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**From tangled banks to toxic bunnies; a reflection on the issues
involved in developing an ecosystem approach for
environmental radiation protection**

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Abstract

Objectives:

The objective of this paper is to present the results of discussions at a workshop held as part of the International Congress of Radiation Research (Environmental Health stream) in Manchester UK, 2019. The main objective of the workshop was to provide a platform for radioecologists to engage with radiobiologists to address major questions around developing an Ecosystem approach in radioecology and radiation protection of the environment. The aim was to establish a critical framework to guide research that would permit integration of a pan-ecosystem approach into radiation protection guidelines and regulation for the environment.

Conclusions

The conclusions were that the interaction between radioecologists and radiobiologists is useful in particular in addressing field versus laboratory issues where there are issues and challenges in designing good field experiments and a need to cross validate field data against laboratory data and vice versa. Other main conclusions were that there is a need to appreciate wider issues in ecology to design good approaches for an ecosystems approach in radioecology and that with the capture of “Big Data”, novel tools such as machine learning can now be applied to help with the complex issues involved in developing an ecosystem approach.

Keywords: Ecosystem approach, radiation protection, radioecology, radiobiology, systems biology

General Introduction

“It is interesting to contemplate an entangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent on each other in so complex a manner, have all been produced by laws acting around us.”

Charles Darwin

On The Origin of Species by Means of Natural Selection, or Preservation of Favoured Races in the Struggle for Life. London :John Murray, 1859.

The final paragraph of Darwin’s monumental monograph pre-empted our modern understanding of the interconnectedness of an ecosystem, its complexities and the variety of processes at play within it. Understanding the impact of environmental contamination on an ecosystem is a fearsome task, assessing damage still more so, given the subtlety of perturbations that might be later amplified to produce potentially catastrophic changes. In a series of papers, on topics where radiobiology and radioecology interact, of which this is the third, we have attempted to draw from the community a consensus view on how to capture the effects of radioactive contamination on an ecosystem, with the aim of defining ecological parameters that might be used for development of safety and regulatory guidelines, and help us to further understand the fundamental science of radioecology.

This discussion paper results from the 5th workshop organised by the International Union of Radioecologists (IUR) which was held in Manchester UK on 26th and 27th August 2019 during the International Congress of Radiation Research (ICRR). The aim was to stimulate the interaction of radiobiologists (who traditionally study mechanisms of radiation action at the physical, chemical and biological level) and radioecologists (who are more focused on uptake, transfer and effects of radionuclides in ecosystems). The particular hope was to engage both groups in the on-going discussions about approaches to developing an ecosystem approach in environmental radiation protection. By an Ecosystem Approach we mean using methods and concepts within radiological environmental protection, which target populations and their interactions with other biota and abiotic components of ecological systems (Bradshaw et al. 2014). This allows a more holistic focus on the multiple facets that reflect the environment and the complexity of factors and interactions underlying the ultimate outcome for populations living in environments contaminated by radionuclides. The series of workshops stem from an initial consensus recognition that ecosystem approaches are better suited to fulfil environmental radiation protection goals (consensus statements from Miami Symposium, 2015; see Bréchnignac et al. 2016). The 2019 workshop started with a discussion of three “provocative statements” designed to be controversial. These served to determine what was impeding development of an ecosystem approach and what the concerns might be in formulating a conceptual roadmap. The discussion then moved to how we could progress towards an ecosystem approach in a practical sense including consideration of modelling needs, the role of big data, rigor in experimental

design and data sharing, and the feasibility of tackling such a complex subject.

Contributions from each attendee were sought and these have been edited to produce a summary for this paper as a prelude to the core discussions on how to identify and remove the blocks to progress in this field. Because the contributions from delegates provide a fascinating window to the multiple shades of opinion that were represented and aired, they will be published as submitted on the website of the International Union of Radioecologists (<http://iur-uir.org>). A key focus of the workshop was not only to reach areas of agreement, but also to: (a) better understand areas where consensus may not be reached; (b) understand what we disagree on and why; (c) identify what the knowledge gaps are and (d) propose what studies and experiments are required to fill those gaps. This paper summarises the discussions at the workshop and does not necessarily represent agreement by all authors. Where required, some background to these discussions is given, but the paper is not intended to be a comprehensive review of the state of the art in the topic area.

BROAD “PROVOCATIVE STATEMENTS”

Provocative Statement 1

Radiation is not a problem in the environment

Summary answer: The statement clearly begs the question “in which context”, e.g. do we mean in the context of regulated releases or at contaminated sites such as the Chernobyl Exclusion Zone (CEZ) and Fukushima Exclusion Zone (FEZ) but discussions highlighted the different assumptions that might underlie such a point of

view. For example, there could be disagreements as to whether effects have been demonstrated at all in contaminated areas, whether effects seen are due to radiation exposure, either past or present, or whether any changes seen have ecological relevance? While there was a general appreciation that “radiation is not the most important problem in the environment” in light of other environmental threats, the question of when and how it might become a problem was more challenging.

The discussion topics covered a wide range of issues, from effects at the molecular level through to population and ecosystem impacts, from regulation to ethics. Some questioned whether a new system of assessment and management is needed if major effects on ecosystems are not immediately apparent. When rabbits were found to be burrowing close to Dounreay radioactive waste areas on the Dounreay nuclear site, the immediate reaction was to cull the rabbits to reduce risk to the human food chain (Ross, 2003). This was followed by suggestions that it would be sensible to test other animals using the rabbits for food, such as cats and buzzards (Thomson, 2003), and a retort by the neighboring Sandside estate owner that the answer to the problem was the secure containment of nuclear waste and not culling “*poor, innocent rabbits, cats or birds*” (Thomson, 2003).

In this case, one might argue that radiation was at least indirectly damaging to that particular population.

If we are considering the release of radioactivity into the environment through current regulated activities then the statement ('Radiation is not a problem in the environment') is probably correct. However, if we are asking about the likelihood of observable effects of radiation in more highly contaminated areas around Chernobyl, Mayak, and Fukushima, then radiation doses in some areas are such that effects are likely and we do observe them (Geras'kin 2016; Beresford et al. 2020b, 2020c).

However, exactly what effects have been seen (or not) in contaminated regions, as well as their cause and ecological relevance are a matter of contention. Examples were given of papers that reported changes across a whole range of molecular and organism levels, from chromosome aberrations to cataracts, (e.g. Møller and Mousseau, 2007a; Bonisoli-Alquati et al. 2010a, 2010b; Mousseau and Møller 2013; Møller et al. 2012a, 2012b, 2013; Baker et al. 2017). Other papers have reported little or no effect (e.g. Deryabina et al. 2015; Bonzom et al. 2016; Lerebours et al. 2018; Fuller et al. 2019; Goodman et al. 2019).

Other issues raised , with respect to interpreting results from contaminated areas (and especially studies in the CEZ) included the positive impacts from removing the human population, problems with the residual impact of high historic doses, and dose response relationships being driven by observations from areas with extreme high dose rates which may have additional confounding factors because of degraded habitat and slow ecosystem recovery (Beresford et al. 2020b, 2020c)

Some scientists believe changes seen in Chernobyl and Fukushima suggest that environmental risks of radiation are underestimated (Mousseau and Moller, 2013). The intense discussion about this in the workshop and during the construction of this manuscript, captured very well the level of disagreement among scientists.

Related to studies conducted in the CEZ and FEZ one of the key points blocking development of an ecosystem approach (or indeed reaching any consensus on the effect of radiation on the environment), is a general lack of open access data (Beresford et al. 2020b; Lecomte-Pradines et al. 2020).

Potentially confounding factors arise if authorised or accidental releases of radionuclides take place in natural ecosystems that are already under pressure from habitat destruction, invasive species, or chemical pollution. For example, interactions between rapid climate change and radioactive contamination could compromise homeostasis and physiological responses, and potentially impair fitness, reproduction, and development (Noyes et al. 2009). Radiation exposure may further reduce the ability of organisms to acclimate and potentially make them more susceptible to infectious and vector-borne diseases (Dmitriev et al. 2011; Morley 2012).

Finally, harm to the environment caused by radioecologists related to studies conducted in the CEZ and FEZ was discussed but was not considered to be a serious issue. It was however emphasised that it is important to be aware of each other's work, as there is the potential to impact subsequent studies. Examples (known to have occurred) include

trapping out small mammals and so impacting the population dynamics for ecologists coming later, and introducing unexposed individuals into contaminated sites.

To conclude, the type and the magnitude of either direct or indirect effects of ionising radiation depend on ecosystem composition, and many ecological factors can be more important than radiation. On the other hand, ignoring population or higher-level effects, and focusing only on individual-level endpoints may lead to inaccurate risk assessments and errors in environmental management decisions. It should be noted here that population and higher-level protection are the stated aim of radiological protection – (ICRP 2008; IAEA 2014). Therefore, the build-up and use of ecological knowledge is essential for understanding responses of populations and ecosystems to radiation, including the potential for changes in interaction between species. In light of this, perhaps a more reasonable statement, than that proposed for this discussion, would have been that “radiation is not the most important problem in the environment”

Provocative Statement 2

“Not all change is bad, but no observable change may not indicate that nothing bad is going on. Focus on detection of negative effects can miss the detection of adaptive and protective effects operating at the ecosystem level.”

Summary answer: This statement was generally agreed with, but it was not seen to advance the discussion of developing approaches in regulation where preventing harm is the main objective. In developing an ecosystem approach, the key should be, measuring change without assigning “good” or “bad” descriptors.

This statement was designed to address the role of adaptation and evolution. Biota can adapt in response to new stimuli and invoke protective mechanisms that are essentially heritable and conserved, and molecularly predicated, in the interest of survival. Such adaptation could occur in response to environmental exposure to low-dose radiation (Audette-Stuart et al. 2011; Mothersill et al. 2013; Lampe et al. 2017; Beresford et al. 2020b). These processes draw attention to the fact that the current environmental radiation protection regulation adapted from the human framework essentially measures or models dose and compares this to benchmarks below which impacts on individuals (mortality, morbidity, fecundity) likely to lead to population level effects are not anticipated (Howard et al. 2010). This can miss long-term processes playing out over generations in populations. It is difficult for humans to make the conceptual jump from individual-level short-term effects to very long-term ecosystem-level effects.

Discussions focused on the difficulties of extrapolating between different levels of effect (molecular, individual, population), as well as the types of mechanisms that could underlie these types of responses. Some changes at the ecosystem level might also be misinterpreted. For example, an increase in prey population density may be interpreted as a positive ecosystem level effect when it actually results from the eradication of the predator partner, perhaps signalling a decline in the condition of the ecosystem. Rabbit populations might increase if foxes or buzzards decrease, which might in turn bring about a decrease in abundance of a plant species, both of which would be indirect rather than direct effects of exposure.

Long-term field studies carried out on different plant species including winter rye (*Secale cereal* L.) and wheat (*Triticum aestivum* L.), spring barley (*Hordeum vulgare* L.) , oats (*Avena sativa* L.) , Scots pine (*Pinus sylvestris* L.), Japanese red pine (*Pinus densiflora* Siebold & Zucc.), wild vetch (*Vicia cracca* L.), crested hairgrass (*Koeleria gracilis* Pers.) in various radioecological situations (nuclear weapon testing, the Chernobyl and Fukushima accidents, uranium and radium processing) have shown that in most cases strong effects at the molecular level (increased rate of mutations, changes in gene expressions) turn into moderate effects at the physiological level (enzyme activities, changes in the phytohormonal balance), and into slight effects at the organismal and population levels (morphological abnormalities, reproductive ability, radioadaptation) (Geras'kin et al. 2013; Boubriak et al. 2016). Evidence from field observations shows development of cytogenetic abnormalities in several generations of progenies distant from the initially irradiated surviving parental generation (Geras'kin et al. 2003) and there is a rich literature in radiobiology concerning delayed *de novo* appearance of cellular effects such as lethal chromosomal aberrations or lethal mutations after many normal generations *in vitro* and *in vivo* (reviewed in Mothersill and Seymour 2019).

In a number of studies, it was noted that parental acute exposure to radiation affects both exposed organisms and their unexposed offspring (Streffer 2006, Sarapultseva and Dubrova 2016, Sarapultseva et al. 2019). At the same time, fertility and survival were restored by the second generation. Thus, it is important to use at least two generations of test design that analyze the long-term effects of ionizing radiation, as has been

shown, for example, for the test organism *Daphnia magna* (Barata et al. 2017). However this raises the question of the long-term significance of genetic effects observed over generations; might there be long-term consequences of epigenetic changes in gene expression resulting in alterations in the genetic structure of populations (Nishikawa and Kinjo 2018)?

The Adverse Outcome Pathway (AOP) approach may help elucidate possible links and consequences across different levels of biological organisation (Ankley et al. 2010; Villeneuve et al. 2014). This is a conceptual framework that gathers and organizes data from studies of organisms using a variety of endpoints. The data are then analysed using pathway analysis methods to determine molecular initiating events and to link these to adverse outcomes seen at higher levels of complexity. By dissecting the problem into smaller organisational levels (from sub-cellular to organism), with each level potentially controlling the structure and function of the level above, may help us to reach a better understanding of how individual exposure responses may be expressed at higher levels of biological organisation i.e. population and ecosystem.

However, it should also be appreciated that there is no reason why a dose-response relationship observed at one level of organisation may be the same at other levels of biological organisation (i.e. a dose response seen for an individual may not be mirrored at the population level). The responses, with latent periods, get attenuated at each level of organisation and are influenced by many confounding factors in the complex environment. There is also the possibility of emergent properties as the system

becomes more complex (Jha 2008). If the underlying mechanisms of adaptation were triggered by the responses at the molecular or cellular level, then adaptation would be strongly dependent on the dose/dose rate, and the radiosensitivity of the species at the life stage that experienced the exposure. Any population level adaptation therefore needs to be explained at molecular and cellular levels in different species. As an example, low dose hypersensitivity first seen as a response in radiotherapy is now thought to be a widely encountered integrated biological response to stress involving apoptosis, autophagy and mutations (Rodrigues-Moreira et al. 2017). Variation in stress response might underlie variation in low dose radiosensitivity within populations and among species.

Populations inhabiting radioactively contaminated territories may become physiologically adapted (i.e., acclimated) to chronic exposure through phenotypic plasticity or epigenetic changes, or even evolutionarily adapted through natural selection (e.g., Ruiz-Gonzalez et al. 2016; Horemans et al. 2019). Investigations of environmental adaptation have mainly focused on single species, often overlooking the symbiotic context of the organisms under study (Exposito-Alonso et al. 2018; Schuman and Baldwin 2018; Song et al. 2019a). Symbiotic relationships, however, are ubiquitous in nature and it becomes increasingly clear that development, growth and health of macro-organisms is influenced by the complex microbial communities they host (Simon et al. 2019; Song et al. 2019a). Many examples of adapted mutualists conferring stress tolerance are available including rhizobia enhancing chickpea plant tolerance to desiccation (Bano et al. 2010), ectomycorrhizal (EcM) *Suilloid* fungi involved in pine tree

metal tolerance (Krznaric et al. 2009) and common mycorrhizal network-connected *Nicotiana attenuate* plant communities showing an enhanced systemic defence responses to plant herbivory (Song et al. 2019a).

Sometimes we observe signs of adaptation to radiation exposure in affected populations (Galván et al. 2014), sometimes not (Geras'kin et al. 2013). While evidence of increased resistance is inconclusive, two arguments support this hypothesis. First, instances of rapid evolution – spanning a few to a few tens of generations – are increasingly documented in the eco-evolutionary literature (e.g., Pespeni et al. 2013; Oziolor et al. 2019). The only precondition seems to be that the drivers of selection are strong enough. Second, such rapid evolution is predicted to be even faster when the toxicant is responsible for generating new variants among which to select, as is the case for mutagenic ionizing radiation. However, an adapted populations' fitness might also decrease when its organisms move to uncontaminated territories (Hickey and McNeilly 1975; Levinton et al. 2003). So, what is the cost of adaptation? Such a fitness cost may be related to the use of metabolic energy to produce the adaptive trait (e.g., the DNA repair mechanism) even when that trait is not required (although it was disputed that the cell population would run such a tight energy budget). Moreover, the selection of genotypes for radio-resistance can lead to the loss of radiosensitive genotypes with valuable properties (Glazko 2001; van Straalen and Timmermans 2002; Hancock et al. 2019a). Since adaptation may play an important role in the response of populations to radiation exposure in natural settings, mechanistic information about population-level drivers of fitness and the role of adaptive responses, is directly applicable to predicting a

radionuclide's impacts at the population level and hence for developing ecological risk assessment.

Even with a reduction in reproductive success of the organisms negatively affected by radiation, exposure may not result in population declines. Under ecologically realistic scenarios, reduced reproductive rate and survival of exposed organisms creates an opportunity for individuals of the impacted species to better exploit resources and hence reproduce. There is some evidence for compensation; embryonic mortality of fish was found to be accompanied by larger broods leading to no net effect on population numbers (Blaylock 1969). Ecological space left open by exposed and affected organisms, can also be filled by organisms immigrating from the outside, which would mask any deleterious effect on the resident population. Source populations can compensate for sinking numbers in that location, in a classic source-sink meta-population fashion.

Finally, some effects observed at affected sites could potentially result from non-targeted effects of acute exposure during the first period of the accident. In particular, historically-induced genomic instability leads to a phenotype with a greater tolerance for mutation than normal. This means that in addition to the mutation burden attributable directly to the ambient dose there may be a contribution due to the historically-induced genomic instability, which is not directly induced by the ambient dose but is a consequence of the increased mutation tolerance (genomic instability) in the population. This concept is discussed in Mothersill et al. (2017) and has been applied to datasets from CEZ and Fukushima (Omar-Nazir et al. 2018; Geras'kin et al. 2019; Hancock et al. 2019b, 2020). Therefore, a clear understanding of exposure history is of

fundamental importance for interpreting field effects studies and subsequently for predicting how populations and ecosystems recover from radiation exposure.

Provocative Statement 3

“Mechanistic studies need to employ systems biology and be field-based to be useful (field v laboratory studies reveal discrepancies)”

Summary Answer: Both field and laboratory data have a role, and laboratory studies can be used to substantiate field observations under controlled conditions. But laboratory data should be validated in the field, whenever possible, before being used to develop ecosystem models. The corollary of this is that suspected field effects can and should be confirmed in the laboratory

The aim of this statement was to explore the suggestions that field-derived radiation sensitivities have been reported to be up to 10-fold greater than laboratory-based values, based on a comparison of between field and laboratory data (Garnier-Laplace et al. 2013). There are multiple reasons for the difference including stress, other pollutants, predation and disease, which could compromise survival of organisms from contaminated environments. However, the reason for the question was to discuss whether ONLY field data should be considered when developing an ecosystem approach or whether laboratory data has merit. A secondary issue is whether or not the difficulties in obtaining good field data, present a major block to developing an ecosystem approach?

The discussion centred mainly on the types of mechanistic analysis that might help in probing laboratory-field discrepancies, including the influence of neurological, immune and humoral responses and DNA damage. Although there is increasing evidence to suggest that ionising radiation, like chemicals, can induce a variety of biological responses e.g. in the nervous, immune, endocrine or inflammatory systems (Jha 2004, 2008), these systems are relatively poorly understood in natural biota. Radiation is known to generate oxidative stress in humans, laboratory animals (Hurem et al. 2017; Maremonti et al. 2019) and wild populations (Einor et al. 2016; Volkova et al. 2017), by increasing oxidative damage and decreasing antioxidant defenses. If the key physiological systems mentioned above are impacted by redox imbalances under chronic exposure conditions, they could eventually impair the reproductive fitness of the organisms and the populations in a stressed ecosystem (Jha 2008). For example, sub-lethal effects can impair homeostatic or physiological conditions, and inflammation has been linked to a range of aging-related pathologies.

DNA damage is a recognised outcome of radiation exposure, together with a variety of key processes such as epigenetic modifications, including DNA methylation and transcriptomic (mRNA) and post-transcriptomic (small RNA and long non-coding RNA) measurements (e.g. Schofield and Kondratowicz 2018). However their ecological relevance is a matter of debate. DNA damage poses a direct threat as a precursor of mutation. Mutations are typically neutral to mildly deleterious. Most of the time they will cause no effect, - due to redundancy in the genetic code and the large proportion of the genome that does not perform any known function, - or have effects that (a) are

non-lethal, and (b) only reduce performance of developmental, behavioural and physiological systems, rather than total impairment of those systems. DNA damage could also affect reproduction and survival because DNA repair requires a diversion of energy from other activities (Roff 2001). Epigenetic variation may however be expected to have a potentially more significant effect as epimutation is in its nature pleiotropic and affects the expression of many genes (Schofield and Kondratowicz 2018). Epigenetic changes have the potential to mediate toxicological, and transgenerational deleterious effects of exposure to ionizing radiation when they suppress the expression of genes otherwise useful for the organism. In principle, however, epigenetic changes can also favour a plastic response to ionizing radiation that can later be accommodated into an evolutionary one (Bossdorf et al. 2008). The translation of these organism-level effects to the population level is however complex. For example, higher mortality can be compensated by immigration from outside of the contaminated areas, and countered by reduced intra-specific competition and lower predation pressure. Time is also an important factor. In a laboratory, organisms are usually measured a short time after exposure, whereas field studies are often conducted years after the contamination event.

It is also difficult to relate or transfer the results of laboratory experiments on transgenerational effects and genomic instability to field conditions. While there is strong laboratory evidence for the manifestation of epigenetic transgenerational instability in the progeny of irradiated males, other studies suggest that low-dose, low-LET exposures do not destabilise F_1 (Mughal et al. 2012) or that lab-results are not

always comparable to observations in the field (Horemans et al. 2019). In contaminated environments, both parents and their offspring/progeny of exposed populations are continuously exposed. This means that *in situ* analysis of mutation rates among the offspring cannot provide any evidence for the manifestation of transgenerational effects, since it would be impossible to distinguish between the direct effects of offspring exposure to ionising radiation and transgenerational instability. However, the historically-induced genomic instability phenomenon referred to earlier, would suggest that there will be a component of the total mutational load induced by genomic instability. This is suggested by the dose reconstruction modeling done by Hancock et al. (2019b). To determine the extent of the additional mutational load experimentally, parents could be exposed in laboratory settings to the mix of radionuclides, similar to that on the contaminated territories. Subsequently, their offspring could be transferred to the clean environment, and the genome stability of non-exposed first- and second-generation offspring of irradiated parents could be analysed. The results of such studies would provide a definitive evidence for the manifestation of transgenerational genomic instability in nature.

The major conclusions were that systems biology approaches to understanding the consequences of exposure to ionizing radiation at low doses under ecological conditions require thinking about in terms of the complex lives and demands of wild organisms. While laboratory experiments can yield useful insights into possible mechanisms, the population-level effects could be fully realized only when a systems biology approach is

used within field-based studies. Mesocosm studies could also be useful in allowing 'more controlled' field observations.

How to make progress in developing an ecosystem approach

The second part of this paper deals with breakout discussions centred around practical approaches which need to be considered if progress is to be made. The three topics are

- 1. What is stopping the development of an ecosystem approach?*
- 2. What can be gained from "big data" and modelling approaches*
- 3. How can we design "good" experiments giving robust data?*

1. What is stopping the development of an ecosystem approach?

The premise of the question is that ecosystem approaches are not being employed. Although this is correct from a regulatory perspective, it is incorrect for radiation ecology and ecology in general. Several examples of research that used ecosystem endpoints are available from Chernobyl and Fukushima accident sites as well as at nuclear facilities in the USA (Savannah River, Oak Ridge, Fernald, and Hanford), in Russia (Mayak), and microcosm studies (Bonzom et al. 2016; Geras'kin 2016; Fesenko, 2019; Hevrøy et al. 2019).

To inform an ecosystem approach, a study needs one or more endpoints that pertain to ecosystem structure or function. Ecosystem endpoints that are gaining popularity are those enumerated in various lists of ecosystem services as defined in the US EPA document published in 2016 (US EPA 2016). For example litter degradation is an

ecosystem function that ensures the service of recycling nutrients and protecting from fire. Ecosystem services can be synonymous with ecosystem functions and as such can become confusing if not defined explicitly. The definition in Wikipedia is as follows:

“They (Ecosystem services) include natural pollination of crops, clean air, extreme weather mitigation, human mental and physical well-being. Collectively, these benefits are becoming known as ‘ecosystem services’, and are often integral to the provisioning of clean [drinking water](#), the [decomposition](#) of wastes, and resilience and productivity of food ecosystems.”

Central to the definition of an ecosystem approach is that interactions among biotic entities must be considered. This derives from seminal work performed in ecology as far back as the 1960s exploring the regulation of populations within various communities (Slobodkin et al. 1967; May 1973; Pimm 1982; Brown and Munger 1985; Karr 1992; Hunter and Price 1992). Emerging in these research efforts were two alternative mechanisms that control ecosystem dynamics, namely a bottom-up control (one that is driven by the producer community) and a top-down control (one that is regulated by top predators). A salient point of these works is that efforts that focus on a single taxon are likely to have limited predictive capacity because the various positive and negative feedback loops cannot be anticipated in isolation of interacting taxa.

Taking an ecosystem approach must begin with asking the questions – what are we trying to understand? What is our focus? Then, rather than attempting to assess or evaluate all possible components of the ecological system one looks one hierarchical

level above the focal level for context and one hierarchical level below the focal level to explore mechanisms (Wu and Loucks 1995). This means that if one is interested in a population of a particular tree species, one would look at the dynamics of the forest association in which that population resides (context) and at autecological endpoints of the tree species for mechanisms governing the population. The focus could be on population structure of interacting taxa or guilds, productivity, energy flow, nutrient cycling, pollination services, etc. The complexity of ecological systems calls for multidisciplinary, even transdisciplinary teams that match the likely dynamics that will be encountered.

Although there are parallels that can be drawn between radiological stressors and other stressors, perhaps the most challenging problem is the accurate measurement or estimation of cumulative dose an organism might encounter in a complex environmental setting. The challenge relates to different emitters (alpha, beta, gamma) and determination of internal versus external dose. Beaugelin-Seiller et al. (2020) discusses approaches to improve the estimate of absorbed dose in field studies.

Many papers on radiological effects in biota pertain to alteration of DNA. These studies provide great insights into mechanisms of action at the molecular level. This knowledge helpful to understand cellular anomalies and has been shown to be important in epigenetic modifications that transmit to subsequent generations (Schofield and Kondratowicz 2018). However the importance of non-targeted effects such as genomic instability and bystander effects continues to grow as more mechanistic information becomes available (Mothersill and Seymour 2010) There is current enthusiasm about

the utility of adverse outcome pathways (AOPs) first proposed by Ankley et al. (2010), in radioecological studies (e.g Song et al. 2019b; Beresford et al. 2020b). The limitation that seems to be overlooked in this conceptual framework, is that there are currently no population models that can effectively forecast the population dynamics in a field setting. This imposes severe limitations on the use of AOPs in an ecosystem approach.

There has been some use made within radioecology of ecosystem endpoints that are common in mainstream ecology, such as ecosystem services, pollinators, and decomposers, level of resilience or objective measurements of interactions (e.g. Mousseau et al. 2014; Bonzom et al. 2016; Newbold et al. 2019; Beresford et al. 2020b). However, many of the concepts used in radiobiology are not integrated into radioecology and some of these might help progress towards developing an ecosystem approach. One suggestion was to have a “dose and dose rate effectiveness factor” or DDREF” for the organism resilience -how sensitive is an organism and how adverse would it be for ecosystem instability if the organisms stopped functioning in a healthy way? A way to bridge from reference animals and plants to an ecosystem approach might be to develop reference values for organism resilience. This could be done by strategically combining mathematical models, laboratory and field study data of organisms’ ability to deal with radiation stress and other pressures to establish a resilience factor (RF). The RF need only be based on a few species of each genus, and our knowledge of their role/interconnection to each other.. However, an argument was put that an absolute resilience factor could not exist, as it would be context-dependent.

That said, there are biological features that make species more or less vulnerable, and more or less resilient. Among these are the reproductive strategies (see MacArthur and Wilson 1967) for K or r-selected species with r-selected species (e.g. mice) 'bouncing back' faster, and population connectivity (with more connected populations receiving immigrants that help the recovery).

Similarly, if you know and understand an animal by studying it in the laboratory you can better predict their interactions and contributions in the environment. This could help to model and inform our understanding of ecosystem resilience, which is based on empirical evidence of a particular ecosystem to resist change or to recover following a perturbation. This considers complexity of the system in terms of biodiversity, redundancy, and flows of information within the system that enable the system to retain basic functions. The concept of resilience and stability was developed by Holling (1973). Each ecosystem is unique and diverse, and to understand its response to a stress it should be mapped in a way which involves modelling the pathways and processes of organisms interacting with each other and their environment. This can be done by explaining the web of direct effects (express/measured at an individual level) and indirect effects (effects mediated/transmitted through interactions) in an ecosystem. If one organism is impacted, how will it ultimately affect the other organisms and the environment, for example if a predator becomes unable to hunt this may result in an imbalance and overpopulation of prey species.

2. Discussion on big data, machine learning, informatics and modelling as new approaches for radioecology.

The effects of low dose radiation on ecosystems, such as in Chernobyl or Fukushima remains poorly understood. Although both field and laboratory studies synergistically contribute to understanding and evaluating the basis for environmental assessments, the complex nature of interactions on a population or ecosystem level make it harder to extrapolate the radiation effects observed in the laboratory-based studies. One approach to study this, at least qualitatively, is by using mathematical and computational modelling techniques that are motivated and formulated based on the insights gained from both laboratory and field experiments.

Mathematical and computational modelling approaches are widely used to understand and study ecological interactions. Novel concepts like multiscale modelling (Powathil et al. 2015), and bio-energetic model-based network analysis (Yodzis and Innes 1992) can be very beneficial for exploring the population dynamics and other ecological interactions under multi-stress scenarios, including radiation stress. Beyond these modelling approaches, current developments in Artificial Intelligence (AI) and Machine Learning (ML) techniques offer novel opportunities to gain insights from large data sets (see Jarry et al. (2003) or Feng et al. (2018) for an example in radiation oncology) and the availability of complex and large datasets in ecological studies permits the application of a large range of data-driven modelling approaches.

Machine learning in particular provides several main benefits: classifiers may be derived for data using supervised learning and requiring large training datasets, unsupervised learning can be used to find patterns in complex data and relationships between entities or measurements that had not been suspected. Convolutional artificial neural networks can find feature representations for datasets to facilitate, for example, the application of similarity metrics or both supervised and unsupervised learning for classification. These approaches are in many ways related to established techniques of data-driven model building as they provide functions for describing datasets. Both can use data collected in the field as training material or to derive functions in modelling, such as estimation of the rate of change of occupation of state spaces (position of a population in a multidimensional space such as that defined by population size, nutritional status and age distribution), but can also use synthetic data, discussed below.

A recent review (Christin et al. 2019) succinctly captures the range of recent data types and applications of machine learning in ecological studies which range from identifying individual bird calls from environmental recordings (Potamitis 2016) to wild animal counts (Norouzzadeh et al. 2018), tree defoliation (Kalin et al. 2018) and diversity assessment (Salamon et al. 2017). The use of drones to capture elements of the landscape (Richter et al. 2008; Gauci et al. 2018) is also amenable to ML analysis to search for changes which themselves can be used as inputs to ML problems.

All of these examples are potentially useful for radioecology but none to our knowledge have been applied at any scale with the exception of established population modelling methods (Vives i Batlle et al. 2012; Alonzo et al. 2016), or studies of Ra-226

characterization (Varley et al. 2015). These tools are only recently being applied to the area of nuclear science and radiation protection (Gomez-Fernandez et al. 2020)

There are several questions:

1. How much information is needed for effective application of machine learning?
How large do populations need to be to provide sufficient information? For example in projects collecting meta-barcoding of earthworms for population diversity measures are complicated by poor numbers naturally occurring in Chernobyl (because of natural soil conditions) but there are high numbers of species and populations in Fukushima.
2. How many measurements are needed to make an estimation of time dependent changes? Collections may not be carried out at enough time points to do time series analysis
3. How rich, how defined and how reliable is the collection of defined phenotype data in existing databases?
4. Dosimetry is critical so that effects can be related to accurate estimates of dose.
To quote Hansen et al. (2019)

“When effects data are presented versus absorbed dose rates and accumulated doses, and with information on the type of exposure, dose-response results from different experiments or situations can be compared. When this information is missing, it is

difficult to interpret results from exposures, to compare results with literature data and to put these results into context”.

5. How critical are the metadata on environmental parameters such as precipitation, temperature etc. when collecting complex data of a wide range of different types (high dimensional data)?

The advantages of applying machine learning or similar approaches are:

- Often no “control” data are needed to determine patterns, just variation; highly dimensional data does not need “clean” controls to be useful.
- Sonic and visual data are highly amenable to convolutional ANN (Artificial neural networks) representation for clustering and classifying
- For ML and model building there is the possibility of using synthetic data, developing the model and then iterating with data collection in the environment and rebuilding the model – bootstrap approach. As of yet this has not been explored in a radioecological context.

A key message here is that the collection of data, the types of data and the analytical methods available are not specific to radioecology, but are certainly all applicable to the assessment of the ecological impact of contamination. Dosimetry is probably the most important issue and this is discussed at length elsewhere in this commentary. Of particular interest in radiation ecology however is time series analysis and machine learning has been successfully applied to longitudinal time series to predict future

events (eg. Rammer and Seidel 2019 and review therein). Modelling applications such as these are potentially of great value when comparing historical and post-contamination time series as a way of identifying deviations from, for example, predicted changes in population size or complexity. This last example does raise another rather specific problem to the collection and use of big data in the context of radiological contamination, which is the likely lack of historical data on the contaminated site, requiring that a control site be used as a surrogate (Geras'kin et al. 2018). Identification of control sites is fraught with difficulty, a problem noted in several studies in Chernobyl.

Data scale and quality is important but there may be data available now such as birdsong and environmental soundscape recordings, which can be amenable to these approaches. The Internet of Things (IoT) is also being explored for its application to environmental monitoring (Lin and Liaw 2015; Muniraj et al. 2017).

Use of drones to capture elements of the landscape (Richter et al. 2008; Gauci et al. 2018) is also amenable to ML analysis to search for changes which themselves can be used as inputs to ML problems.

How can we use such large-scale data collection and analysis to define and quantify the damage caused to the ecosystem by any exposure (including ionizing radiation)?

A suggestion was to focus on diversity including not only the number of species but also intraspecies genetic variation. It has the advantage that it can be quantified by genome sequencing (which has become cheaper and cheaper). However, the main reason to

focus on diversity is that it is characteristic of the ecosystem not its individual members, and that it is also a crucial property of the ecosystem determining its stability (McCann 2000), resilience and capability to cope with future stresses. A point was made that any small disturbance of the ecosystem can be harmful, because the physiological costs of the repair/adaptation might decrease the chances to reproduce or survive the next stress. However, it is important to recognise that organisms are almost always exposed to different stresses including for the resources within the population. From this point of view, exposures affecting the ecosystem homogeneously (causing similar and not too strong stress to all of its populations) do not cause big changes. However, if a population or a species is much more sensitive than others, it may lead to its elimination from the ecosystem or from the biosphere. Maybe, it is worth mentioning that if we focus on diversity, then the population size and the number of species in the ecosystem are crucial parameters describing its sensitivity. This also illustrates that, while useful, diversity estimates are univariate descriptors that cannot capture the complexity of biological communities. It is easy to imagine a community maintaining the same degree of species diversity while species composition drastically changes.

A much more ambitious aim would be to generate very large models from multiple species and non-biotic data using an agent-based approach. While widely used in ecology and socio-ecological contexts these have not yet found use in radioecology (DeAngelis and Grimm 2014) and have the potential to integrate the functions derived from ML for different components of an ecosystem to better define its structure and dynamics (DeAngelis and Diaz 2019). Such systems have the ability to develop stochastic

rather than deterministic models of ecological interactions. These might have a rather specific sensitivity to the individual variation in response to radiation of species members and may approach the holistic ecological modelling for which we search.

3. Discussion on making experiments useful

Most scientists will have received formal training on research design, often as an early course within their undergraduate studies. Robust research design is fundamental to the scientific method. Without it, we cannot be sure that the conclusions drawn are valid. It is logical to assume that the professional scientific community, formally trained in research design principles, would draw on this in planning their own research and also when peer reviewing the work of others. Unfortunately, there are many examples where this is not the case. When we attempt to use research findings from radiation effects studies, either radiobiological or radioecological, to inform the development of benchmarks for use in environmental radiation protection, we often find that the research findings cannot be used in a meaningful way (cite benchmark derivation papers). This is especially true when we are considering the applicability of research findings to inform the implementation of an ecosystem approach.

A good research approach is to develop a hypothesis and then undertake studies to test that hypothesis. We may postulate a very general null hypothesis to direct research that could inform radiation protection in the context of an ecosystem approach: *Low dose radiation does not impact the environment*. This may appear to be a straightforward hypothesis and is certainly key to understanding radiation within the

broader context of an ecosystem approach. However, it immediately raises three questions that must be considered if we are to ensure that research delivers meaningful results:

- What do we mean by 'low dose'? UNSCEAR (2010) defines low doses as 'those of 200 milligrays (mGy) or less and low dose rates as 0.1 mGy per minute (averaged over an hour or less) for radiations such as external Xrays and gamma rays' (UNSCEAR, 2010). Research publications variously refer to low doses as being in the order of a few μ Gy through to cGy. There is a need to determine the relevant dose range over which we need to be targeting research effort from an environmental protection perspective and also to decide whether the focus is life-time accumulated dose or dose rate. Here we consider low dose to be environmentally relevant doses, including those resulting from authorised discharges and those likely to be encountered at contaminated sites. If the focus is accumulated dose then what constitutes low dose/low dose rate may also be species- and life stage- specific.
- What do we mean by 'environment'? – This is a question that has both a scientific and a social context. It requires a clear articulation of the goal(s) of protection and, for research findings to be widely applicable, the definition of environment in this context must encompass the varied goals and values of society. By viewing 'environment' through two lenses, population sustainability and ecosystem functioning, it is expected that species-specific through to

ecosystem services goals would be addressed (assuming that habitat itself is also sufficiently protected).

- What do we mean by ‘impact’? – To some researchers ‘impact’ is any measurable change resulting from radiation exposure, often at the sub-cellular level. Others view impacts as being changes that are observed at the whole organism level and above. Similarly, some researchers consider impacts as deleterious effects whereas others view impact as either beneficial or deleterious effects. Focusing on indicators of population sustainability and ecosystem functioning would enable radiation-induced changes, be they beneficial or deleterious, to be studied in relation to dose or dose rate.

Testing this hypothesis and understanding any apparent radiation effects will require a combination of laboratory and field-based studies. This is an approach that has been adopted in various projects over recent years, including the UK-based TREE project (<https://tree.ceh.ac.uk/>) and the EC COMET project (<https://radioecology-exchange.org/>), and there is a community consensus on the need to continue pairing laboratory and field studies into the future (Brechignac et al. 2016; Beresford et al. 2020b). However, it is important to recognise some of the challenges in translating research findings between field and laboratory. For example, the response of organisms maintained under ‘ideal’ conditions within a laboratory to a particular radiation dose may be different to the response of organisms receiving that same dose in the field setting where radiation is but one of a range of stressors. Considering radiation in the context of other stressors is an essential step towards the potential integration of

radiological assessments into an ecosystem approach. There are also differences in exposure in field situations. Chronic exposure conditions in a laboratory are generally stable over time, whereas chronic exposure in the field is variable both spatially (e.g. Aramrun et al. 2019) and temporally (e.g. due to seasonal changes in food sources and habitat utilisation (Stark et al. 2017)).

Both laboratory and field-based research on radiation effects require appropriate dosimetry. Where the absorbed dose is not quantified for the organisms under investigation, it is impossible to draw meaningful conclusions on the relationship between dose and effect. Many studies in both the Chernobyl and Fukushima Exclusion Zones have purportedly shown effects of radiation on wildlife but the effect has simply been related to an ambient air dose rate. Whilst this may provide a reasonable approximation of the above-ground external gamma dose to which an organism would be exposed at the location where the measurement is taken, the total absorbed radiation dose (considering both external and internal exposure) may be very different for some species (e.g. Aramrun et al. 2019; Beresford et al. 2020a).

A potential dosimetric challenge when pairing laboratory and field studies is the difference in exposure conditions. Laboratory exposures are often delivered using an external gamma source, whereas field exposures are a combination of both external and internal. Depending on the radionuclides within the organism, the dose deposition may be relatively homogeneous (e.g. radiocaesium) or highly localised (e.g. radioiodine). Consequently, for field studies focussing on radiation-induced changes at the sub-organism level, it will be important to consider whether a whole-organism dose or an

organ-specific dose is the most appropriate measure against which to compare a response. For example, in an environment contaminated with radioiodine, relating whole-body dose to changes in thyroid cells may lead to quite different conclusions than in an environment where radiocaesium is the main contaminant. The ongoing development of voxel phantoms for various organisms provides the capability to determine doses for specific organs (e.g. Ruedig et al. 2015; Caffrey et al. 2017).

Given that our ultimate aim is protection of the environment, there is a need to further develop knowledge on the effects of radiation in complex systems. This may be both direct effects and indirect effects (e.g. a change in predator or prey abundance). For laboratory studies, the consensus view is that more use should be made of mesocosm studies (Haanes et al. 2020). For field studies, it is important to ensure that effects are not being masked by immigration of organisms from neighbouring patches and also that other potential stressors and site history are considered.

Workshop participants were asked to suggest what field-based research should be prioritised if resources for research (both financial and personnel) were unlimited. There was a recognition that there was a need to effectively create mesocosms within the environment so that some of the confounding factors or the intra/interspecies interactions that influence interpretation of field-based radiation effects studies could be controlled. The CEZ has already been recognised as a radioecological observatory site (Steiner et al. 2013; Beresford et al. 2020b) although any CEZ observations need to be interpreted in the context of the contribution of absence of humans. The creation of a network of sub-observatories across the CEZ was proposed, covering a dose rate

gradient and key habitats. Aquatic sub-observatories could be ponds which are geographically isolated from each other. In the terrestrial environment, each sub-observatory would be a large enclosure (perhaps 150m x 150m, with solid 1m high walls extending to a depth of at least 0.5m below ground). The walls of the enclosure should minimise the movement of smaller ground dwelling organisms (invertebrates and small mammals) between the enclosure and neighbouring areas. Replicate sub-observatories would be required within each ambient dose rate band and habitat category; the number of replicates would need to be determined based on consideration of the statistical power of subsequent studies, mediated by the practicalities of locating suitable replicate locations to establish sub-observatories. There would need to be a community consensus on the sub-observatory siting. The sub-observatories should each be fully characterised from both an ecological and radiological perspective and instrumented to monitor climatic and soil parameters. These sub-observatories would be of particular interest to study smaller organisms like small mammals and their interactions (e.g. their food) but cannot be used to study e.g. large carnivores, birds or migratory animals.

The sub-observatories would become a focus for radioecological research in the CEZ, with multiple research groups using the same, well-characterised sites and openly sharing data (as required by many funders and recommended in the radioecology literature, e.g. Beresford et al. 2020b). Populations in these sub-observatories would then be monitored over generations, although it is acknowledged that by enclosing populations within sub-observatories this may change the dynamics of the populations

over multiple generations. Of course one needs to take into account that the long-term follow-up of populations in these sub-observatories might be restricted by the fact that they live in a kind of confinement that potentially affects the natural course of ecological processes e.g. reduced ecological pressure on the population due to lack of big predators. Hence, it is as always essential to see if the experimental design matches with the hypothesis you want to test. Clearly there would be a need to ensure overall coordination of research in these sub-observatories to avoid studies by one research group impacting on studies by another group. An important aspect of such studies would be the ongoing development of non-lethal methods in radioecology (Wood et al. 2011), enabling quantification of internal radionuclide activity concentrations and biological responses without the need to kill the animal. The recent development of a field-portable radiation detector that enables quantification of Cs-137 and Sr-90 through live monitoring of animals (Fawkes 2019) is a significant step in this direction. For animals such as small mammals, this allows a robust internal dose estimation to be made for each individual studied and the animal can then be released back into the sub-observatory.

Whilst the sub-observatories would each be located within a specific ambient dose band, radionuclides will be heterogeneously distributed in three-dimensional space within each sub-observatory resulting in a complex external exposure situation. There will be spatial variation in radionuclides at the soil surface, differences in depth distribution profile and, potentially, an additional above ground contribution to external dose field from Cs-137 that has transferred into vegetation. Developments in direct

measurement of external exposure (e.g. Bonisoli-Alquati et al. 2015; Hinton et al. 2015; Aramrun et al. 2018, 2019) enable more accurate quantification of an individual's external dose within heterogeneously contaminated environments, especially for mobile organisms.

The act of creating sub-observatories, with physical boundaries constructed around them in the terrestrial environment, would be expected to have an influence on the dynamics of the system enclosed within them. For example, enclosure walls may lead to localised shading, micro-climatic variations, reduce grazing and predation, restrict access to resources and influence the movement patterns of more mobile organisms (e.g. small mammals) within the sub-observatory. However, there was broad agreement amongst workshop participants that the proposed sub-observatory approach would still enable more robust, coordinated field studies on radiation effects to be undertaken.

From this discussion the thoughts can be summarised as follows:

- Experiments should be 'hypothesis' based, with rationale and adequate planning, good design (including statistical approach).
- The experiments should consider the benefits to the stakeholders, societal benefit and consider the public confidence.
- Before performing the field studies, techniques/ assays should be properly validated under laboratory conditions.

- For both laboratory and field studies - use appropriate controls defining the 'baseline'; generate 'historical control' data recorded before the event; select appropriate 'pristine' sites i.e. historic reference control sites having zero contamination as opposed to just not having radiation contamination while comparing the results from contaminated sites.
- Results should be compared with available previous studies- explore if laboratory and field studies support each other.
- Construct a 'dose-response' curve for different qualities of radiations, measuring different parameters that are sensitive, reproducible and reliable. Repeat the experiments if possible.
- Consider time and dose rate as important variables leading to differences between field studies and the more short term laboratory approaches
- Consider development of techniques / assays which could be translated across species (e.g. DNA damage, oxidative stress).
- In the event it is not possible to conduct validation studies under laboratory conditions (e.g. experiments involving large mammals, trees etc.), studies aimed at identifying key mechanisms carried out in other model species could perhaps be of use to inform field studies.
- Identify the most sensitive species/ life stages and most sensitive individuals (genotype) for the protection of environment. Know the biology of the species adequately.

- Consider different confounding factors which could influence the experimental outcomes (e.g. age, sex, seasonality, temperature, exposure to multiple stressors etc.).
- Consider appropriate measures while comparing the results of laboratory versus field studies (e.g. exposure to external radiations in laboratory exposures compared to exposure to radionuclides and diversity of radiation qualities in field conditions; define routes of exposures (e.g. water, food) and environmental realism).
- Consider ethical issues of using animals for experiments (i.e. 3Rs principles).
- Do not over-extrapolate the results, being aware of the limitations of the study and reporting them in unbiased manner.

Summary conclusions and potential way forward

The workshop covered an extensive range of topics but the overriding conclusion was that an interdisciplinary approach is needed for what was described as a “wicked problem”. It was recognised that at this stage there is still a need for specific well-focused experiments studying ecological relations and ecosystem responses (where possible, drawing on risk assessment/pollutant/chemical studies for inspiration in set-up) because while there has been a lot of talk on ecosystem approach there are relatively few studies that fall under this term. Hence, we really need to encourage these ecosystem experiments – at both small and large scales.

In terms of a “way forward” the conclusion was that while it is necessary to pursue development of an ecosystem approach to understand radiation action in ecosystems and to be able to detect early signs of issues, these are unlikely to be solely due to radiation. An ecosystem approach would be inappropriate for regulation as it would be too complex but it would be useful in providing the evidence on which regulation is based. At the practical level, the interaction of radioecologists and radiobiologists was seen as central to the development of useful biomarkers at all levels of organisation. Time was seen as a key component that is missing in much of the existing analysis, which is very much concerned with ambient dose rates and effect, while ecosystem level effects develop over extended time periods and require change over time to be a central parameter.

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