Identifying toxic impacts of metals potentially released during deep-sea mining – a synthesis of the challenges to quantifying risk

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Abstract

In January 2017, the International Seabed Authority released a discussion paper on the development of Environmental Regulations for deep-sea mining within the Area Beyond National Jurisdiction (the 'Area'). With the release of this paper, the prospect for commercial mining in the Area within the next decade has become very real. Moreover, within nations' Exclusive Economic Zones, the exploitation of deep-sea mineral ore resources could take place on very much shorter time scales and, indeed, may have already started. However, potentially toxic metal mixtures may be released at sea during different stages of the mining process and in different physical phases (dissolved or particulate). As toxicants, metals can disrupt organism physiology and performance, and therefore may impact whole populations, leading to ecosystem scale effects. A challenge to the prediction of toxicity is that deep-sea ore deposits include complex mixtures of minerals, including potentially toxic metals such as copper, cadmium, zinc, and lead, as well as rare earth elements. Whereas the individual toxicity of some of these dissolved metals has been established in laboratory studies, the complex and variable mineral composition of seabed resources makes the *a priori* prediction of the toxic risk of deep-sea mining extremely challenging. Furthermore, although extensive data quantify the toxicity of metals in solution in shallow-water organisms, these may not be representative of the toxicity in deep-sea organisms, which may differ biochemically and physiologically and which will experience those toxicants under conditions of low temperature, high hydrostatic pressure, and potentially altered pH. In this synthesis, we present a summation of recent advances in our understanding of the potential toxic impacts of metal exposure to deep-sea meio- to megafauna at low temperature and high pressure, and consider the limitation of deriving lethal limits based on the paradigm of exposure to single metals in solution. We consider the potential for long-term and far-field impacts to key benthic invertebrates, including the very real prospect of sub-lethal impacts and behavioural perturbation of exposed species. In conclusion, we advocate the adoption of an existing practical framework for characterising bulk resource toxicity in advance of exploitation.

1) Introduction

Whilst the technological challenges of mineral recovery from deep water are significant (e.g. Collins et al., 2013; Glasby et al., 2015), they are no longer regarded as insurmountable obstacles to the exploitation of seabed resources by deep-sea mining (DSM) (Hannington et al 2017). Consequently, the start - and subsequent expansion - of DSM will soon become contingent on: a) a viable economic assessment (see Petersen et al., 2016; Hannington et al., 2017) and, b) an appropriate legislative framework.

Legislatively, under the UN Convention on the Law of the Sea (UNCLOS), legal responsibility for mineral exploitation in the 'Area Beyond National Jurisdiction' (or the 'Area') rests with the International Seabed Authority (ISA). Early in 2017 the ISA published draft Environmental Regulations for deep-sea resource exploitation within the Area (ISA, 2017). This draft code advocates the Precautionary Principle in the initial pursuit of deep-sea mineral resources and a framework of Adaptive Management to monitor and regulate exploitation by licenced contractors. However, these two concepts present challenges for the ISA, as have been recently reviewed by Jaeckel (2016) and Le et al. (2017). These reviews have argued that key challenges remain with the identification of environmental risks of DSM, relevant to the application of the Precautionary Principle, and in the development of appropriate monitoring technologies with which to support the Adaptive Management of exploitation.

Recently, great progress has been made in constraining the toxic risks of mineral extraction under the unique conditions of high pressure and low temperature, as well as presenting these risks in an operationally appropriate manner to support decisions on exploitation licencing. At this key time for the development of DSM exploitation legislation, this synthesis paper provides a summation of the scientific community's latest understanding of the challenges in quantifying the absolute toxic risks of metal exposure to benthic organisms (meiofauna to megafauna). In part, this synthesis presents the key conclusions of the ‘Ecotoxicology’ working group of the EC FP7 Project ‘Managing Impacts of Deep Sea Resource Exploitation’ (MIDAS; https://www.eu-midas.net/), placed within the wider context offered by the research developments of other teams.

Deep-sea ore deposits comprise complex mixtures of potentially toxic elements including, for example: copper, cadmium, zinc, and lead, as well as rare earth elements, such as lanthanum and yttrium (Petersen et al., 2016). These ore deposits form in distinct geological settings, as has been extensively and recently reviewed (Petersen et al., 2016). *In situ*, the surface of these ore deposits are weathered and present minimal toxic risk. Indeed, nodules and massive sulphides can provide valuable substrate for a diverse faunal assemblage (e.g. Wang et al., 2013; Amon et al., 2016; Vanreusel et al., 2016). Deep-sea mining (DSM), whether of seafloor massive sulphides (SMS), polymetallic nodules, or cobalt-rich crusts, may break up these ore deposits and may release toxic concentrations of metals into the environment at distinct phases of the mining cycle, affecting organisms within different marine compartments (Fig 1). Metals may be released in different physical phases (in solution, as ground particulates of different size and shape, or adsorbed onto the surface of particulates (see: Fallon et al., 2017 and references therein), which will affect their bioavailability to organisms. For example, metals in solution may be taken up across permeable tissues such as gill or gut surfaces, whilst particulate or adsorbed metals might be taken into the gut of deposit feeders. In the longer term, metal accumulation in tissues presents a further toxic risk for predatory species higher in the food chain, as they can consume contaminated prey items.

It is expected that the mining of massive sulphides or cobalt-rich crusts will involve crushed ore being pumped as a slurry from the seafloor mining tool, or via a collector after stockpiling on the sea bed (Fig 1, B), to the surface mining support vessel via a riser pipe (Hoagland et al., 2010). Whilst polymetallic nodules may be collected intact (Fig 1, A), nodules in the Clarion-Clipperton Fracture Zone in the Pacific Ocean are friable and readily crumble with handling. Consequently, for all resources, there is a risk that extraction will release metals in dissolved and fine particulate phases into the water column as a plume over potential scales of 100s to 1000s of km^2 (Oebius et al., 2001). Current operator plans include the transfer of bulk ore from the mining support vessel to a barge for transfer to shore and processing (Fig 1, C) and this slurry will require dewatering before and/or after transfer (Hoagland et al., 2010). Dewatering the ore slurry at the surface may also release metals into the marine environment in the return water (Fig 1, D). Releasing return water into the ocean at the surface or mid-depth (Fig 1, D and E, respectively) will disperse potential toxicants widely (100s to 1000s of kilometres) and may impact organisms within the water column. Return water, potentially at above-ambient temperature, that is discharged above the seafloor may disperse as a thermally buoyant plume over a large area, carrying toxicants with it (Fig. 1, F). The impacts of these potential metal releases must be understood for their potential to cause ‘serious harm’ to deep sea marine ecosystems, the avoidance of which is a fundamental legal remit of the ISA (Levin et al., 2016).

There is increasing evidence of the need for incorporation of an Ecosystem Service (ES) approach into the environmental management of the deep sea, and specifically DSM (Danovaro et al., 2017, and Le et al., 2017, respectively). Since the publication of the

Millennium Ecosystem Assessment in 2005, the role of ecosystems in providing resources and services for the benefit of human kind has been recognized, and this is applicable to terrestrial as well as shallow and deep-sea ecosystems (Le et al., 2017). Consideration of ES incorporates a holistic view of species' distributions, abundance and diversity (Ecosystem Structures), as well measures of species' activity (Ecosystem Functions/Supporting Services) (Le et al., 2017; see also Fig 2).

Whilst, our knowledge of deep sea ecosystems and the diversity of life that they contain has been rapidly growing in recent years (Ramirez-Llodra et al., 2010), it is clear that we have only just begun to constrain the true diversity of life throughout the deep ocean. Deep sea communities are typified by species with slow growth and delayed maturity; characteristics which make them particularly susceptible to anthropogenic impacts or other environmental perturbation. These joint considerations: a current paucity of available data on deep sea community structure and the life history characteristics of known deep sea species, mean that there is a high probability that, should large scale deep sea mineral exploitation be prosecuted in the near future, we may in fact irreversibly impact unique biological and genetic resources even before they have been identified (Thornburg et al., 2010; Harden-Davies, 2017).

Abyssal benthic environments support biologically diverse species assemblages of meio-, macro- and megafauna (note, microfauna are explicitly not considered in this synthesis). These biologically diverse communities are comprised of populations with low overall abundance as a result of the absent primary production over the vast majority of the abyssal sea floor, and a complete reliance upon periodic phytodetrital nutrient supply from the surface ocean thousands of metres away. This unique placement renders them energy limited (Smith et al., 2008), therefore restricting overall species abundance.

Nonetheless, recent studies in the Clarion Clipperton Fracture Zone have identified diverse epifaunal communities of filtering feeding organisms, including hexactinellid sponges, crinoids, hydroids, and soft corals, that are reliant on the nodules as substrate for attachment (Amon et al., 2016; Vanreusel et al., 2016). Dominant mobile megafauna associated with abyssal nodule fields include key benthic bioturbating groups, such as holothurians (sea cucumbers) and ophiuroids (brittlestars) (Amon et al., 2016).

In contrast, biological communities that colonise massive sulphides at hydrothermal vents are potentially supplied with a source of energy to support chemosynthetic primary production. These systems can also occur at shallower depths, receiving greater carbon flux from the surface ocean (Van Dover, 2000). As a result, active vent communities are normally comprised of a restricted diversity of fast-growing species that can occur at locally very high abundance. Faunal assemblages typically include crustaceans, molluscs and polychaete worms although with distinct biogeographic provinces (e.g. Van Dover et al., 2002), many of which indirectly rely on the vent fluid as an energy resource (Boschen et al., 2016; and references therein). Hydrothermal vent communities can show high endemism with high rates of speciation recorded; certain taxa being geographically restricted to a single, or small number of active vent sites (e.g. Wolff, 2005). As a consequence, whilst a single vent system might be seen as a productive and robust system, it may represent the only instance of that community within the deep ocean. Moreover, inactive, or relic, vents that might be first targeted for the exploitation of SMS, may support a more diverse faunal assemblage, including organisms that are not tolerant of vent conditions (e.g. relatively warmer waters and higher metal concentrations) or that do not rely on the vent fluids as a source of energy (Marsh et al., 2012; Sarrazin et al., 2015).

Water column, or pelagic communities, that might be impacted by plume release at mid-depths (Fig 1D and E; Ellis, 2001) mediate the transfer of energy and organic carbon from primary producers in the euphotic zone to the sea floor (Robison, 2009), but also from hydrothermal plumes on the deep sea floor (Phillips, 2017). Already studies have demonstrated that metals can exert a toxic effect to marine phyto- and zooplankton species (e.g. Hirota, 1981; Hu 1981; Moraitouapostolopoulou and Verriopoulos, 1982; Caroppo et al., 2006; Fuchida et al., 2017) and can lead to metal accumulation (bioaccumulation) in higher trophic levels of food chains (Amiard Triquet et al., 1993). In contrast, others have noted that some metals (including for example: copper, zinc, iron) represent essential micronutrients and that long-term exposure to low concentrations might create positive effects in pelagic communities through the improved function of some planktonic species (e.g. Barathi et al., 2005).

The bathypelagic zone (below ~1000m and above 4000m) has been regarded as the largest ecosystem on the planet (Robison, 2009) and represents approximately 75% of the volume of the ocean (Ramirez-Llodra et al., 2010). In addition to deep water fish that are exploited commercially (Gordon, 2001), this ecosystem supports a diverse assemblage of gelatinous and other zooplankton, which also play a pivotal role in the sequestration of carbon from the surface ocean to the deep sea floor, in so doing contributing to the global regulation of atmospheric carbon dioxide and thence the Earth’s climate (Packard and Gomez, 2013; Teuber et al., 2014).

These different deep water habitats provide considerable Ecosystem Services for humankind; services that need to be afforded protection in the long term as they are both unique and also non-restorable (Le et al., 2017) (See Fig. 2). Indeed, the provision of Ecosystem Services has been argued as one standard for assessing ‘serious harm’ in the context of DSM Environmental Impact Assessments (EIAs) (Le et al., 2017), because these services link environmental health to human well-being. Provisioning Services include supporting fisheries for human nutrition, whilst Regulating Services include the absorption of carbon from the atmosphere and its subsequent sequestration with the deep ocean interior and within the seabed. At the sea floor diverse and stable biological communities represent a biodiversity resource which provides a reservoir of biological and genetic resources that might present the source of future natural products of further benefit to human kind (Harden-Davies, 2017).

The continued provision of Ecosystem Structures and Functions by marine communities in the vicinity of DSM requires the species assemblage to persist into the future. Aside from the direct removal of settlement surface (or substrate) on which larvae may settle, the introduction of excess metals into the environment may exert a toxic effect on the resident fauna or may lead to organisms avoiding those areas exposed to high metal concentrations (Fig 2; see below). In either case, the disappearance of organisms from a system, or a reduction in the performance of a species or organism within a system, may result in a reduction in biodiversity and potentially increase ecosystem instability, which will present a risk to the continued provision of Ecosystem Services into the future.

However, it should be borne in mind that impacts to Ecosystem Services may represent a relatively slowly responding metric, one that integrates complex interactive effects (not just metal toxicity) over large spatial scales, and may therefore not be relied upon to provide an early or sensitive indication of toxic effects from released metals. It can be argued that the identification of any significant adverse impact, impacts that may precede any change in Ecosystem Services, should also constitute ‘serious harm’ to the marine environment, and therefore should be appropriately quantified before large-scale mineral exploitation is licenced.

2) Mineral resource toxicity to individual organisms cannot be reliably predicted.

For many commonly-studied metals, existing acute toxicological data (lethal concentrations (LC_{50}) and effective concentrations (EC_{50})) are available, but only for shallow-water biological species (e.g. Crompton, 1997). These data identify concentrations of metals which are either lethal, or 'effective', for 50 % of the exposed population over a designated period, conventionally 72 or 96 h. Alternatively, more recent toxicological studies have adopted a variable exposure duration that matches a desired sub-lethal endpoint (Simpson et al., 2017). The US EPA ECOTOXicology Database (ECOTOX; https://cfpub.epa.gov/ecotox/) is an online resource that summarises all available metadata included within each ecotoxicology publication, and this database is updated quarterly. However, interrogation of this database at the end of 2013, at the start of the MIDAS project, identified that no data were available for any deep-sea taxa.

Through DSM, the toxic effects of metals will act potentially at *in situ* deep-sea temperatures and pressures (high pressure - up to 60 MPa, low temperature - down to 2^{o}C), which are very different from those of laboratory exposures reported in the ECOTOX database (conventionally set at standard conditions of a temperature of 20 ^{o}C and a pressure of 0.1 MPa) (Mestre et al., 2014). Brown et al. (2017a) have contrasted the toxic limits of metals in solution at low temperature (10 ^{o}C) and high hydrostatic pressure (10 MPa) with those recorded under standard conditions of temperature and pressure (20 ^{o}C and 0.1 MPa). These initial experiments made use of the shallow-water shrimp *Palaemon varians* as an experimental model; a species that has a close phylogenetic relationship to deep-water hydrothermal-vent shrimp species (Tokuda et al., 2006). These initial experiments showed that both copper and cadmium toxicity were significantly reduced at low temperatures at 96 h, but that the effects of high hydrostatic pressure were more complex. Whilst copper toxicity was significantly increased at high hydrostatic pressure, cadmium toxicity was not. Consequently, copper toxicity was lower than cadmium toxicity at 20°C but greater than cadmium toxicity at 10°C, and remained greater than cadmium toxicity at 10.0 MPa (Brown et al., 2017a).

Similar results were found in an acute copper toxicity study with the experimental model organism *Halomonhystera disjuncta* GD1 (Mevenkamp et al., 2017); a nematode that is phylogenetically closely related to the deep-sea species *H. hermesi* (Van Campenhout et al., 2014; Tchesunov et al., 2015) that inhabits cold-seep ecosystems. In *H. disjuncta* GD1, cold temperatures (10 °C) reduced copper toxicity, whilst toxicity was increased when nematodes were exposed to high hydrostatic pressure (10 MPa) (Mevenkamp et al., 2017).

Whilst these observations of temperature-mediated toxicity are not new (e.g. Lewis and Horning 1991, Heugens et al., 2003, Khan et al., 2006, Prato et al., 2008, Barbieri et al., 2013), to the extent that authors have suggested the use of a correction factor in applying LC_{50} values for different temperature environments (Wang et al. 2014), the moderating effects of temperature may not be consistent within the real world (see: Chapman et al., 2006). Moreover data indicate that the determination of any 'temperature correction factor' will be different at high hydrostatic pressure for each metal in question and would have to be empirically determined for each case, and potentially for each biological species (see also: Kiffney and Clements 1996, Gonzalez-Rey et al., 2007, and Wang et al., 2014; for example).

A further issue of using existing data to regulate mining activity is that many assessments of metal toxicity are based on a single metal presented at a single oxidation state. Mineral ores represent complex mixtures of metals that are site-specific (e.g. Hering 1971; Scott 2001; Glasby et al., 2015; Petersen et al., 2016) and that change with mineral weathering (Koschinsky et al. 2003). It is therefore extremely difficult to predict the exact toxic potential of a mineral resource *in situ* from laboratory studies on single metals, or even metal mixtures (see also the recent discussion of Belzunce-Segarra et al., 2015).

As evidence of this complexity, laboratory studies of the toxicity of copper and cadmium mixtures in the decapod crustacean *Palaemon varians* were assessed by testing deviation from independent addition reference model (IA) predictions (see: Jonker et al., 2005; Pan et al., 2015). Brown et al. (2017a) assessed 96-h lethal toxicity in a mixture of copper and cadmium based on the predicted LC_{29} of both metals (derived from the modelled mortality responses to individual metals in each temperature/pressure treatment), and compared with mortality in a control treatment and in individual LC_{29} exposures to copper and cadmium. IA predicted that mortality in a binary mixture with LC_{29} of both constituents would be 50%; however, the interaction of copper and cadmium appeared potentiating. Most importantly, whilst temperature did not significantly affect the interaction of the two metals when exposed in combination, high hydrostatic pressure significantly increased the toxic effect. These data emphasise the potential key role of pressure in mediating the toxic effect of plumes generated through DSM (Brown et al. 2017a).

The complexity of metal mixture toxicity has recently been reviewed (Pan et al. 2015, and references therein) and a detailed discussion is not presented here. Nonetheless, it is clear from our studies and those of other groups, that the scientific community is not in a position to reliably predict the combined toxic effect of metal mixtures *in situ* at mining sites. Ultimately, there are fundamental differences in metal uptake from solution, and the subsequent toxic effect in organisms, that are mediated by low temperature and high pressure. These findings confound the principle of regulating DSM on the basis of exposure thresholds established under standard conditions. We conclude that existing toxicological data of metals in solution established at standard laboratory conditions should not *necessarily* be applied to the context of DSM.

We argue that to continue to establish lethal limits for single metals, or simple combinations of metals, in the laboratory will only produce incremental progress in our understanding of ‘real world’ toxicity of mineral resources. If the scientific community continues with this approach, progress in this field will be too slow to be incorporated usefully into recommendations for contractors (see also, the arguments in Jager et al., 2006). As an alternative, we propose that it will be necessary to assess the ‘bulk toxicity’ of each mineral deposit to identify *a priori* the potential toxic risk of each mineral resource to be mined within a licence area (e.g. Harris et al. 2014, Fallon et al., 2017, Simpson et al. 2017; see also discussion of the ‘Weight Of Evidence’ approach below). In practice, it actually may not be necessary to quantify the individual toxicity of each metal within each resource (although assessment of mineral composition is undoubtedly integral to resource classification; e.g. International Council on Mining and Metals, 2013). It may only be necessary to determine –

under controlled, ecologically relevant, conditions – the bulk lethal toxicity of that resource for a number of different locally-relevant biological proxy organisms. However, assessments of bulk toxicity should be considered for all relevant life cycle stages of the proxy organisms, including larval stages which are known to be more sensitive (e.g. Rainbow, 2007, Simpson and Batley, 2007, Casado-Martinez et al., 2009, Camusso et al., 2012, Hedouin et al., 2016), and should consider all appropriate physical phases for the metal (e.g. in solution/aqueous, as particulates of relevant size and shape, or adsorbed onto the surface of particulates; see below). A similar approach could be adopted to determine the bulk lethal toxicity of any return waters from surface dewatering before any discharge takes place, potentially as part of a contractor’s Environmental Impact Assessment (ISA, 2017).

3) The physical state of the metal toxicant is important

Any metals released during the mining cycle will occur in different physical states (Simpson and Spadaro, 2016). Metals may enter solution/aqueous phase and be taken up across the gills, body wall and digestive tracts of exposed animals. Alternatively, crushed mineral particles, and dissolved metals that may adsorb onto sediment particles or flocculates may be ingested; this may particularly be the case for metals released during dewatering of ore slurry (for example: Campana et al. 2012, 2013; Camusso et al., 2012). These various exposure routes have implications to predictions of toxicity as well, not least because the vast majority of data listed within databases such as the US EPA ECOTOX database are based on organisms exposed to metals in solution.

For example: Simpson and Spadaro (2016) have identified that metals in solution have greater potential toxicity than in particulate phases. Exposure to particulate copper, in the form of chalcopyrite (100% $CuFeS_2$) or chalcocite (80% Cu_2S), resulted in a reduced absolute mortality in both bivalves *Spisula trigonella* and benthic amphipods *Melita plumulosa* compared to copper in solution. Similarly, Farkas et al. (2017) have recently reported minimal toxicity in the pelagic copepod *Calanus finmarchicus*. exposed to 5 g l^{-1} of the fine-grained fraction of marble processing tailings. However, Farkas et al. (2017) do note that this pollutant did constitute a sub-lethal energetic burden to exposed copepods that took these fine-grained particulates into the gut (energetic implications and sub-lethal impacts are considered further in section 4).

These recent studies reinforce the necessity to determine the bulk toxicity of the mined resource in all relevant phases prior to the extraction of metals under an exploitation licence.

4) Sub-lethal impacts of chronic exposure should be considered

The holistic view of organisms within their ecosystem providing the basis for Ecosystem Structures, Ecological Functions and Services (see earlier discussion, and Le et al., 2017), is in marked contrast to the paradigm of acute 'lethal toxicity'. As discussed, acute toxicity is conventionally assessed in terms of the, rather artificial, construct of '96-hour LC_{50}'. However, DSM - particularly of polymetallic nodules - may continue within a licence block for years to decades and the potential anthropogenic impacts may take place at unprecedented temporal and spatial scales (Glover and Smith, 2003; Selck et al., 2017). Key seabed organisms at considerable distances from the mined site may be subject to chronic metal exposures that are orders of magnitude lower than a lethal dose, but for very extended periods (Newman 2010, Simpson et al., 2017).

A considerable body of research has identified the mechanisms by which metals in the marine environment might exert toxic effects in marine species (e.g. Hollenberg, 2010 and references in that special issue; Wu et al., 2016; Fig 3). Briefly, redox-active metals including iron, copper, cobalt, chromium and nickel, can catalyse the formation of reactive oxygen and reactive nitrogen species that can bind with lipids and cause lipid peroxidation and damage to cell membranes (Stohs and Bagchi, 1995; Ayala et al., 2014). Redox-inactive metals, including cadmium and zinc, also exert toxicity by binding with the sulfhydryl groups of proteins (Valko et al., 2016). Excess metals may also lead to the production of reactive species, including superoxide ($O_2^{-}\bullet$), peroxides (ROOR), singlet oxygen, peroxynitrite ($ONOO^{-}$), hydroxyl radicals (•OH) and nitric oxide (NO), which may also produce damage in animal tissues (Stocker and Keaney, 2004; Aitken and Roman, 2008; Martinez-Finley and Aschner, 2011). In response, organisms have multiple different mechanisms for the detoxification of reactive oxygen or nitrogen species to minimise damage (Fig 3). For example, organisms can produce thiol-rich metal binding proteins (e.g. metallothioneins; e.g. Hardivillier et al., 2004) that can either be sequestered as inclusion bodies or, if forming soluble complexes, can be secreted from the cells. Redox-inactive metals can be bound to glutathione to form non-toxic metal complexes. Reactive species are variously detoxified by enzymes including: superoxide dismutase (SOD), catalase (CAT), and peroxidases (PEROXs) (e.g. Company et al. 2004, 2006a, b, 2007, 2008, Bebianno et al. 2005, Gonzalez-Rey et al. 2008). Recently, Auguste et al. (2016), Martins et al. (2017) and Mestre et al. (2017) have assessed the potential sub-lethal impacts of exposure to dissolved metals specifically in a range of deep sea species, including molluscs and echinoderms that do not inhabit metal-rich environments, and molluscs and crustaceans from hydrothermal-vent habitats; habitats that are naturally metal rich. For example, copper concentrations from the mussel *Bathymodiolus azoricus* habitat on the Mid-Atlantic Ridge

(MAR) have been recorded between 0.17 µM at ~850m water depth at Menez Gwen to 2 µM at ~2300m water depth at Rainbow (Kádár et al., 2005) hydrothermal vent sites.

Auguste et al. (2016) established the effects of copper exposure on the expression of tissue metallothioneins and lipid peroxidation as well as effects of the activity of key antioxidant enzymes in different tissues of the hydrothermal vent shrimp *Rimicaris exoculata* collected from the TAG hydrothermal vent field on the MAR and maintained at 30 MPa and 10 ^{o}C. Shrimp were held in solutions of either ~25 µg l^{-1} or ~250 µg l^{-1} copper for 72 h, which were at least two orders of magnitude less than the 72-h LC_{50} concentration of ~35000 µg l^{-1} for *Palaemon varians* at 0.1 MPa and 10 ^{o}C reported by Brown et al. (2017a). Auguste et al. (2016) demonstrated that even shrimp that have evolved to live in the metal-rich environment of a hydrothermal vent field (*R. exoculata*) are sensitive to copper exposure in solution. Similarly, copper exposure in hydrothermal vent mussels *Bathymodioulus azoricus* collected from the 'Lucky Strike' hydrothermal vent site on the MAR and maintained at 17.5 MPa at ~6 ^{o}C for 96 h caused elevated lipid peroxidation at copper concentrations in excess of 300 µg l^{-1}, indicating lipid membrane damage within these tissues.

In these studies, however, data showed that effects of sub-lethal metal exposure were not consistent across all tissues within each organism. In the mussel *B. azoricus*, although lipid peroxidation was identified in several tissues, gills were more affected whilst mantle and digestive gland were found to be more resilient (Martins et al., 2017). This differential response may be related to the prominent role of the gill in harbouring chemosynthetic symbionts and located at the interface between the internal milieu and external vent environment. Similarly, whilst the induction of metallothionein was significant in *Rimicaris* *exoculata* gill tissue after 72 h, this was not the case in muscle tissue (Auguste et al., 2016). In this latter case the differential response seen in different tissues of the shrimp was considered to result from differences in the route of uptake of the metal over the time course of the experiment.

Clearly, deep-sea species do have the ability to upregulate detoxification pathways in response to metal exposure and this can occur at relatively low concentrations of metals in solution. However, even with the upregulation of detoxification pathways, damage to tissues evidenced as lipid peroxidation can still be observed. Importantly, it is also clear that even those deep-sea species that inhabit metal-rich environments of active hydrothermal vents are physiologically responsive to metals in solution, although the relative sensitivity of vent and ‘off vent’ species, and how this sub-lethal toxic stress might be phenotypically manifest in deep sea species, remains to be fully constrained.

As discussed, cellular protective mechanisms certainly respond to metal exposure, with upregulated metallothioneins, glutathione, molecular chaperones, antioxidants and/or cellular repair pathways increasing basal metabolic demand (Fig 3; see also: Calow, 1989, 1991; Cherkasov et al., 2006; Ivanina et al., 2008; Ivanina and Sokolova, 2008; Sokolova and Lannig, 2008). Whilst successful detoxification might allow for organisms to survive chronic exposure to metals in solution and associated with sediment plumes, the expression of these pathways consumes energy. Basal metabolic rates will increase in association with detoxification and damage repair, as extensively reviewed by Sokolova et al. (2012). Elevated basal metabolic rate will ultimately reduce the energy available for other processes including growth, reproduction and locomotion (e.g. associated with foraging). These impacts may have significant further implications for the role and persistence of species (see Fig. 2), even outside of the immediate mining footprint, that should be incorporated into the assessment of exploitation licence applications. This is especially the case when the area being considered is adjacent to existing areas of exploitation. For example, in resource/food limited environments such as abyssal plains (Smith et al., 2008), or in environments that are routinely hypoxic (Peña et al., 2010), organisms may not have sufficient energy reserves or aerobic scope to meet the metabolic demand of continued tissue detoxification. Alternatively, at key times of the year – for example periods of reproduction or spawning – benthic organisms may be very susceptible to exposure to metal concentrations that would 'normally' be considered to be sub-lethal (e.g. Martins et al, 2011; Levin et al., 2016).

5) Behavioural avoidance by mobile organisms may indicate toxic impacts in real time

As reviewed above, the maintenance of Ecosystem Services within a system impacted by mining is dependent on the species that remain, and in their individual performance and ecological role - itself an integration of physiology and behaviour – in that system (Simpson et al., 2017).

Detailed electrophysiological studies have demonstrated that the vent shrimp *Mirocaris exoculata* and the phylogenetically close shallow-water shrimp *Palaemon elegans* have the sensory ability to detect hydrothermal fluid cues like the hydrogen sulphide, as well as food solutions (Machon et al., 2016). They therefore at least possess the potential to detect and respond to changes in their immediate environment, either to remain in close proximity to vent fluids or to move away from high concentrations of metals.

These sensory abilities extend beyond the crustacean taxa. Bivalve molluscs and gastropod snails can withdraw into their shells and either hold the two valves tightly shut or close their opercula opening to avoid detrimental conditions (e.g. Kádár et al., 2001). For example, the fresh water clam *Corbicula fluminea* can close its valves in response to threshold concentrations of 5.6 or 19.5 µg l^{-1} copper for response times of 300 and 30 minutes, respectively (Jou et al., 2016). Indeed, Hartmann et al. (2016) have argued that mussel behaviour could be used as a biomarker for toxicological applications, and a similar case has been made for gastropod molluscs exhibiting avoidance behaviours when presented with repellent solutions (Hagner et al., 2015).

Of direct relevance to abyssal polymetallic nodule mining, Brown et al. (2017b) have reported consistent avoidance behaviours in echinoderms presented with copper contaminated sediments. In 96-h laboratory exposures at 4 ^{o}C the shallow-water holothurian *Holothuria forskali* consistently avoided sediments contaminated with copper at concentrations of 5 mg l^{-1} by climbing onto the side of the treatment tank (Brown et al., 2017b). These behaviours resulted in no significant induction of antioxidant enzyme activity. These behaviours were mirrored by the abyssal holothurian *Amperima* sp. exposed to copper-contaminated sediments at a depth of 4167m in the Peru Basin (Brown et al., 2017b) and were also sufficient to avoid induction of antioxidant enzymes in the bulk tissue extracts.

These data demonstrate that macro- and megafaunal species have the sensory capacity to detect metals in the environment and that, in at least some species, this can result in the expression of avoidance behaviours to help protect the organism from toxic effects. It can be concluded that mobile species exposed to contaminated plumes have the potential to demonstrate chronic impacts by moving away from areas of contamination during exploitation. These migrations may produce long-lived direct and indirect (through species interactions) changes in the biological community structure at sites far field from the immediate mining site, creating downstream effects on system Ecological Functions and ultimately Ecosystem Services (Fig 2).

Moreover, avoidance behaviours do not necessarily constitute a permanent solution to toxicant exposure in marine organisms. For example, valve closure in molluscs represents only a temporary avoidance behaviour as bivalve molluscs must open their valves periodically to irrigate their gills with oxygenated water (Byrne et al., 1991). Also, for many benthic species, including those at bathyal and abyssal depths, the speed at which a behavioural response can be elicited may be too slow to represent a viable means to avoid toxicant exposure (Ward et al., 2013). In addition, the potential variability in the ‘typical’ behavioural response to toxicant exposure, which may impede their application as biomarker responses, has been identified by other research teams (e.g. Garcia et al., 2008; Melvin et al., 2017). Behavioural avoidance is also not possible for sedentary or attached species. As recently reported (e.g. Amon et al., 2016; Vanreusel et al., 2016), polymetallic nodules support a diverse assemblage of sedentary epibenthic fauna, including sponges and soft corals, which cannot move or otherwise avoid environmental exposure. These sessile fauna create vertical habitat for mobile species (Buhl-Mortensen et al., 2010) and their removal from the abyssal system through mining would create downstream impacts for other mobile fauna, impacting Ecosystem Structures and Functions.

6) A way forward? A holistic assessment of potential toxicity using the established Weight Of Evidence (WOE) Approach to quantify the toxic risk of deep-sea mining to biological species and communities.

Based on current information, including the results of the MIDAS Project Ecotoxicology working group, we argue that it is not possible to predict *a priori* the absolute toxicity, and therefore identify exposure thresholds, of mining different seabed resources at bathyal and abyssal depths. Indeed, to propose absolute exposure limits based on an incomplete understanding of the mineral resource composition, exposure route and duration, and biological species involved, would be scientifically flawed and would run counter to the ethos of the Precautionary Principle recently advocated by the ISA.

However, it is possible to adopt a Weight Of Evidence (WOE) approach to quantify the risk associated with mining a particular resource. Weight of Evidence approaches have been advocated for quantifying the toxic risk of exposure to metals in sediments in the absence of absolute data on lethal or effective concentrations (Chapman et al., 1998). Since this time the use of the WOE has been refined to address further uncertainties in the methodology and recommendations on their refinement have since been advocated (e.g. Batley et al., 2002). They have since found application in the assessment of risk in terrestrial (e.g. Milton et al., 2003; Semenzin et al., 2008) and aquatic systems (e.g. Schiesari et al., 2007; Martín-Díaz et al., 2008; Kellar et al., 2014).

Using this approach, multiple Lines Of Evidence (LOE), including a characterisation of the mineral resource (chemical composition, grain size), the accumulation of metals within organism tissues, the organism tissue biomarker response, and additional bioassays (MICROTOX, or similar), it is possible to quantify the risk associated with mining (Piva et al., 2011; Benedetti et al., 2012; 2014; Regoli et al., 2014; Bebianno et al. 2015; Mestre et al., 2017).

Applying this approach, with multiple LOE to locally relevant 'canary' species in the vicinity of the mining site or validated shallow-water ecotoxicological proxy species, would provide a mechanism for regulators and contractors to develop a holistic overview of the toxic risk of mining and resource. Appropriate 'canary species' could be identified as either: a) biomass dominants within the local biological community (e.g. abyssal sponges or holothurians, or hydrothermal vent shrimp and mussels), or b) key species necessary to maintain Ecosystem Services (e.g. abyssal holothurians, abyssal meiofauna), or c) species which could be captured and caged for routine monitoring during exploitation (e.g. bivalve molluscs such as *Bathymodiolus azoricus,* which is phylogenetically close to the deeper-dwelling *B. puteoserpentis,* and which naturally inhabits hydrothermal vent environments but which can survive at atmospheric pressure rendering it tractable for laboratory experiments). Ultimately, the precise selection of appropriate canary species will be habitat dependent, and still requires much more comprehensive datasets of the resident biological communities in potential mining locations, particularly in the case of nodule fields at abyssal depths.

Using the WOE approach it will be possible to identify high-risk resources or high-risk communities within a licence block during contractor exploration or contractor Environmental Impact Assessments prior to exploitation licences being issued by the regulating authority – the ISA in the case of the mining within the Area. Exploitation of these high-risk areas could be restricted until appropriate mitigation is in place (through operating procedure, or through mining tool design) or could be used to identify lower risk areas within a licence block that could be prioritized for exploitation.

The WOE approach has been applied and validated within the shallow waters of Portmán Bay, Spain during the MIDAS project (Mestre et al., 2017). Nevertheless, further testing and validation of the WOE is required before this approach can be universally recommended to the ISA for use with the Area. The full implications of adopting an *a priori* WOE approach at bathyal and abyssal depths should be tested in the field, potentially by adopting independent scientific advisors/agencies into a flexible and staged implementation of the Adaptive Management of pilot mining or as part of the first exploitation contracts issued by the ISA (Jaeckel, 2016).

In conclusion, the combined results of the EC MIDAS Ecotoxicology workgroup and other teams reviewed here identify the important, but as yet unpredictable, interacting roles of temperature and pressure in mediating the toxicity of metals to marine invertebrate fauna. As a consequence of these interactions, combined with the variable metal composition of different mineral reserves, it is not possible to predict acute thresholds for marine invertebrates that may be exposed to toxic concentrations of metals during DSM operations. Moreover, we conclude that acute exposure limits represent an artificial measure of possible impact, which do not identify sub-lethal and longer-term chronic responses that may disrupt Ecological Functions and Ecosystem Services within the deep sea. Our data indicate that future regulation of DSM exploitation should consider the potential for the perturbation of normal behaviours of deep-sea species, changes which may also affect the provision of Ecosystem Services in the long term. In order to quantify the toxic risk of mineral exploitation, we advocate the incorporation of existing 'Weight of Evidence' approaches into environmental impact assessments. We identify the potential for these approaches to be further developed and validated through continued collaboration of scientific researchers and mining contractors during any future pilot mining operations, according to the principles of Adaptive Management.

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CONFLICTS OF INTEREST

None

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FIGURE LEGNDS

Figure 1. Schematic representation of potential deep-sea mining operations (not to scale), identifying stages at which toxic metals may be released to the environment. Metals may be released in particulate or dissolved phases associated with localised plumes from mining collectors (A), or individually controlled mining tools and collectors (B) operating in tandem. Dewatering ore slurry at the mining support vessel or transportation barge (C) may introduce toxic concentrations of metals into the euphotic zone, impacting photosynthetic primary producers or zooplankton communities and potentially affecting the sequestration of carbon dioxide in the surface ocean, as well as pelagic food webs driving the flux of carbon to the sea floor. Mid-water discharge (D), whilst away from the productive euphotic zone, may impact vertically migrating zooplankton – again altering the vertical flux of carbon to the sea floor. Whilst the discharge of water near the seabed (E) will limit mid-water impacts, the release of thermally altered and buoyant water may disperse contaminants over a much larger area than that immediately impacted by the mining tools.

Figure 2 Representation of how behavioural, sub-lethal and lethal impacts of metal exposure on individual organisms can, over time or with increasing magnitude of the metal release, scale to produce impacts on Ecosystem Structures, Ecological Functions and thence to Ecosystem Services (adapted from Le et al., 2017). Shown from left to right is the effect of increasing metal concentration, or increased proximity to the metal release. Behavioural modifications or sub-lethal effects will occur at low metal concentrations or far from a mining site whilst lethal impacts at high metal concentrations or at locations close to metal release will occur. Sub-lethal exposure can result in a reduction in organism performance that cause direct impacts to Ecological Function (grey line) or, in combination with other physiological stressors (e.g. hypoxia, restricted diet, post-spawning) can result in organism mortality that cause direct impacts to Ecosystem Structures. Note that the relative positions and relative order of behavioural and sub-lethal effects is variable.

Figure 3 Simplified synthesis of the routes of metal toxicity and detoxification in animal tissues. Metals in the environment that enter animal tissues can be sequestered by various metal binding proteins, including metallothioneins and other thiol-rich peptides, and either stored or excreted from those tissues. Metallothioneins can also detoxify hydroxyl radicals within cells. Redox active metals can drive the formation of superoxide anions (O2-•) via hydroxyl radicals (OH•) that, if unregulated, can lead to lipid peroxidation and damage to cellular membranes. Superoxides can be enzymatically converted to hydrogen peroxide by superoxide dismutase (SOD). Hydrogen peroxide (H2O2) is enzymatically converted to water by catalases and peroxidases (CAT and PEROXs, respectively) or by the action of glutathione peroxidase (GPx) and glutathione disulphide reductase (GR). Synthesis adapted from: Stocker & Keaney (2004), Aitken & Roman (2008), and Martinez-Finley & Aschner (2011).

Figure 1

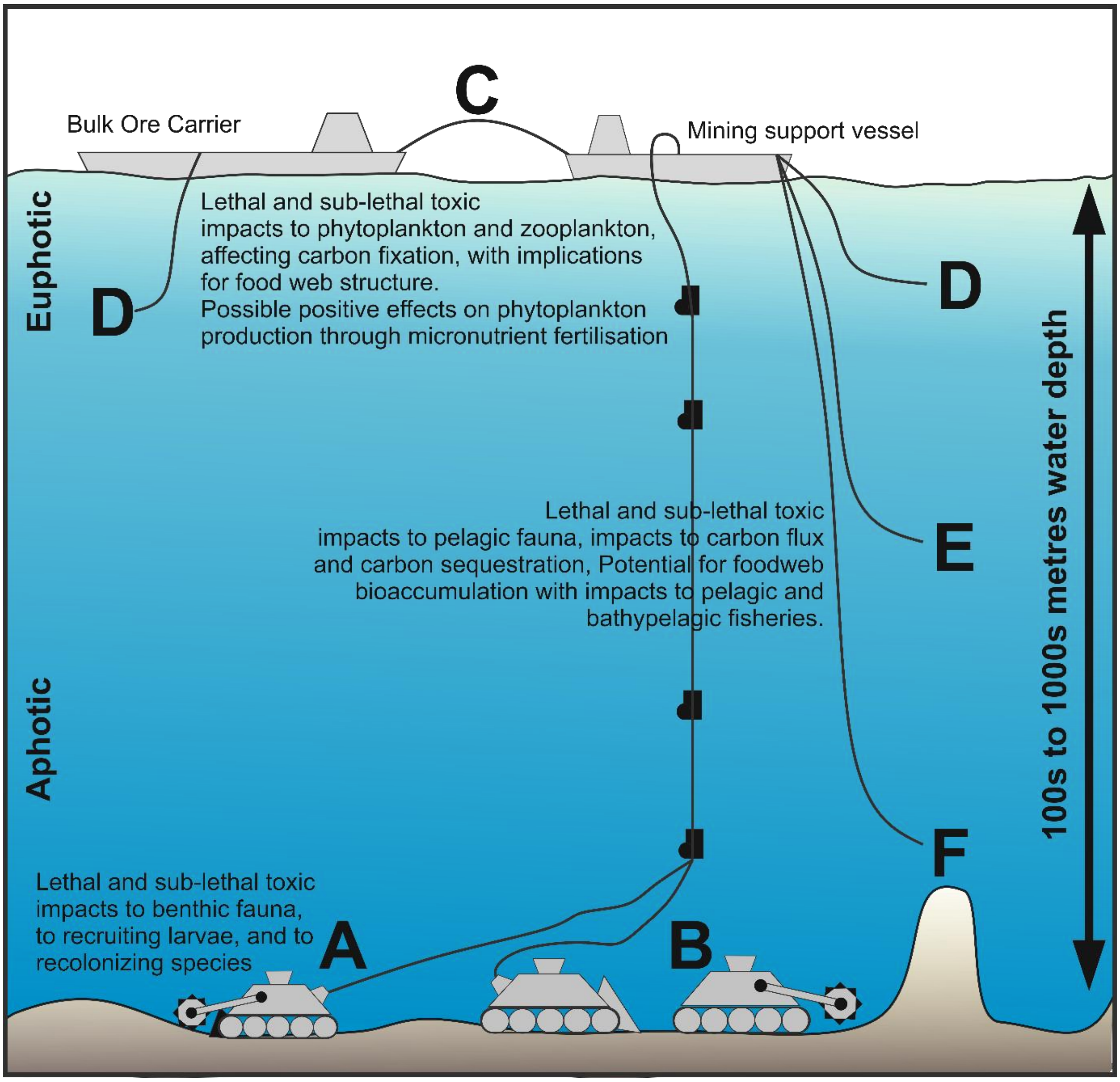

C
Bulk Ore Carrier
Mining support vessel
Euphotic
D
Lethal and sub-lethal toxic impacts to phytoplankton and zooplankton, affecting carbon fixation, with implications for food web structure.
Possible positive effects on phytoplankton production through micronutrient fertilisation
D
Lethal and sub-lethal toxic impacts to pelagic fauna, impacts to carbon flux and carbon sequestration, Potential for foodweb bioaccumulation with impacts to pelagic and bathypelagic fisheries.
E
100s to 1000s metres water depth
Aphotic
F
Lethal and sub-lethal toxic impacts to benthic fauna, to recruiting larvae, and to recolonizing species
A
B

Figure 2

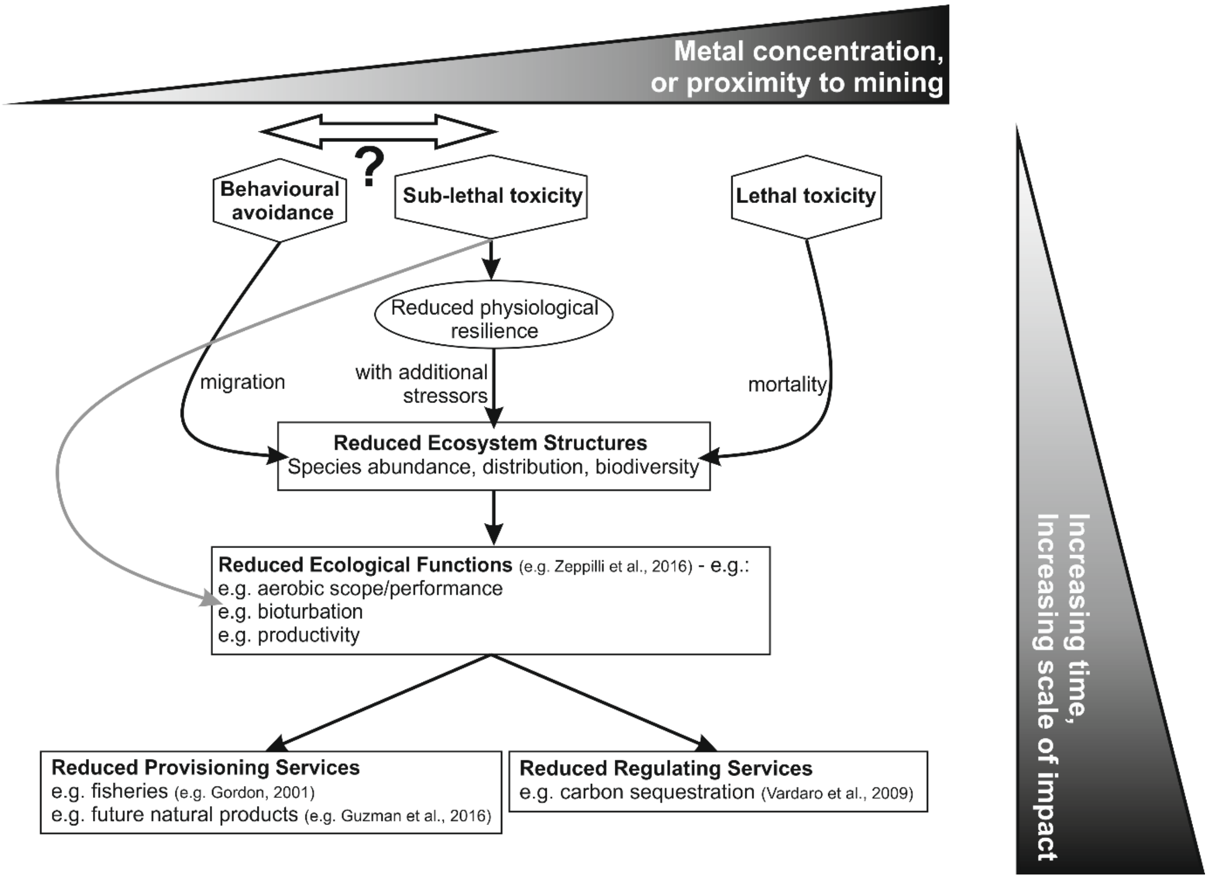
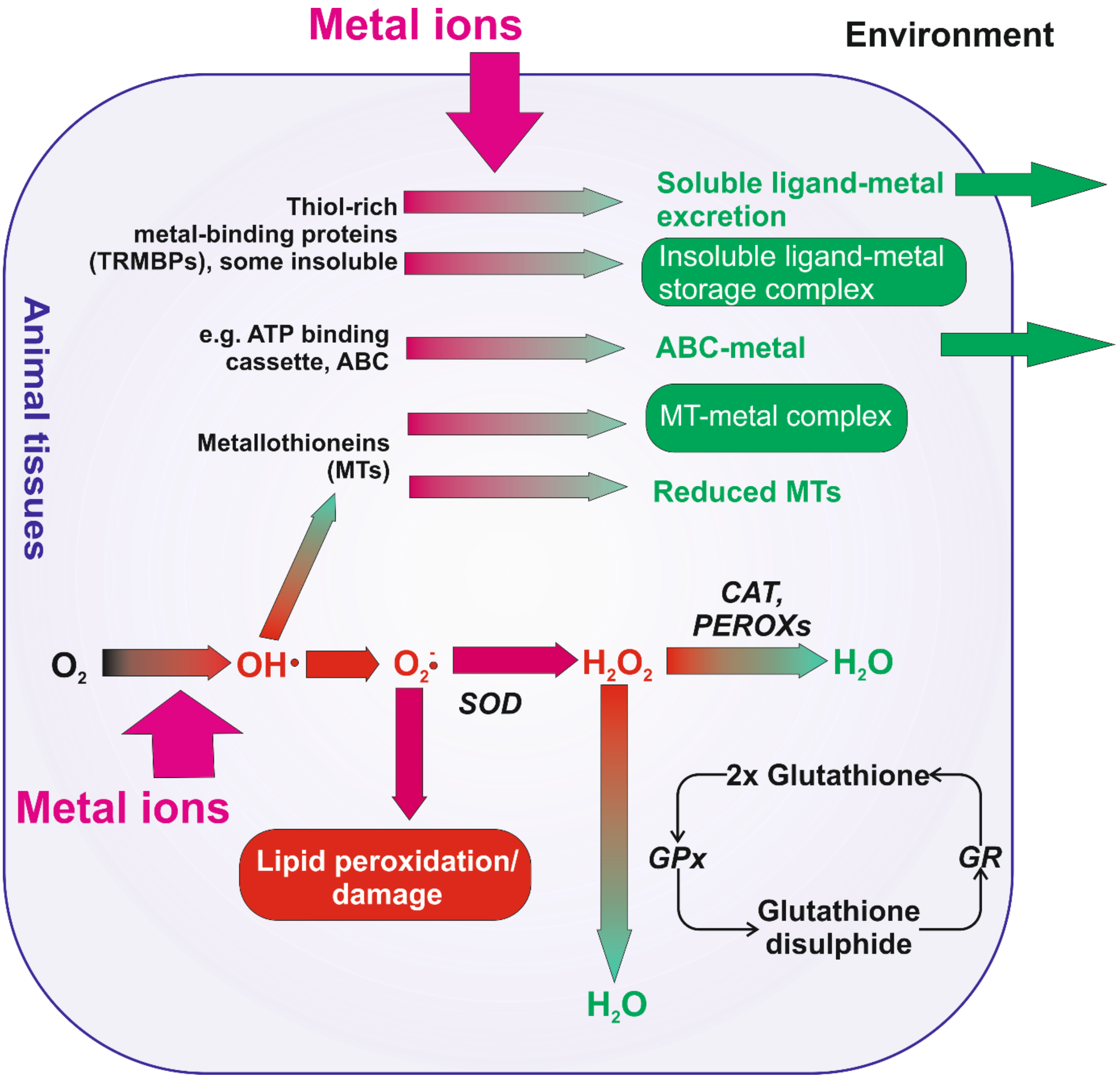

Metal concentration, or proximity to mining
?
Behavioural avoidance
Sub-lethal toxicity
Lethal toxicity
Reduced physiological resilience
migration
with additional stressors
mortality
Reduced Ecosystem Structures
Species abundance, distribution, biodiversity
Reduced Ecological Functions (e.g. Zeppilli et al., 2016) - e.g.:
e.g. aerobic scope/performance
e.g. bioturbation
e.g. productivity
Reduced Provisioning Services
e.g. fisheries (e.g. Gordon, 2001)
e.g. future natural products (e.g. Guzman et al., 2016)
Reduced Regulating Services
e.g. carbon sequestration (Vardaro et al., 2009)
Increasing time,
Increasing scale of impact

Figure 3


Metal ions
Environment
Thiol-rich metal-binding proteins (TRMBPs), some insoluble
Soluble ligand-metal excretion
Insoluble ligand-metal storage complex
e.g. ATP binding cassette, ABC
ABC-metal
Animal tissues
Metallothioneins (MTs)
MT-metal complex
Reduced MTs
CAT, PEROXs
O_2
$OH^{\bullet}$
$O_2^{\bullet -}$
H_2O_2
H_2O
SOD
Metal ions
Lipid peroxidation/ damage
2x Glutathione
GPx
GR
Glutathione disulphide
H_2O