



# Exploring New Frontiers in Marine Radioisotope Tracing – Adapting to New Opportunities and Challenges

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Radioisotopes have been used in earth and environmental sciences for over 150 years and provide unique tools to study environmental processes in great detail from a cellular level through to an oceanic basin scale. These nuclear techniques have been employed to understand coastal and marine ecosystems via laboratory and field studies in terms of how aquatic organisms respond to environmental stressors, including temperature, pH, nutrients, metals, organic anthropogenic contaminants, and biological toxins. Global marine issues, such as ocean warming, deoxygenation, plastic pollution, ocean acidification, increased duration, and intensity of toxic harmful algal blooms (HABs), and coastal contamination are all impacting marine environments, thereby imposing various environmental and economic risks. Being able to reliably assess the condition of coastal and marine ecosystems, and how they may respond to future disturbances, can provide vital information for society in the sustainable management of their marine environments. This paper summarizes the historical use of radiotracers in these systems, describes how existing techniques of radioecological tracing can be developed for specific current environmental issues and provides information on emerging issues that would benefit from current and new radiotracer methods. Current challenges with using radioecological tracers and opportunities are highlighted, as well as opportunities to maximize the application of these methods to greatly increase the ability of environmental managers to conduct evidence-based management of coastal and marine ecosystems.

**Keywords:** radionuclides, radiotracers, radioecology, ecosystem condition, marine, coastal

## INTRODUCTION

Many of today's environmental challenges that threaten the health and viability of coastal and marine ecosystems are caused or heightened by a plethora of anthropogenic stressors that are exacerbated by a changing climate and ocean (Dwight et al., 2005). Global marine issues, such as ocean warming, deoxygenation, plastic pollution, ocean acidification, increased duration and

intensity of toxic harmful algal blooms (HABs), and coastal contamination are all impacting marine environments, thereby imposing various environmental and economic risks (Turley and Gattuso, 2012; Speers et al., 2016; Yagi, 2016; Beaumont et al., 2019). Being able to reliably assess the condition of coastal and marine ecosystems, and how they may respond to future disturbances, can provide vital information for society in the sustainable management of their marine environments.

The use of a suite of radioactive isotopic tracers (radiotracers) both in controlled laboratory experiments (reviews by Fisher and Reinfelder, 1995; Fowler et al., 2004; Stewart et al., 2008; Metian et al., 2019a) and in field settings (Swarzenski and Porcelli, 2003; Fry, 2006; Baskaran, 2011; Fowler, 2011; Harmelin-Vivien et al., 2012) has been invaluable to advance our understanding of marine and coastal ecosystems. These experiments (lab and field) have enabled efficient analyses of the uptake and retention of diverse contaminants in aquatic organisms. The analysis of radioisotopes is rapid, relatively inexpensive, and enables experimentation with trace concentrations of elements, well below concentrations typical for most elements in natural waters. Consequently, this methodology has been useful to determine a contaminant's bioavailability (proportion of the total contaminant exposure that is available for uptake into biota), bioaccumulation (uptake of a contaminant into an organism from water and diet), bioconcentration (contaminant concentration in an organism relative to that in the ambient water), and, mostly for organic contaminants or organometallic compounds, biomagnification (in which the contaminant's concentration in the tissues of a predator exceed that in the tissues of its prey).

Nuclear techniques have opened up new perspectives on the pathways and rates of uptake (e.g., bioaccumulation) and biomagnification processes of radioactive and non-radioactive contaminants. Similarly, these techniques have been used for rapidly identifying and quantifying biochemical toxins in seafood, for assessing the impacts of sustained ocean acidification on diverse calcifying organisms, and for evaluating metabolic processes under increasing ocean temperature. Similarly, isotopes have allowed the reconstruction of the geochemical evolution of Earth have resulted in the temporal reconstruction of key environmental processes and rates.

The goal of this paper is to identify advantages that are conferred by using radioisotopes to address existing and emerging environmental processes in marine ecosystems. The paper is organized into four parts: (1) an introduction that reviews the historical use and success stories of radiotracers in coastal and marine science; (2) a section summarizing the development of existing techniques using radiotracers; (3) a section that identifies new tools and techniques that often are multidisciplinary, covering existing coastal and marine issues that would benefit from the use of radiotracers to assess ecosystem condition; and (4) a section describing the challenges and opportunities in this field going forward. The innovative use of radioisotopes in coastal and marine radioecology can help United Nations (UN) Member States work toward and achieve their respective UN Sustainable Development Goal (SDG) targets. The UN has further proclaimed 2021–2030 as the Decade of

Ocean Science for Sustainable Development<sup>1</sup> to promote global coordination and support for ocean science and the coastal communities that depend on a healthy and sustainable ocean. Radiotracers can be used in experimental and field applications to provide unique information and perspectives on how, for example, subtle changes to ocean chemistry over time may influence essential biological systems with far-reaching impacts.

## HISTORICAL USE OF RADIOTRACERS

The exploration and use of radioisotopes in environmental science has advanced our understanding of natural processes. From developing a chronology of Earth's differentiation and planetary evolution using Pb isotope systematics (Patterson et al., 1955) to using products of legacy nuclear weapons testing to derive sedimentary age models, the utility of radioisotopes has evolved continuously over the last 60 years as analytical methods have been established and vastly improved. Indeed, the Suess effect, using radiocarbon (<sup>14</sup>C) dendrochronology, became the cornerstone for supporting anthropogenic global warming and helped determine CO<sub>2</sub> exchange rates between the atmosphere and oceans (Revelle and Suess, 1957), and was further developed into more contemporary (and not dependant on legacy atomic weapon testing) <sup>13</sup>C growth trend analysis in corals (Swart et al., 2010).

Radionuclides can be either natural or artificial and have been used to trace many processes in the marine sciences (see following examples). Indeed, radionuclides are characterized by their rate of radioactive decay, or loss that can be used as a clock to trace the rate of a variety of processes, making them powerful time pieces. Atmospheric nuclear weapons testing during 1945 to 1980 (peaking in 1963) and the development of civil use of nuclear energy has led to the release of many artificial radionuclides to the marine environment such as <sup>3</sup>H, <sup>90</sup>Sr, <sup>137</sup>Cs, <sup>129</sup>I and <sup>238</sup>, <sup>239</sup>, <sup>240</sup>Pu (Benitez-Nelson et al., 2018a). Almost at the same time, due to improvement in analytical chemistry and instrumentation, there was an increasing use of elements belonging to the classic three U-Th series radioactive decay chains to investigate marine processes (Benitez-Nelson et al., 2018a). The GEOSECS (Geochemical Ocean Sections) program was one of the pioneering efforts to make use of radionuclides to study basin-scale processes (Broecker and Peng, 1982).

Natural and artificial fallout radionuclides have been used since the early 1970s for dating recent sediment and determining sedimentation rates (Robbins and Edgington, 1975; Appleby, 2008). In the 1960s and 1970s, numerous studies began to characterize the sorption of radionuclides to marine sediment and the bioaccumulation of radionuclides in aquatic biota; these in turn led to compilations of sediment partition coefficients (K<sub>d</sub>s) and bioconcentration factors (BCFs) of long-lived radionuclides in marine sediments and organisms (IAEA, 2004). These K<sub>d</sub>s and BCFs have been used in modeling efforts to evaluate the cycling and potential impacts of radionuclides in marine ecosystems (European Commission, 1990; ICRP, 2008).

<sup>1</sup><http://oceandecade.org>

In addition, numerous studies explored the application of natural and artificial radionuclides to evaluate processes, such as: geochronology of sediment (e.g., Swarzenski et al., 2006; Appleby, 2008); contaminant uptake, retention and trophic transfer through organisms, including model predictions relative to results from independent field observations (e.g., Fisher et al., 1988, 2000; Roditi et al., 2000; Baines et al., 2002; Luoma and Rainbow, 2005; Baumann and Fisher, 2011); sediment plume dynamics (Swarzenski et al., 1995); carbon flux (Buesseler et al., 2006); water mass ventilation (Matsumoto and Key, 2004), GEOTRACES/GEOSECS/JGOFS programs (Broecker and Peng, 1982; Bowles and Livingston, 1997; Moffett and German, 2018); runoff; and groundwater discharges (Charette, 2001; Moore, 2006; Swarzenski, 2007; IAEA, 2010). Furthermore, by coupling radiotracers (e.g.,  $^{222}\text{Rn}$  and  $^{226}\text{Ra}$ ) with stable isotopes of water (e.g.,  $\delta^{18}\text{O}$ ), one can discriminate source terms of submarine groundwater discharge and distinguish fresh groundwater discharge from saline, recirculated groundwater. This information is vital for both groundwater resource managers that are tasked in developing potable water budgets as well as marine resource managers that are interested in the delivery of SGD-borne nutrients and contaminants to coastal ecosystems (Rocha et al., 2016).

## DEVELOPMENT OF EXISTING TECHNIQUES USING RADIOECOLOGICAL TRACERS

The use of radioecological tracers helps in improving our understanding of contaminant transfer in marine organisms under various environmental conditions and also to better assess biogeochemical cycling of both organic and inorganic particles and dissolved species in a global change context. This section provides some specific examples of where radioecological tracers have been developed or can be developed for certain marine management scenarios.

### Contaminant Biodynamics for Fisheries Management

As noted by Fowler et al. (2004), our understanding of the processes involved in the transfer of contaminants through coastal marine food chains can be greatly improved using nuclear techniques. Specifically, the ability to radioanalyze live organisms and the increased sensitivity of radiotracer detection allows for: (1) biological variation of individual organisms to be captured in experiments relative to conventional ecotoxicology studies not using radiotracers; (2) measuring contaminant biokinetics over the long term in a limited number of individuals; and (3) distinguishing dietary from aqueous sources of contaminants for marine that cannot be easily investigated using standard analytical techniques (Wang and Fisher, 1999). Furthermore, nuclear techniques allow experiments to be conducted using contaminant concentrations that are either well below or approximately close to those present in the natural waters. The uptake of contaminants by aquatic animals predominantly occurs

via uptake of soluble elements through respiration (e.g., via gills) or, to a greater extent for most contaminants, uptake of solid-phase contaminants associated with diet via ingestion (Fowler and Fisher, 2005). Once internalized, it is important to understand how the organism processes the contaminant, either through detoxification/sequestration or release through excretory products back to the environment. If neither of these processes occur effectively, adverse effects/toxicity may ensue. Data on contaminant loss rates from an organism are a crucial part of biodynamic modeling (i.e., balance between uptake and loss), that can be quantified using radiotracers. Much of what is known about depuration rates has been evaluated through the use of radiotracers, but loss rates are usually assessed from individual whole animals rather than specific tissues or organs (with a few exceptions, including Cresswell et al., 2017a), so definitive predictions for loss rates for specific contaminants from different animals remain elusive. A more comprehensive assessment to explain loss rates of contaminants, using radiotracers, would enhance modeling efforts to describe the main uptake pathway of contaminants (e.g., Metian et al., 2016) or their transfer in aquatic food chains, and potentially improve predictions of contaminant concentrations in seafood.

In addition, assessment of depuration rates in commercially relevant seafood species could reduce the risk of consumer exposure to contamination by maintaining organisms a certain period of time in clean open-circuit water systems after the harvesting phase. Indeed, biokinetic parameters, such as biological half-life, inform the time needed to reduce the quantity of contaminant in seafood by half, compared to its initial levels.

### Effects of Abiotic and Biotic Factors on Metal Speciation and Bioavailability

It has long been recognized that the chemical and physical speciation of metal contaminants can influence their bioavailability to aquatic organisms, including plants (e.g., phytoplankton) and animals. This has been extensively studied for metals dissolved in freshwater and seawater (Luoma and Rainbow, 2008; de Paiva Magalhães et al., 2015). However, quantification of the bioavailability of metal contaminants associated with sediments has remained a challenge, particularly in terms of the influence of metal speciation within sediments on bioavailability for benthic animals. While numerous studies have evaluated the bioaccumulation of metals from sediments (Luoma, 1989; Bryan and Langston, 1992; Wang et al., 1999), few have quantitatively assessed how metal speciation in sediments affects bioavailability to benthic fauna (Baumann and Fisher, 2011). Since redox conditions in sediments can change with eutrophication, particularly in coastal sediments, the speciation of metals, particularly those bound to iron and manganese oxides, may change seasonally, and hence affect their bioavailability for bottom-dwelling organisms.

The bioavailability of  $^{137}\text{Cs}$  from contaminated sediments off Fukushima, Japan following the accident at the Fukushima-Daiichi nuclear power plant, has resulted in higher levels of this contaminant in benthic fish than in pelagic fish in Japanese coastal waters (Buesseler et al., 2017; Wang et al., 2018). The

speciation of sediment-bound Cs in those regions has not been fully explored, but evidence suggests that Cs can build up in benthic food chains through assimilation of Cs in deposit-feeding worms that assimilate it from contaminated sediments and transfer it to fish or macroinvertebrates (Wang et al., 2016). In addition, physical factors have changed in the world's oceans (temperature, seawater pH) and these may also affect the bioaccumulation of metals and radionuclides. The extent to which such physical factors may influence contaminant bioavailability remains to be determined. While it is difficult to replicate field conditions in controlled laboratory sorption experiments, radiotracers offer a rapid method for understanding the factors governing contaminant partitioning between the sediment, solution and suspended particulate phases that can aid in the interpretation of field data (Payne et al., 2004).

### Role of Microbes/Plankton in Contaminant Uptake

The role of microbes in marine ecosystems and biogeochemical cycles are often assessed using radiotracers. The classic C-14 assimilation method for measuring phytoplankton primary productivity (Nielsen, 1952) is commonly used in oceanography due to its sensitivity, allowing for high resolution measurements of relatively small volumes of seawater. Microbial biomass production is also similarly measured using compounds labeled with  $^{14}\text{C}$  or  $^3\text{H}$  that are incorporated into DNA or proteins, two biosynthesis pathways that scale with growth. The two most commonly used methods are thymidine and leucine incorporation (Fuhrman and Azam, 1980; Kirchman et al., 1985), which trace DNA and protein synthesis, respectively. Additional radiotracers, such as  $^{33}\text{P}$  (Perry, 1976),  $^{35}\text{S}$  (Jørgensen and Fenchel, 1974), and  $^{55}, ^{59}\text{Fe}$  (Hutchins et al., 1993) have been used to measure microbial uptake and turnover of macro- and micro-nutrients, as well as alternative respiratory pathways like sulfate reduction in both seawater and marine sediments. In addition to rate measurements, the individual organisms involved in the uptake and cycling processes can be identified, visualized, and enumerated by combining autoradiography with fluorescent *in situ* hybridization (FISH) using targeted probes (Ouverney and Fuhrman, 1999).

In addition to assessing 'natural' microbial processes in the marine environment, the uptake of contaminants by microbes can also be explored using radiotracers (Fowler and Fisher, 2005). Due to their large surface area to volume ratios, phytoplankton efficiently accumulate metals and radionuclides in seawater through passive surface adsorption and absorption across cell membranes. Zooplankton similarly adsorb contaminants from seawater but can also accumulate them through ingestion of food. These micro-organisms then serve as vectors for the transfer of metals and radionuclides up into the marine food chain or deeper into the water column through particle transport. Laboratory experiments often label phyto- and zooplankton with radiotracers to serve as dietary vectors for contaminants of interest to higher order marine organisms (Willis and Sunda, 1984). In the field, size-fractionated measurements of contaminants on particulate matter via plankton nets and/or

filtration can indicate the partitioning of these pollutants into the microbial component(s) of marine foodwebs and ecosystems (Buesseler et al., 2012). However, generally fewer radionuclide data exist for phytoplankton at the base of the food chain, compared to zooplankton and larger organisms.

### Specific Contaminants of Concern: Hg/Methyl-Hg, $^{210}\text{Po}$

There are a number of contaminants that are capable of building up in marine food chains. This is particularly true for methylmercury (MeHg) which displays clear evidence of biomagnification (Morel et al., 1998; Reinfelder et al., 1998; Mathews and Fisher, 2008). Unlike inorganic Hg, MeHg is highly assimilated by animals from their diet and is lost from animals at very low rates. Consequently, MeHg tends to increase significantly from one trophic level to another (i.e., biomagnification), and reaches very high concentrations in tissues of old, large, slow-growing predators (e.g., swordfish, tuna, sharks, whales). Given its toxic effects in seafood consumers (mostly neurological effects), there is considerable public health interest in following the bioaccumulation and trophic transfer of MeHg in aquatic food chains. Application of the beta/gamma-emitting radioisotope  $^{203}\text{Hg}$  (both inorganic and organic species) has been undertaken to study the biodynamics in marine food chains (Lee and Fisher, 2016, 2017) and may continue to elucidate how MeHg cycles in aquatic ecosystems.

Another important radionuclide that can accumulate in marine biota animals is the naturally occurring  $^{210}\text{Po}$ . This radionuclide is the last of the radioactive daughter products of  $^{238}\text{U}$ , which is ubiquitous in the world's oceans.  $^{210}\text{Po}$  is an alpha-emitting radionuclide (5.3 MeV) that primarily associates with proteins in animals (Heyraud and Cherry, 1979; Cherry and Heyraud, 1981, 1982). It is greatly enriched in phytoplankton at the base of marine food chains (Fisher et al., 1983; Stewart and Fisher, 2003) and can be efficiently transferred to zooplankton, and subsequently, larger animals along short food chains (Stewart et al., 2008). The resultant radiological dose to marine fish generally exceeds that of anthropogenic radionuclides (e.g.,  $^{137}\text{Cs}$ ) (Aarkrog et al., 1997), even in most fish exposed to  $^{137}\text{Cs}$  in waters off Fukushima (Fisher et al., 2013). Given the potential importance of  $^{210}\text{Po}$  dose to marine biota as a benchmark against which doses from anthropogenic radionuclides need to be assessed, it is important to better understand how  $^{210}\text{Po}$  speciates in water and how it binds to specific proteins in marine organisms. While important inroads have been made through the pioneering work of Cherry and Heyraud (1981, 1982), clearly more research is warranted on the marine geochemical cycling and biochemistry of this radionuclide.

### U/Th Series Isotope Tracers to Examine the Marine Carbon Cycle

$^{238}\text{U}$ - $^{234}\text{Th}$  and  $^{210}\text{Po}$ - $^{210}\text{Pb}$  isotope pairs have been used extensively to estimate the seasonal to annual particulate organic carbon (POC) export and remineralization in the oceans.  $^{234}\text{Th}$  ( $t_{1/2} = 24.1$  d) is produced from the alpha decay of  $^{238}\text{U}$  in the ocean.  $^{238}\text{U}$  is a highly soluble and conservative element

in seawater, whereas  $^{234}\text{Th}$  is highly particle reactive. Similarly,  $^{210}\text{Po}$  ( $t_{1/2} = 138$  d) is produced from the alpha decay of its grandparent  $^{210}\text{Pb}$  ( $t_{1/2} = 22.3$  y) via  $^{210}\text{Bi}$  ( $t_{1/2} = 5.01$  d). Both  $^{210}\text{Po}$ - $^{210}\text{Pb}$  are variably particle reactive and  $^{210}\text{Po}$  is highly reactive to biogenic particles (Stewart et al., 2008; Jones et al., 2015; Bam et al., 2020). The daughter and parent radioisotopes reach secular equilibrium (i.e., comparable activity concentrations; Bq/g or Bq/mL) when parents are much longer-lived relative to the daughter in a closed system and there have been multiple daughter half-lives experienced (Benitez-Nelson et al., 2018b). However,  $^{234}\text{Th}$  and  $^{210}\text{Po}$  are preferentially adsorbed onto sinking particles which 'disrupts' the parent-daughter secular equilibrium and can result in preferential isotopic ratios, which provides an estimate of the particle flux. Also, the scavenging of  $^{210}\text{Po}$ - $^{210}\text{Pb}$  is driven by the difference in the affinities of the nuclides for active biological uptake versus passive adsorption (Nozaki et al., 1998). The flux of the radioisotope is calculated simply as follows:

$$F = \lambda_D \int_0^z (A_P - A_D) dz$$

where  $F$  is the flux ( $\text{dpm m}^{-2} \text{d}^{-1}$ ) of daughter radioisotopes,  $z$  is the depth of water column (m),  $\lambda_D$  is decay constant of daughter ( $\text{d}^{-1}$ ),  $A_P$  and  $A_D$  are activities ( $\text{dpm L}^{-1}$ ) of parent and daughter radioisotopes in the water column respectively.  $^{234}\text{Th}$  provides a flux rate integrated over a few weeks (Buesseler et al., 1998, 2001), which coincides with the residence time of biological particles in the upper ocean. Thus,  $^{234}\text{Th}$  based flux is useful to estimate and/or rule out POC-flux due to short-term local blooms, upwelling or temporary disruption.  $^{210}\text{Po}$  provides a flux rate integrated over several months. The export fluxes of these daughter radionuclides can be converted to the POC export flux as follows:

$$F_{\text{POC}} = F_R \left[ \frac{\text{POC}}{R} \right]_{\text{sinking particles}}$$

where  $F_{\text{POC}}$  is flux of POC ( $\text{mg C m}^{-2} \text{d}^{-1}$ ),  $F_R$  is flux of radioisotope ( $^{234}\text{Th}$  or  $^{210}\text{Po}$ ;  $\text{dpm m}^{-2} \text{d}^{-1}$ ), POC is particulate organic carbon concentration ( $\text{mg C L}^{-1}$ ) and  $R$  is the activity ( $\text{dpm L}^{-1}$ ) of radioisotope ( $^{234}\text{Th}$  or  $^{210}\text{Po}$ ;  $\text{dpm L}^{-1}$ ). For example, Maiti et al. (2016) has estimated the carbon flux using both  $^{210}\text{Po}$  and  $^{234}\text{Th}$ . Here, we present the profiles of  $^{234}\text{Th}$ ,  $^{238}\text{U}$  and  $^{210}\text{Po}$ : $^{210}\text{Pb}$  (Figures 1A,B) adapted from Maiti et al. (2016). Net disequilibria of  $^{234}\text{Th}$ : $^{238}\text{U}$  and  $^{210}\text{Po}$ : $^{210}\text{Pb}$  integrated relative to depth can be used to estimate the flux of  $^{234}\text{Th}$  and  $^{210}\text{Po}$ , which is then used to calculate the carbon flux, as shown in Figure 1C.

Multiple studies have shown that the  $^{234}\text{Th}$ -based POC fluxes are similar to  $^{210}\text{Po}$ -based fluxes, falling within a factor of 0.8 to 1.8 (Gulf of Mexico; Figure 1C; Maiti et al., 2016), 0.5 (Equatorial Pacific; Murray et al., 2005), 0.9 to 3.4 (Mediterranean Sea; Stewart et al., 2007), 0.74 to 2.3 (South China Sea; Wei et al., 2011). These differences in the  $^{234}\text{Th}$  and  $^{210}\text{Po}$  based fluxes might due the fact that one must assume: (1) steady state conditions and minimal physical processes; (2) negligible advective and diffusive fluxes; and (3) most importantly, daughter radionuclide deficit is caused due to

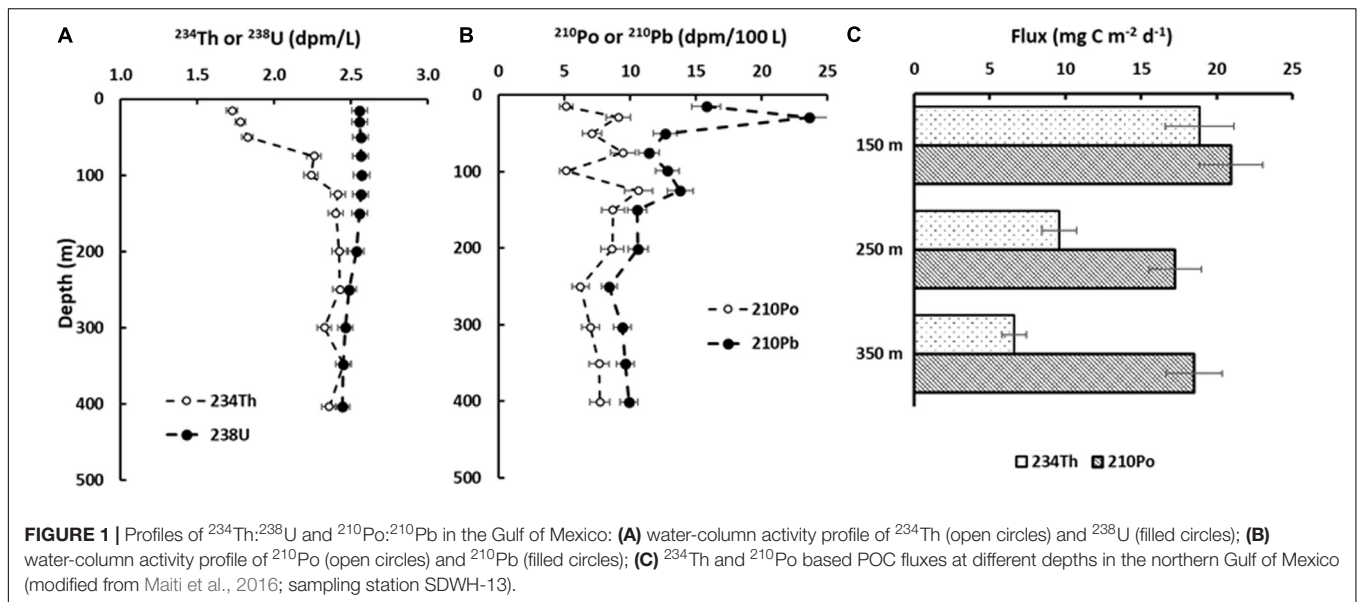
the preferential scavenging of daughter radionuclide relative to parent radionuclide (Maiti et al., 2016; Tang and Stewart, 2019). The adsorption and scavenging of radionuclides are influenced by the concentration and composition of the particles (Roberts et al., 2009; Tang et al., 2017).

The concentration and composition of the particles in the ocean is changing due to higher sediment input from the coastal erosion, high riverine input, changes in terrestrial organic matter input and exponentially increasing amounts of micro- and nano-plastic particles (Rachold et al., 2004; Chen et al., 2012; Jambeck et al., 2015; Basu and Mackey, 2018; Zheng et al., 2018). Further, the ocean chemistry is rapidly changing due to climate change, expansion of oxygen minimum zones and high sediment loads in estuarine and coastal areas. It is estimated that 4–12 million tons of plastic was transferred to the ocean annually in 2010 and it is expected to increase by an order of magnitude by 2025 at the current rate (Jambeck et al., 2015). These changes in particle type, origin, shape and size might influence the way radioisotopes can be used to study the particle fluxes, as the affinity of adsorption and sorption of these radionuclides is different for different particle types and sizes.

Atmospheric  $\text{CO}_2$  is converted into POC by phytoplankton in the ocean (Figure 2). A portion of the POC can be exported to the deeper ocean by sinking particles, while some amount of POC can also be converted to dissolved organic carbon (DOC). Transparent exopolymer particles (TEP) are carbon-rich, extracellular particles formed by the accumulation of DOC and can aggregate and sink along with POC (Arrigo, 2007). Increased  $\text{CO}_2$  in the atmosphere can increase the POC in the surface water and impact ocean acidification. Ocean acidification could lead to increase in the abundance of TEP (Arrigo, 2007; Louis et al., 2017). TEP enhances the particle aggregation and sinking of aggregated particles influenced by the POC export (Mari et al., 2012; Louis et al., 2017). The change in primary productivity, and consequently increases in phytoplankton and algal blooms events, are becoming more frequent in global oceans (Hallegraeff, 2010; Wells et al., 2015; Schulz et al., 2017). The increased sinking of phytoplankton after the bloom events cause high POC export (Kessouri et al., 2017; Roca-Martí et al., 2017).

It is important to understand how plastic gyres, plastics leaching DOC, and biofilms using plastics as substrates will impact the particle dynamics, phytoplankton and zooplankton communities and carbon export in the global ocean. Plastic pellets act as a transport medium for different chemicals and trace metals, such as zinc, copper, cadmium, and lead, via sorption and desorption processes (Mato et al., 2001; Munier and Bendell, 2018), and for the radionuclides,  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  (Johansen et al., 2019b). In future, studies should focus on the kinetics of sorption and desorption of organic matter and radionuclides to plastics to better quantify the carbon export. Further, Romera-Castillo et al. (2018) have suggested that DOC leaching from plastics in the oceans may have unaccounted effects to the marine organic carbon cycle.

Future studies should focus on the how the changing physical, chemical, and biological conditions in the ocean environment change the aggregation, sinking, and export rates of the POC, as well as other sinking particles. The coupled use of radioisotope



tracers and stable isotopes of carbon and nitrogen could be beneficial to better estimate POC export, differentiate the source of POC and DOC (plastic-derived, terrestrial and marine) and understand the remineralization of organic carbon in the ocean.

## Role of Metals in HABs Biotoxin Production

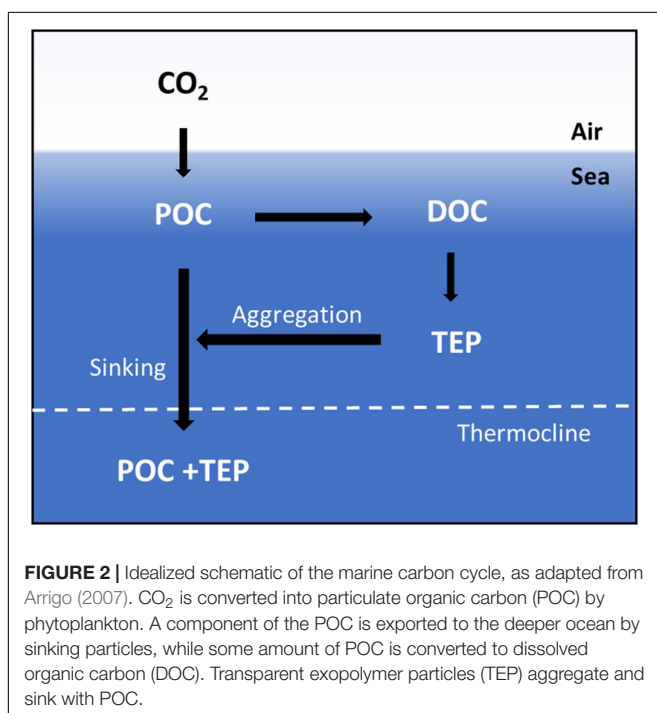
The initiation of harmful algal blooms (HABs) and their production of biotoxins remains an open question, although such blooms almost certainly require specific environmental

conditions (i.e., temperature, light, nutrients). In addition to the influence of trace metals on phytoplankton through nutrient limitation, as well as toxicity, their bioavailability may also be implicated in stimulating toxin-producing HABs (Sunda, 2006). Most well-studied may be the occurrence of *Pseudonitzschia* and the neurotoxin, domoic acid, following iron fertilization experiments or with the addition of copper (Maldonado et al., 2002; Wells et al., 2005; Silver et al., 2010; Trick et al., 2010). Other studies have linked trace elements, such as Li, Se, and Ni, to HAB species (Kudela et al., 2010). Algal-bacterial interactions may also be involved in bloom dynamics, with siderophore-producing bacteria potentially supplying iron to HAB species (Yarimizu et al., 2018). Given the potential links between various trace metals and HAB toxin production, the use of sensitive radiotracers, such as  $^{59}\text{Fe}$ , would enable experiments with environmentally relevant concentrations of elements potentially implicated in HAB initiation, biotoxin production, and physiology. Radiotracer techniques would allow for precise detection of trace elements and potentially identify their location within cells or in the surrounding phycosphere.

## EXISTING AND EMERGING ISSUES THAT WILL BENEFIT FROM CURRENT AND NEW RADIOTRACER TECHNIQUES

### Development of New Applications of Existing Radiotracers to Address Marine Processes

In light of these many and varied applications of radiotracers toward understanding marine ecosystem processes, there are additional areas where existing techniques could be further developed to address under-explored outstanding research questions. Ongoing development in the use of natural abundance radiocarbon ( $^{14}\text{C}$ ) distributions throughout the



marine environment (both inorganic and organic in seawater, sediments, and biota) continues to enhance our understanding of critical aspects of the marine carbon cycle and ocean circulation and ventilation as a whole (Broecker and Peng, 1982; Matsumoto, 2007; McNichol and Aluwihare, 2007). For example,  $^{14}\text{C}$  measurements on DOC fractions and even specific compounds and biomarkers (Close, 2019), are increasingly possible due to analytical innovations leading to decreased sample size requirements. Furthermore, these measurements can be used to probe both age and lability of various components of the DOC pool. Although the systematic increased resolution of dissolved inorganic carbon  $^{14}\text{C}$  measurements, initiated as part of the World Ocean Circulation Experiment (WOCE), has led to unprecedented insight into the carbon reservoir of the interior ocean and its role in regulating climate (McNichol et al., 2000), increased demand for other types of  $^{14}\text{C}$  measurements can drive technical method innovation, resulting in lower costs, smaller required sample sizes, and reduced analysis time. As ocean conditions are changing along with changes in the global climate, existing radiotracers can be applied to new systems within marine environments. For example, radiotracers commonly used to track processes in anoxic sediments can be adapted for use in water column oxygen minimum zones and expanding low oxygen regions of the global ocean. Furthermore, combining radiotracers with other analytical techniques, such as liquid chromatography-mass spectrometry (LC-MS), can link biomarker identification to specific metabolic processes that have biogeochemical implications in marine systems, such as the assimilation of carbon or lipid biosynthesis in marine organisms (Evans et al., 2018, 2019).

## Multiple Stressors; Multiple Contaminants, and Environmental Stressors

Marine organisms and ecosystems are exposed to a wide range of environmental changes due to human activities. Some of the changes are global, whereas others are regional or local. These wide-ranging changes are often referred to as drivers or stressors (Boyd et al., 2018). The multiple drivers framework represents a complex matrix of changing ocean properties that will vary from locale to locale, and may also change with season. A wide range of perturbation experiments can be used to better understand how multiple drivers influence marine life (Boyd et al., 2018). Radiotracers could be used to assess the effect of environmental stressors on the bioaccumulation of an element, including bioconcentration or trophic transfer. In recent years, focus was placed to understand the effect of ocean acidification on metal bioaccumulation in marine organisms such as cadmium (Cao et al., 2018). These studies highlight the main advantage of using gamma-emitter radiotracers including: (1) the ability to radioanalyze the same individual live organisms over time, thereby reducing biological variability between samples; and (2) the possibility to conduct experiments at contaminant concentrations that are below or comparable to those present in the environment. Working with stable metals, analytical protocols commonly require higher, more environmentally

unrealistic experimental concentrations of metals and also require more time for conducting analyses.

Nuclear techniques can also highlight some physiological responses and be considered as “proxies” for physiological change in aquatic species. Radiotracers, such as  $^{65}\text{Zn}$ , have been used in the past, but nuclear techniques, such as nuclear magnetic resonance spectroscopy (NMR), allow the determination of metabolic profiles of tissues, organs or individuals (Viant et al., 2003) under multiple sets of conditions (such as ocean warming, acidification and hypoxia, e.g., Schmidt et al., 2017; Tripp-Valdez et al., 2017; Götze et al., 2020) and with different environmental stressors (e.g., heavy metals, Lannig et al., 2010; Cappello et al., 2016).

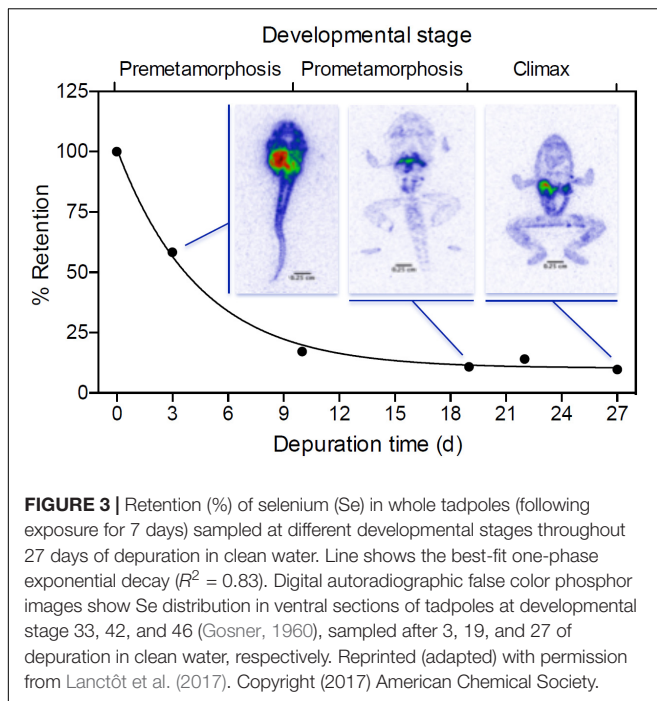
## Impact of Physiological Processes on Contaminant Uptake and Loss

It has been well documented that physiological process can impact contaminant bioaccumulation processes (Luoma and Rainbow, 2008) and the use of radiotracers can greatly enhance the understanding of such processes. It has been well documented that molting (shedding of exoskeleton) in decapod crustaceans can affect the accumulation kinetics of metals using radiotracer. For example, Cresswell et al. (2015) found that cadmium and zinc uptake in freshwater decapods (*Macrobrachium australiense*) returned to an intermolt rate of uptake 2–3 days post-molt, which may be due to the exoskeleton no longer being as permeable by that stage, or due to a decrease in calcium pump rates as the exoskeleton hardens. However, White and Rainbow (1984, 1986) observed that the rate of cadmium and zinc uptake in post-molt marine decapods (*Palaemon elegans*) continued to be enhanced and had not returned to pre-molt rates by the time each study ended, with the time from molt to the end of the studies occurring between 36 h and 8 days. O’Mara et al. (2019) detected an increase in the uptake of manganese and zinc in marine school prawns (*Metapenaeus macleayi*) that had molted, compared to those that did not molt during aqueous exposure.

Radiotracers have also been used to study how significant physiological changes, such as metamorphosis in amphibians, can affect contaminant bioaccumulation. Lanctôt et al. (2017) used radiotracers of selenium ( $^{75}\text{Se}$ ) to study the role of tissue degeneration and remodeling during anuran metamorphosis as a mechanism for examining tissue-specific contaminant burdens. The study with the Australian striped marsh frog, *Limnodynastes peronii*, demonstrated that selenium biodistribution (i.e., organ distribution) varies significantly throughout metamorphosis (Figure 3).

## Integration of Stress/Energy Biomarkers With Radiotracer Contaminant Studies

As mentioned before, an assessment of the effect of environmental stressors on organisms can be conducted by testing their responses using nuclear techniques. For example, in metabolomics (the scientific study of chemical processes involving metabolites), NMR provides a useful technique for profiling metabolites and has been used for assessing the impact of environment stressors on marine organism over almost



20 years (Viant et al., 2003; Lannig et al., 2010; Lankadurai et al., 2013; Tikunov et al., 2014; Cappello et al., 2016). Metabolomics consists of a large-scale study of small molecules, commonly known as metabolites, within cells, biofluids, tissues and/or organisms. Untargeted metabolic profiling, based on NMR spectroscopy, is a powerful approach because of its holistic nature and because metabolites and their concentrations directly reflect the underlying biochemical activity and state of cells and tissues.

Another type of magnetic resonance spectroscopy has been also used toward this aim. *In vivo* magnetic resonance spectroscopy (MRS) is a specialized non-destructive technique associated with magnetic resonance imaging (MRI). This technique is a non-invasive, ionizing-radiation-free analytical technique that has been used to study metabolic changes in human patients in the last century (Lauterbur et al., 1980; Bell and Bhakoo, 1998). However, developments have been made in marine ecology and comparative physiology, more particularly, in the assessment of physiological (energetic) status of marine organisms, such as mollusks or fish, under changing environmental conditions (Bock et al., 2002, 2019). Mainly, two *in vivo* techniques have been used: proton magnetic resonance spectroscopy ( $^1\text{H}$ -MRS) and  $^{31}\text{P}$  magnetic Resonance Spectroscopy ( $^{31}\text{P}$ -MRS).  $^1\text{H}$ -MRS is able to quantify the abundance of neurochemicals, such as neurotransmitters and metabolites like amino acids, but also anaerobic end products, such as lactate, and is also used for fat and lipid analysis (Bock et al., 2017; Wernter et al., 2018).  $^{31}\text{P}$ -MRS measures phosphorus-containing metabolites that play an essential role in physiology. The most important are the high-energy phosphates, ATP and phosphagen (creatine phosphate or phospho-l-arginine) and their end product, inorganic phosphate, to address the energy

status. Furthermore, the  $^{31}\text{P}$ -NMR signal of inorganic phosphate can be used to follow acid–base regulation of cells and tissues in the same spectrum (Bock et al., 2019). Recently, *in vivo*  $^{13}\text{C}$ -NMR spectroscopy was introduced to study metabolic pathways in marine organisms under environmental stress (Tikunov et al., 2014).

NMR has also had substantial utility in phosphorous speciation studies, and has resulted in increased understanding of the modern phosphorous cycle in both terrestrial soils and aquatic sediment. For example,  $^{31}\text{P}$ -NMR has been used successfully in studying the speciation of phosphorous in Chesapeake Bay to better understand nutrient contamination and dynamics (Li et al., 2015) and  $^1\text{H}$ - $^{31}\text{P}$  NMR correlation spectroscopy has been used to characterize the organic phosphorous fractions in soil which has resulted in an increased understanding of phosphorous biogeochemistry (Vestergren et al., 2012). All of these NMR spectroscopy techniques are quite unique and could be used for the future development of nuclear techniques toward the assessment of the physiological impacts of environment stressors.

Other nuclear-derived techniques, such as the polymerase chain reaction (PCR), reverse transcription PCR and enzyme-linked immunosorbent assay, are important tools to rapidly and efficiently identify and characterize environmental stress caused to marine organisms (Boutet et al., 2002; Peck et al., 2011; Huo et al., 2018).

## Development of Radio-Assays for HAB Biotxin Detection; Adapting Nuclear Receptor Binding Techniques for a Range of Marine Biotoxins

The frequency and scales of today's HAB constitute a growing worldwide problem, negatively affecting aquatic ecosystems, public health and local economies. HAB events have been shown to cause oxygen depletion, phycotoxin production, mucilage, reactive oxygen species, and polyunsaturated fatty acids. HAB can also trigger systematic damage to tissue and cells of marine organisms and can even cause catastrophic mortalities in commercially important fish species, as well as marine mammals and sea turtles (Anderson, 2017). Worldwide, the occurrence of HAB appears to have increased in frequency, geographic extent and intensity, which may be due to heightened nutrient discharges, but also climate change impacts, such as warming and water column stratification (Heisler et al., 2008), as well as the introduction of exotic species (van den Bergh et al., 2002). Most of the HAB species are dinoflagellates, accounting for as much as 100 taxa in the marine environment (Mouestrup et al., 2009).

Paralytic shellfish toxins (PSTs) and ciguatoxins can be effectively and quickly analyzed in marine biota using the receptor binding assay (RBA) technique (IAEA, 2013). Briefly, the RBA method quantifies the toxin potency by determining, through a scintillation counter, the concentration of tritiated toxin standards, which compete with the toxin from sample extracts for binding to voltage-gated sodium channels in a rat brain membrane preparation (Van Dolah et al., 2012).



The establishment of effective HAB early warning systems, the adoption of robust molecular techniques to improve rapid identification methods of HAB-forming species, and necessary expansion into freshwater ecosystems should all be developed and strengthened to ameliorate potential deleterious impacts of new HAB events (Cuellar-Martinez et al., 2018).

## Maternal Transfer of Contaminants, Including Intergenerational Transfer

Among the different exposure pathways studied separately in the past using radiotracers, there was water, food, and sediment (Casado-Martinez et al., 2009; Metian et al., 2010; Wang, 2011; Cresswell et al., 2014), but episodically, a fourth pathway has been investigated: the maternal transfer of contamination (viz. transfer of contaminants from the mother to the eggs or the juveniles; e.g., Lacoue-Labarthe et al., 2008; Jeffree et al., 2015). It has been shown that in some marine organisms, maternal transfer is metal-dependent and while eggs can concentrate some elements, juveniles might be safe due to protective mechanisms (Lacoue-Labarthe et al., 2008). Gamma-emitting radiotracers are the ideal tools to assess such transfer. In the same vein, it is also possible to look at more than one generation transfer (i.e., transgenerational), but this requires some specific species with a short life-cycle (such as *Daphnia* spp.; see Lam and Wang, 2006).

Radiolabeled organic compounds are also part of the radiotracer toolkits to examine the bioaccumulation of organic compounds in aquatic organisms. Although they are mainly labeled with  $^{14}\text{C}$ , some tritium-labeled compounds are also used. A large range of compounds exist, from polychlorinated biphenyls (PCBs) to benzo(a)pyrene (BaP) and include surfactants (e.g., linear alkylbenzene sulfonates; Metian et al., 2019b), pesticides and polycyclic aromatic hydrocarbons (PAHs) (Danis et al., 2005; Wang and Wang, 2006; Berrojalbiz et al., 2009; Renaud et al., 2014). Radiolabeled organic contaminants are usually beta-emitters, and unlike gamma-emitters, radiocounting individuals over time is impossible due to the analytical methods usually used to detect organic radiotracers (scintillation counting). This means that the description of kinetics requires destruction of the samples and a higher number of organisms. Nevertheless, the main advantage is the high degree of sensitivity of radio-detection methods compared to standard analytical techniques (Fowler et al., 2004), which allow investigating the bioaccumulation of organic contaminants which occur at very low environmental concentrations, such as individual PCB congeners or organic metabolites.

## Instrument Improvements for the Analysis of Environmental Samples

The general low level of radionuclides in environmental samples and/or the small sample sizes available have required the use of efficient techniques. As outlined by Povinec (2017), the transition from counting of radioactive decays to counting atoms using mass spectrometry methods (Accelerator Mass Spectrometry-AMS, Inductively Coupled Plasma-Mass Spectrometry-ICP-MS, Resonance Ionization Mass Spectrometry-RIMS, Secondary Ion Mass Spectrometry-SIMS, Thermal Ionization Mass

Spectrometry-TIMS) is a major paradigm shift in radioanalytical technology. Several of these latter methods have been adapted over the last decades to cover a wide span of environmental samples and radionuclides. Such analyses include: the quantification of key radionuclides, such as  $^{129}\text{I}$  at very low levels at the Savannah River site in the United States, where speciation studies of iodine were conducted using AMS (Garcia-Leon, 2018); the analysis of  $^{240}\text{Pu}/^{239}\text{Pu}/^{241}\text{Pu}$  isotope ratios in waters, sediments, terrestrial soils and marine and terrestrial biota using AMS to understand the fate of Pu and other radionuclides from former nuclear weapons testing in the Montebello Islands, Australia (Johansen et al., 2019a); the development of ICP-MS and coupling with linear quadrupole, time of flight (TOF), and Fourier transform ion cyclotron resonance (FTICR) to analyze environmental samples for radionuclides in a relatively cost-effective way (Roos, 2008), especially for  $^{135}\text{Cs}/^{137}\text{Cs}$  ratios via ICP-MS with the development of interference separation methods (Russell et al., 2015); and the determination of  $^{99}\text{Tc}$  in environmental samples through the development of chemical separation, combined with traditional radioanalytical techniques and mass spectrometric measurement techniques, to undertake safety assessments and decommissioning of nuclear facilities, as well to study water mass movement, exchange and circulation in oceanography (Shi et al., 2012).

These advances in analytical instrumentation to detect ultra-trace concentrations of radionuclides, down to 0.1 fg levels (López-Lora and Chamizo, 2019), is providing a deeper understanding of the fate of contaminants and water mass movement in marine systems.

## CHALLENGES AND OPPORTUNITIES

While the use of radioecological tracers has been practiced for nearly 60 years, today there is a real risk that these techniques will be lost, as fewer and fewer scientists are active in this field. This is partly due to the high regulatory and safety requirements for the shipment, storage, use (including personnel training) and disposal of radioactive materials, and partly to the lack of training in radiochemical and radiobiological methods in recent decades. A new generation of scientists with radioecological skills is needed to evaluate nuclear accidents (such as at Fukushima), advise on siting and potential impacts of new nuclear installations, and advise on decommissioning of aging nuclear power plants (Fisher et al., 2015). Further, it is important for the environmental science community to continue to support and train new scientists in the field of radioecological tracers to develop new techniques to apply to emerging coastal and marine ecosystem issues. By combining conventional environmental biological and chemical techniques with radioecological tracing techniques, a greater understanding of the coastal and marine ecosystem condition can be achieved. The development of radioecological tracer techniques should focus on addressing specific environmental issues, such as changes in animal physiology in response to changing climatic conditions and anthropogenic stressors, especially in combination with emerging 'omic' techniques (e.g., to detect genes, mRNA, proteins and

metabolites in biological samples). This will greatly increase the ability of environmental managers to conduct evidence-based management of coastal and marine ecosystems.

One problem in working with radioisotopes is the difficulty in obtaining permits to conduct experiments in field situations. While some studies have taken advantage of using radioisotopes to explore how metals behave in natural settings (Dahlgard, 1986; Fisher et al., 1996), increasingly, such studies have become difficult due to legal and administrative constraints. The few experiments that have compared lab and field behaviors of radionuclide processing by marine biota have indicated small differences, so laboratory simulations may be generally applicable to field situations. Moreover, modeling of metal bioaccumulation in aquatic animals that use key laboratory-generated criteria (uptake rate constants, assimilation efficiencies, efflux rate constants) has consistently shown that predictions closely match independent field measurements of metal bioaccumulation in diverse invertebrates and fish (Fisher et al., 1996, 2000; Wang et al., 1996; Roditi et al., 2000; Baines et al., 2002; Luoma and Rainbow, 2008; Mathews and Fisher, 2009; Baumann and Fisher, 2011; Dutton and Fisher, 2014). Thus, it appears that laboratory generated data can be applicable to real-world situations for many metals and metalloids. However, experimental work with large animals typically preclude laboratory experimentation and it is therefore often difficult to make comparisons between modeled tissue concentrations for these animals based on lab measurements and field measurements.

The use of radioecological tracers can increase the complexity of the experimental design (i.e., study contaminant mixtures during multiple environmental stressors such as pH and

temperature), increase data representativeness and quality through studying the fate of contaminants in individual organisms rather than pooled organisms, reduce the number of organisms required in experimental assays (Cresswell et al., 2017b), and reduce the requirement for destructive use of animals to align with initiatives, such as the National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs<sup>2</sup>); and allow for more rapid and more accurate detection of experimental analytes. This is especially the case where such analytes exist in high background concentrations, such as zinc.

## AUTHOR CONTRIBUTIONS

All authors participated in a planning meeting for an IAEA Cooperative Research Project, held in Monaco in December 2018. All authors prepared content for their associated sections. TC compiled content from all authors and prepared the final version. All authors have reviewed and agreed to the submission of the final version.

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<sup>2</sup>www.nc3rs.org.uk

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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