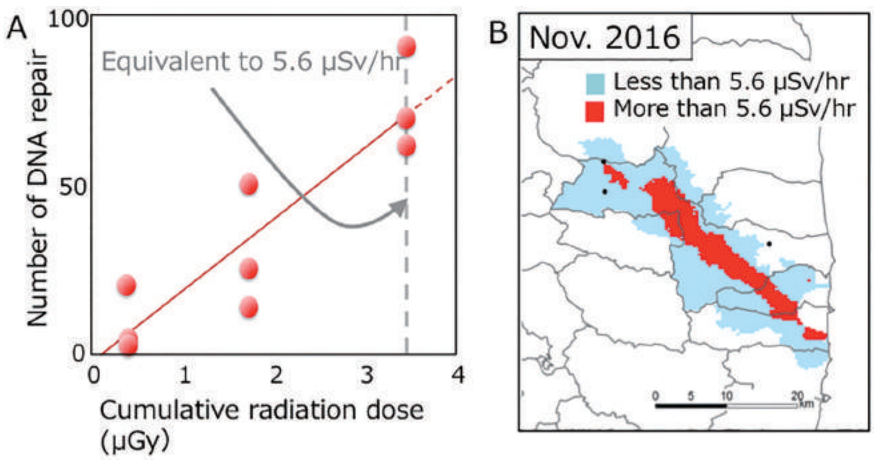

Figure 5. A. Correlation between cumulative radiation dose and number of DNA repair spots using cultured cells. B. Risk map of DNA mutation accumulation in difficult-to-return zone.

Hall, 31.9 ± 16.2 spots/gFW for Tsushima Elementary School, and 78.0 ± 13.7 spots/gFW for Teshichiro Community Hall. We found a significant positive correlation between cumulative radiation dose and number of DNA lesion repair spots (R^2=0.81). This result confirms the feasibility of using cultured cells to quantitatively assess DNA lesion repair in the field.

Can we draw any conclusions from this research as to the likelihood of DNA mutations accumulating in locations with relatively high air dose rates in present-day Fukushima Prefecture? As mentioned above, if radiation-derived DNA damage remains within the range of repair capacity, there will be a positive correlation between radiation dose and the number of DNA lesion repairs, but if the level of DNA damage exceeds DNA repair capacity, the number of DNA lesion repairs will not increase even if radiation dose increases. This will likely result in accumulation of DNA damage and boost the probability of mutation. The results of this study showed a significant positive correlation between cumulative radiation dose and the number of DNA lesion repairs, indicating that the risk of accumulation of DNA mutations has not increased, at least within the scope of the air dose rates investigated for this study. In other words, since the maximum dose rate to which cultured cells were exposed in this study was 5.6 μV/h, the risk of accumulation of DNA mutations will not increase at dose rates lower than this value (Figure 5A). We accordingly used the results of the 11th Airborne Monitoring Survey conducted by the Nuclear Regulatory Commission from September 14 to November 18, 2016 to investigate the distribution of areas within the zone designated as difficult for evacuees to return to in which air dose rate exceeds 5.6 μSV/h. We found that of the 6288 squares of the 250-m grid covering the zone, 5194 were below 5.6 μSV/h (Figure 5B). This indicates that at least as of November 2016, the risk of accumulation of DNA mutations will not increase in 82.6% of the zone.

Future challenges

- We will continue our field experiments, burying cultured cells in soil so as to determine the threshold air dose rate at which DNA mutations will start to accumulate as a result of soil-derived radiation.
- We will develop genetically modified plants and cultured cells that enable detection of DNA lesion repairs while keeping the specimens alive so as to enable long-term assessment of DNA repair in the same individuals.

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LATEST UPDATE ON ENVIRONMENTAL EMERGENCY RESEARCH is a publication aimed at presenting the latest outcomes of the environmental emergency research being pursued at NIES Fukushima Branch to people working on the front lines of efforts to resolve the many different problems caused by disasters. We endeavor to outline the structure of the issues we are tackling, and present leading related research outcomes in an easily digestible format. This publication will hopefully contribute to the resolution of frontline issues related to disasters and the environment, and to the restoration of a safe and stress-free living environment.

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