Studies on Radiation Effects from the Fukushima Nuclear Accident on Wild Organisms and Ecosystems

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Abstract

Many past genetic and ecological studies on wild organisms around Chernobyl have demonstrated significant genetic, physiological, developmental, fitness and population effects resulting from exposure to radioactive contaminants. Similarly, several studies on biological impacts of radiation have also been conducted since the Fukushima accident, including on genetic damage, abnormality rates and population abundance. In Fukushima Prefecture, increased morphological abnormalities have been observed in some wild organisms (e.g., butterflies, gall-forming aphids, firs and red pines). Radionuclide-contaminated soil or marine water has also had physiological impacts on mortality in butterflies and goshawks as well as on blood components in marine birds and Japanese monkeys. Moreover, population censuses of birds, butterflies and cicadas have indicated that the abundance of these species declined significantly with increased levels of radiation. Overall, these impacts on wild organisms from radiation were detected within two to three years after the accident. Apart from the direct impact of radiation, biota monitoring inside and outside the evacuation zone in Fukushima Prefecture has shown that populations of some wild animals and insects have also been affected by decontamination activities and/or the large-scale evacuation. Hereafter, it will be increasingly significant to continue monitoring biota in land and coastal areas of Fukushima Prefecture, and more detailed studies of the impact on wild organisms from radiation are also needed.

Key words: ecosystem, evacuation zone, impact of radiation, wild organisms

1. Introduction

Massive amounts of radioactive materials were released into the environment as a consequence of the so-called “Fukushima accident” that occurred at the Fukushima Daiichi Nuclear Power Plant (FDNPP) following the Great East Japan Earthquake and tsunami (TEPCO, 2012). Most of these materials fell into the Pacific Ocean owing to the wind direction at the time, but some of them showered down on costal land areas. As a result, radionuclide soil contamination occurred, mainly in Fukushima Prefecture (hereafter, Fukushima). Among the radioactive materials deposited on the ground, cesium (Cs)-137 is the major radionuclide of concern since its half-life is relatively long in comparison to other radionuclides released from the FDNPP ($T_{1/2}$ = 30.1 years, Chino *et al*., 2011). In addition, Cs-137-contaminated soil binds strongly to clay, and the migration rate of clay-bound Cs-137 shows low mobility, less than 1 cm per year (Matsuda *et al*., 2015; Takahashi *et al*., 2015), suggesting that the major portion of Cs-137 is distributed in the upper layer of the soil column, within 10 centimeters of the surface. Cs-137 can emit gamma (γ) rays, hence unusually high air dose rates continue over land areas. Furthermore, although the total amount radionuclides released into the coastal area has decreased, they have continued to spread from the FDNPP following the accident through underground water. As a result, all the wild animals and plants there have been left behind in a high-radiation-dose area, and are still exposed to Fukushima radiation. It is, therefore, of high concern whether any adverse effects are being found in wild organisms in the Fukushima area resulting from long-term, low-dose exposure to radiation. Many investigations have tried to determine levels of contamination with radioactive materials or estimate radiation exposure doses in terrestrial organisms living in Fukushima (Garnier-Laplace *et al*., 2011; Strand *et al*., 2014). Yet there are few studies on the impacts of environmental radiation on wild organisms. Moreover, it is well-known that populations of wild organisms are highly affected by human activities. The Japanese government designated “Areas in which residents are not permitted to live” and

"Areas where it is expected that residents will face difficulties in returning for a long time" near the FDNPP that have annual integrated radiation dosages exceeding 20 mSv. The result has been massive, long-term evacuation from these areas. Although radiation levels in most of the evacuation zone are not considered acutely lethal to wildlife (Garnier-Laplace *et al*., 2011), it is thought that changed land use due to decontamination activities and the cessation of daily activities such as agricultural and horticultural work significantly affect wild organisms and ecosystems in these areas. This paper reports on recent advances in research on radiation effects on wild organisms and ecosystems in Fukushima (Table 1). Much space is devoted here in particular to discussing the effects of radiation on morphogenesis and populations of insect and bird species because these issues were studied intensively soon after the Fukushima accident. Studies

Table 1 Overview of radiation or evacuation impacts from the Fukushima accident on wild organisms.

Taxon	Species	Effects	Reference
Insects	Pale grass blue butterfly	Reduced male forewing size and more severe abnormalities found in F1 offspring from female first-voltine than their parents.	Hiyama *et al*., 2012
		More severe abnormalities found in adult butterflies collected in the fall of 2011 than in the spring of 2011.	Hiyama *et al*., 2013
		Recovery of abnormality rate to normal levels after the fall of 2012.	Hiyama *et al*., 2015
		Increased mortality and abnormality rates in larva when fed *Oxalis corniculata* collected from the Fukushima area.	Nohara *et al*., 2014a
		Some abnormal phenotypes considered due to internal exposure inherited by the next generation	Nohara *et al*., 2014b
	Gall-forming aphid	Higher morphological abnormality rate in Fukushima in the spring of 2012.	Akimoto, 2014
	Earthworms	Increased DNA damage found in the species collected from a highly contaminated area (2.85 μSv/h) in the summer of 2012.	Fujita *et al*., 2014
	Butterflies and cicadas	Decreased population abundance with increased radiation dose rate levels.	Møller *et al*., 2013
	Carpenter bees	Abundance of the species lower within the evacuation zone than outside. The difference in abundance may be due to reduced human activity rather than increased radiation doses.	Yoshioka *et al*., 2015
	Small bees, wasps and beetles	These species more common inside the evacuation zone. This may be due to increased wild plants providing food and nesting materials.	Yoshioka *et al*., 2015
Birds	Barn swallow	Reduced population and juvenile fraction, but no DNA damage detected in peripheral erythrocytes.	Bonisoli-Alquati *et al*., 2015
	Streaked seawater	No effect on fledging mass but reduced blood levels of vitamin A observed in the species captured at Mikura Island (approximately 220 km south of Tokyo).	Uematsu *et al*., 2014
	Common birds	Abundance of 14 common bird species decreased with increased radiation dose.	Møller *et al*., 2012b
	Goshawk	Decreased reproduction performance in the North Kanto area (approximately 100 km from FDNPP).	Murase *et al*., 2015
Mammals	Japanese monkey	Lower blood components found in Fukushima population compared to Shimokita population.	Ochiai *et al*., 2014
	Bull	No histological change in sperm cells and testes of bulls abandoned with 20 km of the FDNPP.	Yamashiro *et al*., 2013
	Japanese field mouse	No significant increase in frequencies of apoptotic cells and morphologically abnormal sperm even though the mice were captured in highly contaminated parts of Fukushima (4.1 to 13.9 μSv/h).	Okano *et al*., 2016
Amphibians	Frogs	No abnormalities found in gonadal tissues of frogs captured in contaminated areas.	Matsushima *et al*., 2015
Mollusks	Rock shells	No rock shells found in intertidal zone within 20 km of the FDNPP.	Horiguchi *et al*., 2015
Plants	Japanese fir	Irregular branching at the main axis, resulting termination of the main shoot or forking of lateral shoots.	Watanabe *et al*., 2015
	Japanese red pine	Increased cancellation of apical dominance with increased absorbed dose rate.	Yoschenko *et al*., 2016

of radiation impacts on mammal and plant species are also taken up in this paper. Those studies include a hematological study of wild Japanese monkeys inhabiting forested areas of Fukushima City, a histological study of reproductive organs and cells of bulls within 20 kilometers of the FDNPP and a study of morphological changes in conifer trees (fir and red pine trees). Finally, this paper also presents a summary of ongoing research projects on radiation impacts in wild organisms and terrestrial biodiversity monitoring performed by the National Institute for Environmental Studies (NIES).

2. Estimation of Exposure Doses to Terrestrial and Marine Organisms in Fukushima

Garnier-Laplace *et al.* (2011) reconstructed the radiological doses received by forested land (based on soil samples collected from the area of greatest atmospheric deposition, Iitate Village, measured on March 31, 2011) and marine biota (based on seawater concentrations of iodine (I)-131 and Cs-137 on March 30, 2011, measured 330 m offshore). They estimated that wild organisms inhabiting areas near the FDNPP were exposed to maximum radiation dose rates ranging from 1 to 3.9 mGy/day in terrestrial organisms (lowest among plants and highest among forest rodents) and from 2,600 (benthic biota) to 4,600 (macroalgae) mGy/day among marine organisms during first 30 days after the accident (Fig. 1). Moreover, almost all of the radiation was derived from short-lived radioisotopes such as I-131 (8-day half life). From three months after the accident, the exposure dose rate of radiation in wild organisms decreased to 0.24 to 1.68 mGy/day due to the radioactive decay of short-half-life contaminants (Strand *et al.*, 2014). Thereafter, Cs-134 and Cs-137, that have long half lives, became the main sources of radiation for wild organisms in Fukushima. The International Commission on Radiological Protection (ICRP) defined an agreed upon set of reference organisms and presented information on dose consideration levels for assessing and managing radiation exposures in wild organisms (ICRP, 2008). With reference to the derived consideration reference levels from the ICRP report, radiation exposure doses in the first month after the accident seem to have some influence on terrestrial organisms such as conifers

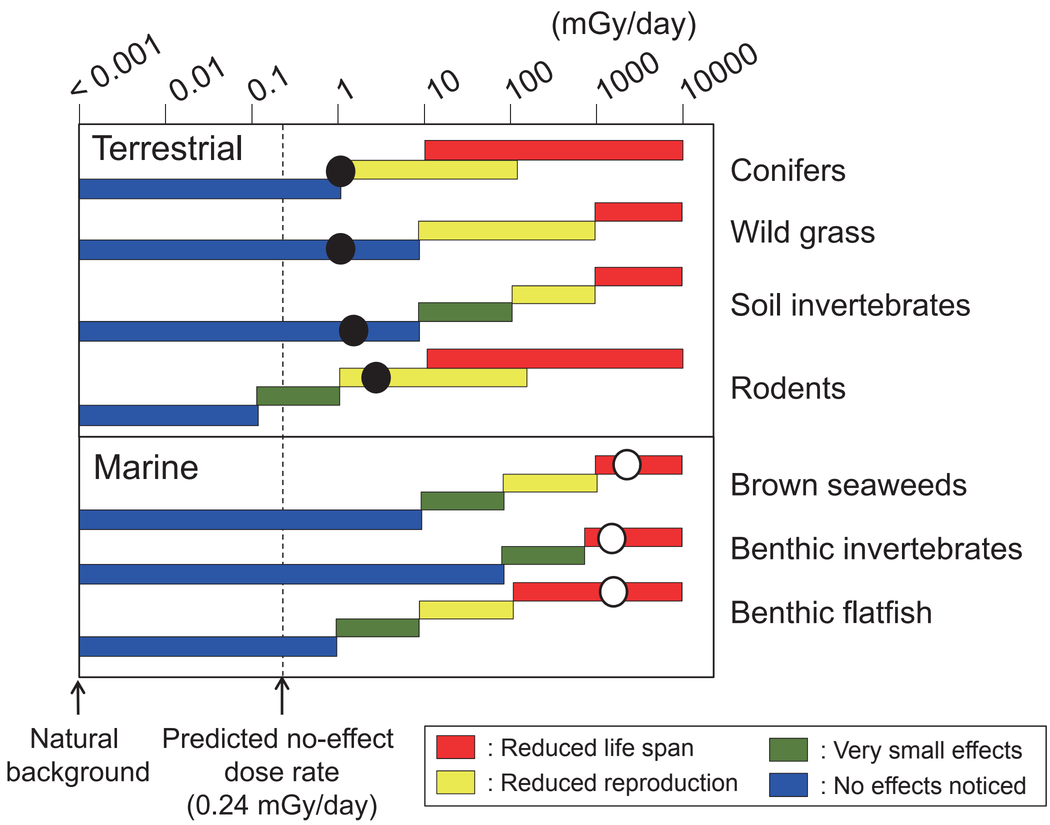

Fig 1: Maximum exposure doses in wildlife during the first month after the Fukushima accident, and ICRP effect levels. Maximum exposure dose rates in soil and seawater during the first month after the Fukushima accident were estimated from radionuclide concentrations in soil and seawater, respectively, and these values were plotted on a scale of potential effects in terrestrial (upper) and marine (lower) biota based on data from the ICRP (2008). Potential effects of radiation on organisms as proposed by the ICRP are represented as follows: no effects noticed (blue bars), very small effects (green bars), reduced reproduction (yellow bars), and reduced life span (red bars). Maximum exposure dose rate values estimated from radionuclide concentrations in soils are represented by closed circles, and those in seawater, by open circles. This figure was redrawn from Garnier-Laplace, *et al.* (2011).

and rodents (Fig. 1). In marine organisms, radiation exposure doses in the first month after the accident reached a level that threatened their life span (Fig. 1). The presumed radiation exposure dose indicates a possibility of severe effects on marine organisms. No such adverse effects on marine biota, however, have been reported, except among the intertidal biota (Horiguchi *et al*., 2015). Detailed results of the field observations of intertidal biota around the FDNPP are described later in this review (see Section 4.2).

3. Impact of Radiation from the Fukushima Accident on Wild Organisms

3.1 Radiation Effects on Insect Species

The pale grass blue butterfly is the wild species most well-researched for radiation impacts on terrestrial organisms in Fukushima. Hiyama *et al*. (2012) showed that the first-voltine adults of the butterflies collected in the Fukushima area in the spring of 2011 exhibited a relatively higher rate of mild abnormalities than those collected in other places, and the male forewing size showed a negative correlation with the radiation dose at the collection site. The first filial generation of first-voltine females showed more severe abnormalities than their parents, suggesting that the abnormalities were inherited by the next generation. Furthermore, adult butterflies collected in the fall of 2011 showed more severe abnormalities than butterflies collected in the spring of 2011 (Hiyama *et al*., 2013). These abnormal phenotypes observed in the field were experimentally reproduced by simulating the breeding conditions under which they occurred, with external γ-ray exposure from Cs-137 and internal exposure through feeding of highly contaminated *Oxalis corniculata* leaves collected from the Fukushima area. From the two studies, Hiyama *et al*. (2013) concluded that artificial radionuclides from the FDNPP caused physiological and genetic damage to this species. It is noteworthy that the abnormality rate in field-collected adult butterflies recovered to its normal state in Fukushima after the fall of 2012 although it peaked in the fall of 2011 (Hiyama *et al*., 2015). This suggests that abnormal phenotypes observed in the butterfly are considered a transitory event just after the accident and may not result in the population of the species deteriorating. As described in the previous section, high levels of exposure to radiation occurred during the first 30 days after the accident. Thus, the high rate of abnormalities in the butterflies found in 2011 is considered to be the result of early exposure to high levels of radiation.

The research group also performed vigorous studies for understanding the biological impacts of ingested radioactive materials (replicating internal exposure). The mortality and abnormality rate in larva of the pale grass blue butterfly fed *O. corniculata* leaves collected from areas in Fukushima increased sharply at the lower dose range of the ingested radioactive Cs (Nohara *et al*., 2014a), and some of the abnormalities found in first generation of the butterfly succeeded in the next generation (Nohara *et al*., 2014b). From these experiments, it is concluded that the ingestion of a radioactive-Cs-contaminated diet has cumulative effects on this species and has the possibility of resulting in deterioration of the population.

In other insect species, morphological abnormalities in two independent gall-forming aphids, *Tetraneura sorini* and *T. nigriabdominalis*, were found in the spring of 2012 (Akimoto, 2014). The abnormality rate of *T. sorini* collected in Fukushima was higher than in those captured in a control area (average abnormality rate of 13.2 percent in Fukushima and 3.8 percent in the control area). The second-generation of larvae produced in *T. sorini* galls from Fukushima, however, showed few abnormalities (0.37%), suggesting that the abnormalities found in the first generation were not inherited by the next generation. Furthermore, the morphological abnormalities in the aphid species collected in the spring of 2013 had significantly improved in comparison to those in the spring 2012 samples. Akimoto (2014) concluded that the improvement in abnormalities found in the spring of 2012 in Fukushima might be caused by the reduction of radiation levels and/ or selection for radiation tolerance. Acquisition of radiation tolerance in gall-forming aphids may be attributable to the high reproductive rate of the species. The other study also demonstrated that earthworms (*Megascolecidae*) collected from an area with a high air dose rate (2.85 μSv/h) in the summer of 2012 exhibited a significantly greater extent of DNA damage than those from a low dose rate (0.28 μSv/h) area in Fukushima (Fujita *et al*., 2014). The extent of DNA damage in wild boars (*Sus scrofa leucomystax*) showed no significant difference between individuals captured in the same regions, suggesting that the risk of DNA damage from radiation varies among species because of differences in foraging habits.

As for the effects of radiation on the abundance of insect populations, censuses of spiders, grasshoppers, dragonflies, butterflies, bumblebees and cicadas were performed at 300 sites in forested areas west of the evacuation zone in Fukushima (Møller *et al*., 2013). The abundance of butterflies and cicadas declined significantly with increased air dose radiation in Fukushima, while there were no effects on the numbers of grasshopper, dragonflies and bumblebees. Interestingly, the abundance of spiders showed a positive correlation with the air dose rate at sites where they were collected. These tendencies toward abundance due to radiation in Fukushima were not particularly seen in Chernobyl, because all of the insects investigated in this study showed a negative correlation in Chernobyl between their abundance and the air dose rate in the area. So far, the cause of these differences in radiation-dose-dependent changes in abundance of insects between Fukushima and Chernobyl remains unclear. It would be especially interesting to study the positive correlation between the number of spiders and the air dose rate found in Fukushima.

3.2 Impacts of Radiation on the Fitness and Abundance of Bird Species

After the Chernobyl accident, the barn swallow (*Hirundo rustica*) was the wild bird species most well-researched for effects of radiation. Radiation-dose-dependent abnormalities were observed in birds in the Chernobyl exclusion zone, including DNA damage in adult birds, partial albinism, tumor formation and reduced brain size (for a review, see Møller & Mousseau, 2014). The population size (Galván *et al*., 2011) and sex ratios within a group of barn swallows (Møller *et al*., 2012a) were also affected by radiation from Chernobyl. Therefore, the barn swallow is one of the best indicators for investigating radiation effects on bird species. Radiation-dose-dependent changes in genetic damage and abundance of barn swallows were also investigated in Fukushima. In contrast to results obtained in Chernobyl, no radiation-dose-dependent increase in DNA damage of peripheral erythrocytes could be detected among birds captured in Fukushima using the neutral comet assay (Bonisoli-Alquati *et al*., 2015). A decreased number of barn swallows, however, and a decline in the juvenile fraction of the birds were observed in Fukushima among individuals with higher levels of radioactive contamination, indicating resulting lower survival and reproduction rates in Fukushima. Bonisoli-Alquati *et al*. (2015) therefore speculated that DNA damage among the birds does not account for the decline in barn swallows in contaminated areas, and further studies, such as for finding a proximate mechanism for the demographic effects on the birds, are needed to grasp the cause of the contradictions.

Because most of the radionuclides that leaked from the FDNPP disseminated into the western North Pacific Ocean, high levels of radioactive contamination of fish occurred. Indeed, 53 percent of the fish captured between April and June 2011 in Fukushima Prefecture exceeded the safety standard value for food (100 Bq/kg; Fisheries Agency of Japan, 2015). These indicate that marine birds may have consumed highly radionuclide-contaminated fish during the spring of 2011, with potentially adverse effects on the viability of marine birds. Therefore, the physiological responses of the streaked shearwater (*Calonectris leucomelas*) collected from Mikura Island, located approximately 220 km south of Tokyo and affected by the Fukushima nuclear plumes, were investigated. Seabirds collected from Birou Island, located far from the extent of the Fukushima nuclear plumes' impact, were used as a control (Uematsu *et al*., 2014). Although fledging mass did not differ between Mikura Island and Birou Island, levels of vitamin A in the blood of the fledglings of the birds sampled in Mikura Island colonies were significantly lower than in birds from Birou Island colonies. The reduction rate of vitamin A in the blood from Mikura Island was only 13 percent compared to that in the control area and there was no significant relationship between vitamin A content in the blood and fledgling mass in the streaked shearwaters, suggesting that the effects of vitamin A reduction at Mikura Island on the viability of the marine bird are still unclear.

The effects of radiation on the abundance of common birds in Fukushima were performed in 2011 and the results obtained were compared to those in Chernobyl that were investigated during four years (2006 to 2009) (Møller *et al*., 2012b). An analysis of 14 bird species common to Fukushima and Chernobyl showed that the abundance of the birds was negatively correlated to radiation dose at the two sites, but the tendency toward negative effects differed between Fukushima and Chernobyl. The negative relationship between abundance of the 14 common bird species and radiation dose was more pronounced in Fukushima than in Chernobyl. These investigations were carried out at the same level of background radiation, thus it would be noteworthy to discuss the difference in radiation effects between Fukushima and Chernobyl. Møller *et al*. (2012b) interpreted it as being because selection from high levels of radiation had already run its course in Chernobyl, because the investigations started in 2006, 20 years after the Chernobyl accident, but such adaptation to radiation had not occurred in Fukushima because the investigation was performed in the early period after the Fukushima accident (from March to July, 2011). Indeed, s previous study showed that 16 bird species exposed to low levels of radiation for 24 years in the Chernobyl exclusion zone exhibited high levels of antioxidant (e.g., GSH—a reduced form of glutathione) production and a high redox state (GSH/ oxidized glutathione), resulting in the reduction of reactive oxygen species and DNA damage (Galván *et al*., 2014). From the perspective of adaptation to radiation, long-term field investigations are needed for evaluating the effects of radiation on the abundance of bird species in Fukushima because the study by Møller *et al*. (2012b) was performed shortly after the Fukushima accident.

Field observations on the reproductive performance of the goshawk (*Accipiter gentilis fujiyamae*) were carried out for 22 years, including before and after the Fukushima accident, in the northern Kanto area (approximately 100 km from the FDNPP). The results obtained indicate that the bird's breeding potential attenuated markedly after the accident compared with the pre-accident years, and it progressively declined during the three post-accident years (Murase *et al*., 2015). The decline in reproduction performance seems to be caused primarily by increased air dose rates rather than by other factors. The decreasing trend in reproduction performance continued at least until three years after the Fukushima accident although external radiation exposure rates were decreasing yearly, indicating that internal radiation exposure from prey species may have also contributed to the reduction in goshawk reproduction activity. Thus Murase *et al*. (2015) concluded that internal exposure, as well as external exposure, may have an important role in the reproductive performance of the goshawk. On the other hand, nutrient deficiencies, which result from decreased abundance of goshawk prey species, could also affect the reproduction activity of the species. Indeed, some studies have reported a reduction in the abundance of prey species near Fukushima (Møller *et al*., 2012b; Møller *et al*., 2013), so further field research and DNA analysis are needed to clarify the factors that have led to reduced goshawk reproduction.

3.3 Impacts of Radiation on Mammals

Currently, few studies have been performed on mammals to estimate the potential biological consequences of exposure to low doses of radiation in Fukushima. A one-year hematological study of wild Japanese monkeys (*Macaca fuscata*) inhabiting forested areas of Fukushima City (70 km northwest from the FDNPP) and the Shimokita Peninsula in Aomori Prefecture (approximately 400 km north from the FDNPP) was performed in April 2012 (Ochiai *et al*., 2014). The study showed that individuals from Fukushima had lower white and red blood cell counts, lower hemoglobin concentrations and lower hematocrit values than those sampled in Shimokita. Because no radioactive Cs was detected in the muscles of the Shimokita monkeys but was detected in those of Fukushima (78 to 1,778 Bq/kg), Ochiai *et al*. (2014) concluded that the deterioration of blood components found in Fukushima monkeys could have been due to the effect of low-dose radiation. No significant difference in hematological values, however, was observed between two groups of Fukushima monkeys captured in areas with different radiation dose levels. This contradiction may be attributable to large variations in Cs concentration in the muscles. Indeed, Hayama *et al*. (2013) showed that although the muscle radio-Cs concentration in wild Japanese monkeys exhibited significant correlation with the level of radioactive-Cs soil contamination at the capture locations, Cs levels varied with the season (increasing two-to-three-fold in winter compared to summer in 2011) and such variations in radioactive-Cs levels were also observed in experiments, as described above (78 to 1,778 Bq/kg).

The effects of chronic radiation exposure on the testes of bulls abandoned within 20 kilometers of the FDNPP, were investigated, because testes are a relatively radiosensitive organ and involved in reproduction (Otala *et al*., 2004). The calculated maximum internal exposure doses of two bulls were 1.1 to 1.8 mGy (bull 1, during 196 days) and 5.0 to 9.5 mGy (bull 2, during 315 days), and the external exposures of bull 1 and bull 2 were 2.8 mGy and 1.8 mGy, respectively (Yamashiro *et al*., 2013). In the face of high levels of chronic exposure to radiation, the bulls' sperm and testes exhibited no evidence of significant histological changes in testis or sperm morphology. Thus Yamashiro *et al*. (2013) concluded that no adverse radiation-induced effects were observed in bull testes following chronic exposure to radiation for up to 10 months. This study, however, was very preliminary because only two bulls from a relatively uncontaminated part of Fukushima were investigated. Further investigations on the effects of radiation on spermatogenesis are needed, using more bulls abandoned in highly contaminated areas and extended to other animal species.

3.4 Impacts of Radiation on Plant Species

The uptake and accumulation of radioactive-Cs in higher plants from contaminated soil were studied vigorously in the early phases after the Fukushima accident, because phytoremediation, the use of higher plants to clean up soil contaminants, was one of the main issues at that time (Kobayashi *et al*., 2014; Sugiura *et al*., 2016; Tamaoki *et al*., 2016). Unfortunately, these attempts ended in failure because the absorption of radioactive-Cs from soil by higher plants was less than expected, which means that the transfer factor of radioactive-Cs (Cs concentration in plants/ Cs concentration in the soil) in higher plants was less than 1. Subsequently, two cases of biological effects of radiation on plant species were reported. Watanabe *et al*. (2015) showed that young Japanese fir (*Abis firma*) trees growing naturally near the FDNPP exhibited a significant increase in morphological abnormalities than at other sites. These trees showed irregular branching at the whorls of the main axis, with a distinct deletion of the leader shoot, resulting in termination of the main shoot and forking of the lateral shoots. The frequency of these abnormalities corresponded to the ambient dose rate of radiation at the sites observed (four sites with radiation doses from 0.13 to 33.9 μSv/hr), suggesting a possibility that contamination by radionuclides contributed to the morphological changes in Japanese fir trees in the area near the FDNPP. It is interesting to note that the frequency of leader shoot deletions became apparent significantly after the spring of 2012, reaching a maximum in all of 2013. It remains unexplained why the highest frequency of abnormalities was observed in 2013, already two years after the Fukushima accident. Thus, Watanabe *et al*. (2015) concluded that further studies were needed to elucidate the processes of leader shoot deletion arising from radiation at the cellular and/ or tissue level in the plants.

Morphological abnormalities in plants considered to result from high levels of radiation in Fukushima were also found in another conifer species. Field observations at different air dose rates in Fukushima showed that cancellation of apical dominance was observed in young populations of Japanese red pine (*Pinus densiflora*), whereas no morphological effect was detected in mature populations of the tree (Yoschenko *et al*., 2016). The observed abnormality is similar to that found in young Scots pine trees (*Pinus sylvestris* L.) in contaminated areas in Chernobyl (Yoschenko *et al*., 2011). The probability of apical dominance cancellation in populations of Japanese red pine increased as absorbed dose rates increased, suggesting that the abnormalities found in the red pine may be attributable primarily to external radiation. All morphological abnormalities were formed with four years from the beginning of exposure, and about half of all abnormalities detected appeared two years after the beginning of exposure. Interestingly, appearance of the apical dominance cancellation was temporary, and no new abnormalities were observed in the five-year whorl of the trees. Such temporal patterns of morphological change are similar to the cases of Scots pine in Chernobyl (Yoschenko *et al*., 2011) and Japanese fir in Fukushima (Watanabe *et al*., 2015). Taken together, these results indicate that morphological abnormalities found in conifer tree species might be caused by internal exposure rather than external exposure, because the external exposure dose to these trees reached its maximum during the first year. It would be very challenging to attempt through research to understand the underlying mechanism of apical dominance cancellation as well as to determine the temporal pattern of abnormality formation.

4. Ongoing Research Projects in NIES Relevant to Biological Impacts after the Fukushima Accident

In the early phases after the Great East Japan Earthquake, NIES immediately embarked on some research projects to investigate the impacts of the tsunami on beach vegetation (Hayasaka *et al*., 2012) and macro-benthos populations (Kanaya *et al*., 2014; Kanaya *et al*., 2015). An attempt at phytoremediation of radioactive-Cs from contaminated soil using higher plants was also performed as described above (Tamaoki *et al*., 2016). As for the impacts of radiation, NIES started investigating the effects of radiation on wild organisms from 2012, and also started monitoring terrestrial biodiversity a little later, from 2014. Although the impacts of radiation on wild organisms have been studied intensively in Fukushima as described above, there are few or no reports which cover indirect effects on ecosystems in the area (e.g., free from the impact of human activities due to the massive evacuation for a prolonged period). Indeed, the overall effect on the biota in the Chernobyl exclusion zone has been observed to be in favor of biodiversity and abundance of individuals (Sokolov *et al*., 1993). Thus monitoring of biota in the Fukushima evacuation zone by NIES is a unique approach to detecting the impact of the accident on ecosystems.

4.1 Overview of Terrestrial Monitoring in Fukushima by NIES

Terrestrial monitoring was started by NIES to collect knowledge on the biodiversity and ecosystem effects of the large-scale and long-term evacuation of the Fukushima area. The monitoring focused on mammals, birds, frogs and insects to maximize monitoring effectiveness with limited effort (Yoshioka *et al*., 2016). For mammal monitoring, infrared cameras were placed at 46 forest sites in nine municipalities inside and outside the evacuation zone. A map of sampling points is shown on the Biodiversity Web Mapping System (BioWM) website provided by NIES (http://www.nies.go.jp/biowm/index.php?lang=en). Fourteen species of large or medium-sized mammals such as black bears (*Ursus thibetanus*), wild boars (*Sus scrofa*), and Japanese serows (*Capricornis crispus*) were recorded in the evacuation zone by camera-trap monitoring from May to October, 2014. Although the impacts of the evacuation on mammalian fauna populations had remained undetermined until then, the half-year data sets obtained were published as a data paper to enable their use by other researchers (Fukasawa *et al*., 2016). The data were also incorporated into the BioWM website (http://www.nies.go.jp/biowm/index.php?lang=en), where viewers can browse for distributions of mammals in eastern Fukushima.

Monitoring of birds and frogs was conducted at 52 sites in schoolyards of nine municipalities, including the evacuation zone (Yoshioka *et al*., 2016). IC recorders were used to record bird songs and frog calls for 20 min/day around dawn and 10 min/day around PM 8:00 each day from May to June. Data analysis of the monitoring is ongoing, but some of the recorded bird songs were used at an event called “Bird Data Challenge,” collaborating with branches of the Wild Bird Society of Japan in Fukushima. This was a new and important approach to accumulating data on bird abundance and habitats while sharing the work with citizens. As for the frog analysis, an automatic song detection system based on machine learning methods is being constructed to extract frog calls from recorded data sets. Moreover, other researchers at NIES have shown that there were no clear abnormalities in the gonadal tissues of frogs captured from contaminated sites in Fukushima even though there was a positive correlation between radioactive-Cs concentrations in soils and those in frogs (Matsushima *et al*., 2015).

Preliminary results on abundance of insects were obtained by sampling using Malaise traps at the same 52 sites as bird and frog monitoring in 2014 (Yoshioka *et al*., 2015). A total of 48,081 insects were sampled, mostly Hymenoptera and Diptera. It is interesting to note that only the abundance of carpenter bees (*Xylocopa appendiculata*) was lower within the evacuation zone than outside. A potential cause of the reduced abundance of carpenter bees in the evacuation zone might be reduced numbers of garden plant flowers following the decrease in human activities due to the evacuation, because these bees favor large flowers such as wisteria, orchids and passion fruit. On the other hand, some small bees, wasps and beetles were more common inside the evacuation zone. This may be due to increased abundance of wild plants that provide food and nesting materials for these species. Abundance of most of the other collected taxa, including pollinators, did not differ between inside and outside of the evacuation zone. These results suggest that flying insects were not critically affected, so it will be important to monitor the long-term effects of the evacuation at the same sites.

4.2 Radiation-impact Studies on Wild Organisms by NIES

An analysis of calculated dose rates in Fukushima indicated that more severe impacts on wild organisms were expected in the coastal areas adjacent to the FDNPP than in the forested areas (Fig. 1; Garnier-Laplace *et al*., 2011). Thus, NIES launched a multiple-year survey to clarify the ecological impacts of the Fukushima accident in the intertidal zone. Field surveys at 43 coastal sites from Chiba to Iwate prefectures including Fukushima in 2012 showed that abundance and diversity of intertidal species decreased significantly with decreasing distance from the FDNPP, and no rock shells (*Thais clavigera*) were observed within 20 km of the FDNPP (Horiguchi *et al*., 2015). Quantitative investigations in 2013 also showed that biodiversity and habitat density in the intertidal zones were much lower at sites close to the FDNPP than at other sites. Almost all of the investigation spots had been hit by the tsunami, suggesting that the loss of rock shells from around the FDNPP may have resulted from the nuclear accident. So far, there is no clear evidence to attribute the absence of rock shells to radionuclide contamination of the marine ecosystem because many harmful chemicals, such as boric acid and hydrazine, also leaked into the sea together with the radionuclides. Clarification of the main factors contributing to the absence of rock shells and continued field research on temporal changes in the biodiversity and population of intertidal organisms will both be necessary for keeping track of their recovery in the future.

In terrestrial areas, NIES initiated research into the impacts of radiation on wild large Japanese field mice (*Apodemus speciosus*) because the mice are common in Japan and the ICRP had selected rodents as a reference animal for environmental protection studies (ICRP, 2008). NIES investigated the accumulation of radioactive-Cs in the mice, and the chronic effects of radiation on the male genitals, such as DNA lesions, DNA mutations and morphology of sperm cells in testes. The average ambient dose rate at the Fukushima capture sites was 4.1 to 13.9 μSv/h and the median concentrations of Cs-134 and Cs-137 in the Fukushima mice exceeded 4,000 Bq/kg (Okano *et al*., 2016), suggesting that radiation exposure doses to the mice were within the “very low probability of effects” level in the ICRP criteria (ICRP, 2008). There were no significant increases, however, in frequencies of apoptotic cells or morphologically abnormal sperm in Fukushima mice. Thus, it is concluded that radiation caused no substantial male subfertility in Fukushima during 2013 and 2014, and radionuclide pollution levels at the study sites would not be detrimental to spermatogenesis in wild mice in Fukushima.

5. Conclusions

In summary, the Fukushima accident provides a good opportunity to investigate chronic impacts of radiation on wild organisms, as did the Chernobyl accident. Recent advances suggest that adverse effects of radiation on wild organisms could be found in terrestrial and coastal areas of Fukushima in the early-phase aftermath of the accident, but these were rarely detected past two or three years after the accident. Hereafter, impacts on biodiversity and ecosystem services in the evacuation zones due to increased decontamination activities and the abandonment of cultivation will be an issue for consideration. Indeed, wild boars are considered a pest animal in farmland, and preliminary results of mammal monitoring showed that the species’ population inside the evacuation zone was higher than outside the evacuation zone (Fukasawa *et al*., 2016). Therefore, details on and long-term monitoring of changes in wildlife populations and land use in the evacuation zone is of increasing importance for developing efficient plans to return the residents to the area in the near future.

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