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From sea squirts to squirrelfish: facultative trace element hyperaccumulation in animals

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Abstract

The hyperaccumulation of trace elements is a widely characterized phenomenon in plants, bacteria, and fungi, but has received little attention in animals. However, there are numerous examples of animals that specifically and facultatively accumulate trace elements in the absence of elevated environmental concentrations. Metal hyperaccumulating animals are usually marine invertebrates, likely owing to environmental (e.g. constant exposure via the water) and physiological (e.g. osmoconforming and reduced integument permeability) factors. However, there are examples of terrestrial animals (insect larvae) and marine vertebrates (e.g. squirrelfish) that accumulate high body and/or tissue metal burdens. This review examines examples of animal hyperaccumulation of the elements arsenic, copper, iron, titanium, vanadium and zinc, describing mechanisms by which accumulation occurs and, where possible, hypothesizing functional roles. Groups such as the ascidians (sea squirts), molluscs (gastropods, bivalves and cephalopods) and polychaete annelids feature prominently as animals with hyperaccumulating capacity. Many of these species are potential model organisms offering insight into fundamental processes underlying metal handling, with relevance to human disease and aquatic metal toxicity, and some offer promise in applied fields such as bioremediation.

Significance to metallomics

This review examines examples of hyperaccumulation in animals, the mechanisms by which this is achieved, the biological roles that have been proposed for this phenomenon, and identifies knowledge gaps requiring further research. The hyperaccumulation of trace metals such as arsenic, copper, iron, titanium, vanadium and zinc in animal models can offer significant insight into human metal handling disorders and the risks associated with environmental metal contamination.

Introduction

Trace elements are those that are found at relatively low concentrations within the environment, and biologically can be classified as either essential or non-essential. Essential elements such as copper (Cu), zinc (Zn), and iron (Fe) perform a variety of key functions through their association with biomolecules such as proteins.[1] However, even elements that in human biology are considered non-essential, for example the metalloid arsenic (As), still can have important roles in other biota.[2] For most elements, and in most organisms, accumulation is limited, usually through regulation of uptake and/or excretion.[1] This is vital as even essential elements accumulated to high concentrations can cause a variety of deleterious effects. However, there are a number of species that maintain elevated concentrations of elements within specific tissues and/or cellular compartments. This is a particularly prominent phenomenon in plants, wherein approximately 500 taxa can be defined as metal hyperaccumulators.[3-6] While hyperaccumulation has also been widely noted in bacteria, yeast, and fungi,[7,8] it has received little attention in animals. This is somewhat surprising given the potential importance of animal hyperaccumulators as model species for understanding processes critical for ecological risk assessment (e.g. regulatory tools utilizing body burden as a predictor of impact),[9,10] environmental remediation,[11] food safety,[12] and human disease.[13] To address this gap, the current review seeks to summarize the existing literature regarding facultative trace element hyperaccumulation in animals, particularly focussing on trace metals and metalloids.

Hyperaccumulation: defined and refined

In the current review, we define hyperaccumulators as species that concentrate elements to levels greater than 1 000 mg kg^{-1}, on either a whole animal or tissue basis. To place this threshold in context, Luoma and Rainbow conducted a literature survey of bioaccumulation in aquatic organisms, including those from contaminated ecosystems, and found that 88% of trace element concentrations fell between 0.1 and 100 mg kg^{-1}.[14] It is important to highlight that our definition of hyperaccumulation is simplified relative to that used by previous authors,[11] in that it uses the same threshold for all trace elements, rather than making element-specific distinction. Consistent with some definitions of hyperaccumulation in the plant literature,[15] we largely exclude from discussion those animals in which experimental exposures can result in increased tissue element burdens, and also those animals exposed naturally to extreme environmental contamination scenarios. Consequently, we focus on facultative hyperaccumulators. These are animals that strategically concentrate elements, without evidence of a toxicological impact, and in spite of relatively low environmental concentrations. It is notable that for many of these species the functional role of hyperaccumulation has yet to be discerned.

In some studies hyperaccumulation can be a consequence of how animals or tissues are handled for analysis. For example, failure to flush or depurate gut contents can result in artificially elevated tissue burdens by measuring gastrointestinal sediments or prey items that may have elevated metal contents, but which are not accumulated.[16] This will be particularly important where there is an unrecognized environmental enrichment of elements (e.g. contamination and/or metal-enriched geology). An example of this effect is in studies of Fe accumulation in field-collected ascidians, where it was noted that body burdens reduced significantly after animals were held in clean seawater for several days following collection.[17] This was attributed to the flushing of Fe-containing particulate matter originating from the point of collection, from the body cavity. The importance of this artifact is underlined by the drop in Fe body burden, which took the animals from a hyperaccumulator status (as high as 2600 mg kg^{-1}), to values under this threshold (as low as 900 mg kg^{-1}).[17] A similar effect is likely to explain many of the observations of hyperaccumulation in the sponges.[18] Here, the confounding factor is not only the presence of abiotic factors such as sediments associated with sponge tissues, but also the accumulation of trace elements associated with symbionts. For example, the observation of elevated molybdenum (Mo) concentrations in the tissue of the sponge *Halichondria phakellioides*, was attributed to a bacterial symbiont, and not the sponge itself.[19] Similarly, elevated arsenic (As) concentrations in giant clams have been attributed to uptake of the element by commensal algae through mimicry of phosphate in low phosphate waters.[20,21]

A related phenomenon influencing hyperaccumulation designation is the consideration of adsorbed (i.e. adhering to the tissue/animal surface) metal, as accumulated burden. For example, a common biological response to metal exposure in aquatic animals is the secretion of mucus.[22,23] In a laboratory study examining waterborne aluminium (Al) accumulation in freshwater crayfish, mean gill Al concentrations in excess of 1 200 mg kg^{-1} were reported. However, the vast majority of branchial Al was complexed to mucus, and not actually accumulated inside the animal.[24] Similarly, the carapace of crustaceans,[25] and the cuticle of insects,[26] can bind elements such as nickel (Ni) and Fe at levels close to, or greater than, 1 000 mg kg^{-1}. This implies hyperaccumulation even though the elements associated with these tissues are not, technically, absorbed (i.e. taken into the animal).

Another issue in collating hyperaccumulation data is inter-individual variation, and the reporting of burden values as a mean. Many authors have noted that tissue accumulation can vary significantly, even between animals of the same species collected at the same time.[17] The reasons for variations in individual burdens are not always known, but include factors such as fed state, sex, developmental stage/age, and/or reproductive state.[17,27,28] The consequence of this variation is that mean values, the standard measure for reporting burdens, can be below the hyperaccumulation threshold, even though individuals within the species display hyperaccumulation characteristics. This issue could be alleviated by authors detailing ranges of individual tissue burdens, in addition to mean values.

Most hyperaccumulating animals are aquatic and, specifically, marine

Aquatic animals are more likely to be hyperaccumulators than terrestrial animals. This could be a consequence of a greater research focus on metal accumulation in aquatic biota, but there are also environmental factors and organismal physiology factors that are likely to explain this trend. While terrestrial species are exposed to trace elements solely through the gastrointestinal system, aquatic biota are also exposed via the water. Not only does this create an additional route for uptake, it often also increases the duration of exposure. With the exception of behavioral responses (e.g. valve closure in molluscs),[29] waterborne exposure is constant, while dietary exposure is periodic, associated with the transit of a meal. The respiratory surfaces of aquatic animals are also used for mineral uptake and excretion and are exquisitely designed for transport processes, with reduced diffusive distances, large surface areas, and high perfusion rates.[30] Consequently, this extra pathway for absorption may partly explain their greater capacity for accumulation relative to terrestrial species.

Many marine species osmoconform, an important physiological trait that is likely to enhance trace element assimilation. These animals, mostly marine invertebrates, maintain body water and ion contents consistent with their environments, and therefore have a limited need to utilize the integument as a barrier against environmental ion exchange (see Figure 1). Consequently, they display relatively permeable body surfaces, which may perform roles in trace element acquisition.[32] For example, the primitive hagfish, the only known vertebrate ionic and osmotic conformer, has been shown to accumulate Fe and Ni across its skin surface.[33,34] This may, therefore, be a contributing factor explaining the over-representation of marine osmoconformers among hyperaccumulating animals.

Trace element uptake and the importance of chemical speciation

The key first step necessary for trace element hyperaccumulation is uptake across an epithelial surface. The bioavailability of a given element is dependent upon a number of chemical and biological factors that influence the permeability of the epithelium.[10] Chemical factors include element concentration, chemistry of the medium (water or diet), metal speciation, and size fraction of any particulate element. Biologically, uptake will be determined by fundamental characteristics of the transporting surface, including the cell types present, and biological modification of the organism-environment interface (e.g. mucus, acid-base fluxes), with these themselves varying as a function of life history, developmental stage, fed state and environmental characteristics such as salinity, temperature, and dissolved oxygen.[34,35]

As noted above, trace elements are absorbed via one of three epithelial surfaces in aquatic animals: the epidermis, gill or gut. While these surfaces all possess distinct chemical and biological properties, the fundamental pathways of uptake are conserved. Waterborne trace elements are almost entirely absorbed as the free metal ion, through specific membrane transport pathways.[1] Consequently, water chemistries that favor the formation of anionic trace element complexes decrease bioavailability by reducing free metal ion availability and thus access to the transporter.[10] The transporters that comprise these pathways may be dedicated to the translocation of specific nutrient metals, or may be promiscuous and absorb a number of substrates (e.g. divalent metal transporter; DMT-1).[1] It is worth noting that these transporters are also implicated in metal hyperaccumulation in plant and microbial species, suggesting a fundamental conservation of the mechanisms underlying hyperaccumulation between these diverse groups.[6] Some metals are also able to move through epithelia as mimics of other elements, such as sodium and calcium.[1] Under this transport scenario, the presence of enhanced water cation content increases competition for the transporter, reducing uptake.[10] Specific examples of these transport pathways are provided in element-specific sections below.

However, there are other mechanisms of trace element uptake which may contribute to hyperaccumulation in animals. For example, trace elements bound to nutrient ligands may also be bioavailable. The best example of this is the absorption of metals liganded to amino acids, which are subsequently taken up via amino acid transporters.[37,38] This will be more important for transport across digestive epithelia where the concentrations of nutrient ligands are sufficiently high for this to contribute significantly to uptake. However, it should be noted that enhanced elemental transport in the presence of ligands can sometimes be explained by chemodynamic phenomena.[39] Under this scenario the ligand acts to shuttle the trace element to a transporter, and following ligand-element dissociation, uptake occurs via the free ionic elemental form, and not through the nutrient transporter.

Chemical speciation may also result in a third route of uptake. Trace elements that form neutral complexes, be they organic or inorganic, may be able to cross epithelia through simple diffusion.[40] While there is evidence for this as a route of trace element uptake in algae and bacteria,[41] it has not been well described in animals, in part due to an inability to delineate between actual transport of these complexes and the chemodynamic effect of increasing substrate access to epithelial transporters.[42]

Finally, elements bound to particulate matter, in insoluble precipitates, or in nanoparticle form, may be taken up by endocytosis. This is a mechanism that is commonly reported for metals in the digestive system of molluscs.[36] However, there is little evidence for this being an important pathway of metal uptake in the transport epithelia of other hyperaccumulating groups such as polychaetes and ascidians.

Value of studies in hyperaccumulating animals

The study of hyperaccumulating animals has significant utility from both fundamental and applied perspectives. Investigating basic biological function is greatly facilitated by animal models that exhibit extreme phenotypes, an approach in alignment with Krogh's Principle ("For a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied").[43] To this end the study of hyperaccumulating animals can offer significant insight into mechanisms of trace element absorption and intracellular handling. There is particular value in studying the mechanisms by which these species can withstand levels of trace metals that would be highly toxic to other species. Hyperaccumulating animals could also find utility as model systems for human conditions associated with metal accumulation, such as Wilson's disease, hemochromatosis, and the myriad of metal accumulation disorders in neuropathological disease.[13] This utility is conferred by metal transport pathways and cellular handling mechanisms that are largely conserved between humans and animals.[1]

The use of metal hyperaccumulating animals in the remediation of contaminated environments has been proposed. Two groups of organisms have particular promise in this regard. When cultivated in contaminated waters, bivalve molluscs such as pearl oysters can accumulate trace metals to levels that exceed the 1 000 mg kg^{-1} threshold.[11] The second group are sponges, which have been shown to hyperaccumulate Fe.[18,44] These animals are of specific interest in that they bring added value as remediators, through the cultivation of pearls and the extraction of novel bioactive materials, respectively.[11] A related concept is the use of hyperaccumulators to extract precious metals. Species that can concentrate valuable elements in tissues from a dilute source, have the potential to make metal extraction economically viable. For practical reasons, bacterial and plant species,[45] and/or animal waste products (e.g. chicken feathers),[46] are advantageous over the use of animals in these approaches. However, biomimetic technologies based on animal models are being explored. For example, a compound modelled on tunichrome, a key entity associated with vanadium (V) accumulation in ascidians (see *Vanadium* below), has recently been shown to effectively remove gold (Au) from test effluents.[47]

Some researchers have even suggested animals that accumulate high concentrations of metals with important biological properties could find utility as functional foods. For example, the sea cucumber *Aposticophus japonicus* is a luxury food item in parts of Asia,[48] and accumulates organic forms of V in edible tissues following waterborne exposure to this metal.[49] When fed to mice, V-enriched sea cucumber protein was shown to have anti-diabetic properties,[49] a well-established biological effect of V.[50] Organic V species are postulated to have a higher efficacy and lower toxicity than inorganic V forms, and thus specifically cultured V-rich sea cucumbers may have promise as a health supplement.[49]

Trace elements in facultative hyperaccumulators

Arsenic (*As*)

Arsenic is a naturally-occurring metalloid element that can be found worldwide at low concentrations in surface water (~20 nM).[51] However, As concentrations can be enriched through anthropogenic processes such as Cu refining, herbicide production, and wood preservation,[52] and there is a seasonality to waterborne As concentrations associated with geodynamic processes such as sediment cycling.[51] Arsenic exhibits four redox states (-3, 0, +3, and +5), with the dominant state being dependent upon redox potential and pH.[53] In aquatic environments, As typically exists as either trivalent arsenite (As^{III}) or pentavalent arsenate (As^{V}) forms, with arsenite being more common under reducing conditions, and arsenate being more common under oxidizing conditions.[54] Trivalent As is generally more toxic than the pentavalent form.[55] Organic forms of As, such as monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}), are also found in seawater,[56] and are considered less toxic than inorganic As species.[57] While no physiological function is typically associated with As, evidence from rodent studies suggests a possible role for As in methionine metabolism.[58]

A number of polychaete species have been shown to hyperaccumulate As. For example, the cirratulid polychaete *Tharyx marioni* exhibits whole-body concentrations of As that exceed 2 000 mg kg^{-1}, with the palps being especially high in As (up to 13 000 mg kg^{-1})[59] (Table 1). This occurs even when the organisms are living in low ambient As, and regardless of the age of the individuals. A separate study in the Mediterranean fan worm *Sabella spallanzanii* identified mean concentrations of As higher than 1 000 mg kg^{-1} in the branchial crowns of these organisms.[73] Organisms from multiple sites were sampled to eliminate the possibility that body burdens reflected any anthropogenic As inputs.

Arsenic hyperaccumulation occurs principally via inorganic forms (Figure 2). Pentavalent As is a phosphate analogue and as such can be taken up by phosphate transporters in all eukaryotic cells,[74] whereas trivalent As appears to be transported by aquaglyceroporins and/or hexose permeases.[75] Once absorbed, inorganic As is biomethylated, likely as a mechanism of detoxification. [76,77] This process involves the actions of glutathione S-transferase, and arsenic methyltransferase with S-adenosylmethionine as a methyl donor, resulting in the production of mono-, di-, and tri-methylated species.[77] Eventually, in marine biota most As ends up as the relatively non-toxic arsenobetaine.[51] However, the predominant form of As found within the branchial crown tissues of polychaetes is not arsenobetaine, but rather the somewhat more toxic DMA^V.[73]

The retention of DMA^V in polychaete tissues could indicate a role for As hyperaccumulation as an anti-predatory mechanism. The palps and branchial crowns of these organisms are exposed and vulnerable, therefore reducing the palatability of these tissues by accumulating a toxic form of As may serve as protection against predators. Supporting this, feeding experiments using the seabream *Diplodus sargus sargus* revealed that these fish rejected the As-rich branchial crown of *S. spallanzanii*, while accepting the relatively As-poor thorax of the same animals.[73] However, in feeding trials with the two-spot goby *Gobiusculus flavescens*, some individuals repeatedly rejected whole *T. marioni*, but other individuals accepted them without hesitation.[59] Thus a role for As hyperaccumulation as an anti-predatory strategy in polychaetes remains speculative.

Arsenic also provides the only known example of metal hyperaccumulation in a terrestrial animal. The caterpillar of the moth *Callopistria floridensis* can accumulate As to whole body concentrations higher than 4 200 mg kg^{-1} (mean value 1 462 mg kg^{-1}; Table 1), a consequence of feeding on an As hyperaccumulating fern.[60] The hyperaccumulation of As appears to be a specific adaptation, as a closely related species (*Mamestra configurata*) directly dosed with dietary As exhibited whole body burdens less than 1% of those in *C. floridensis*.[78] The accumulation of As in *C. floridensis* could be considered facultative as it permits the exploitation of a feeding resource that would otherwise be unavailable owing to its toxicity. However, it also represents an example of a species that accumulates a trace element owing to its enrichment in the environment, and as such does not strictly meet the criteria for hyperaccumulation as defined in the current review. Similarly, there are some insects that feed on Ni-hyperaccumulating plants leading to tissue Ni levels as high as 700 mg kg^{-1}.[79,80] In this case there is evidence suggesting that this accumulation protects the insect against predation.[81] This indicates that, in at least some situations, trace element hyperaccumulation in insects that feed on hyperaccumulating plants has a strategic benefit, whether or not that accumulation is strictly facultative in nature.

Copper (*Cu*)

Copper is an essential trace element, acting as a cofactor for a number of key proteins.[82] Owing to its flexible redox state, Cu plays a particularly vital role in proteins, such as cytochrome c oxidase, which are associated with cellular respiration.[1] Copper is naturally present in the Earth's crust and as is typically found in seawater within the range of 0.5 to 4.5 nM.[83] Carbonate complexes are the dominant forms of inorganic Cu in seawater, with only a small fraction of Cu being present in the bioavailable ionic Cu^{2+} form.[84] Copper also binds to dissolved organic matter with high affinity,[85] which will further limit waterborne bioavailability. As such, the primary means of Cu uptake in aquatic organisms is dietary.[84] However, Cu transporters have been characterised in both the gill and gut epithelia of fish. These include a high affinity Cu-specific transporter (Ctr), an apical transporter which recognizes Cu in its free monovalent form (Cu^{+}), and which works in association with an epithelial reductase.[1] Basolateral Cu export appears to be achieved by a highly conserved Cu-ATPase. There is also evidence that Cu transport can be achieved via sodium transporters, owing to the physicochemical similarities between Cu^{+} and ionic sodium.[1]

There are several groups of aquatic biota that have species which hyperaccumulate Cu. In fish, two known examples have been characterized. In the striped bass (*Morone americana*)[63] and the mullet (*Mugil cephalus*),[86] some individuals display hepatic Cu levels as high as 2 440 and 1 936 mg kg^{-1}, respectively, although only for the former species were mean tissue Cu levels greater than 1 000 mg kg^{-1} (1 020 mg kg^{-1}; Table 1). A high variation in accumulation between individuals was suggested to relate to differences in age and sex,[63] but there was no relationship between accumulation and environmental Cu concentrations in either study. In striped bass Cu hyperaccumulation was associated with pathological findings, suggesting that this phenomenon might be a piscine equivalent of human Cu storage disorders, such as Wilson's Disease,[63] and thus may not serve a useful biological role.

Several species of cephalopod molluscs accumulate Cu in the digestive gland. The best described example is the squid *Loligo opalescens* (8 370 mg kg^{-1})[61] (Table 1). However, many of the same cephalopods that accumulate Cu will also accumulate Zn,[62,87,88] likely owing to similarities in the physicochemistry of these two elements. Copper hyperaccumulation is also noted in bivalve molluscs, and is especially prominent in oysters. Carpene and colleagues found that oysters accumulated Cu to concentrations two orders of magnitude higher than those in other bivalve species in the same environment (~1 000 mg kg^{-1}; Table 1).[62] Similar to the accumulation of Zn and Fe (see sections below), without environmental measurements of trace elements it can be difficult to distinguish between facultative hyperaccumulators that are strategically concentrating Cu, and those species where hyperaccumulation occurs as a result of exposure to elevated environmental Cu. Certainly it is well-described that very high tissue Cu burdens can result in oysters collected from contaminated waters (e.g. > 20 000 mg kg^{-1}).[89]

Elevated Cu concentrations in molluscs are often associated with the utilization of Cu-containing hemocyanin as a respiratory pigment.[62,80] Hence, in these species the accumulation of Cu is likely to be a reservoir for hemocyanin synthesis.[91]

Iron (Fe)

Although an abundant element in geology, aquatic Fe concentrations are low (high pM to low nM) owing to its generally poor solubility.[92] Iron in its ferric form (Fe^{III}) readily forms insoluble precipitates with hydroxides, reducing its bioavailability to aquatic animals. Consequently, bioavailability of Fe is highest in anoxic waters, as these favor the divalent ferrous form (Fe^{II}). However, irrespective of oxidation state, most dissolved Fe in natural waters is found complexed to organic ligands.[93] Consequently, most aquatic animals absorb Fe via the dietary pathway, for its essential roles as a cofactor of multiple enzymes, and as a component of heme.[1] Epithelial Fe transport is well characterized in mammals, and evidence to date suggests that the entities involved are conserved throughout the animal kingdom. For example, in fish apical uptake of ferrous Fe in its free ion form occurs via DMT-1, with basolateral export promoted by ferroportin (also known as iron regulated protein, IREG).[1]

Distinguishing between facultative hyperaccumulators of Fe and those animals that hyperaccumulate as a consequence of elevated environmental Fe concentrations is especially challenging. Iron is an important and relatively common contaminant, and thus accumulation can often be linked to sources of pollution. For example, sea snakes accumulate Fe concentrations as high as 6 000 mg kg^{-1} in shed skin, likely as a mechanism for eliminating toxicant burden associated with Fe-rich effluents.[94] However, tissue Fe can also be elevated owing to naturally Fe-rich geology.[95] Unfortunately, not all studies that describe Fe burdens in animals associate these measures with analysis of Fe content in water and sediments. In fact, this is true for many of the trace elements considered in the current study (e.g. Cu, Zn, As).

Molluscs are one group for which there is compelling evidence for Fe hyperaccumulation.[96,97] This is particularly notable in Patellidae limpets,[98] where digestive gland Fe concentrations greater than 11 000 mg kg^{-1} have been reported.[65] In this, and other mollusc species, the deposited Fe likely acts as a reservoir for supplying Fe to the radula. This is a tooth-like structure, common to all molluscs except the bivalves, usually used as a digestive tool for scraping algae off hard surfaces and/or grinding hard substrates to release nutrients. This role requires high mechanical strength, a property provided by veneers of biomineralized Fe, embedded in a chitin-based matrix.[99] In fact, the Fe content of radulae can be close to 100 000 mg kg^{-1} (Table 1).[64] The exact mineral comprising the radula differs between mollusc groups, with chitons generally utilizing magnetite (Fe_3O_4), and limpets using goethite (α-FeOOH). Biomineralization of Fe is not restricted to molluscs, however. For example, horny sponges accumulate crystals of lepidocrocite (γ-FeOOH), which are likely to perform a structural role.[27]

Digestive gland Fe in hyperaccumulating molluscs is largely stored in insoluble granules.[97] In this particulate form, Fe is redox inert and thus cannot cause toxicity through the generation of oxidative stress.[1] Storage of Fe within the body as a mechanism to supply the radula, rather than environmental acquisition, is likely a consequence of the evolutionary history of molluscs. The radula of molluscs appeared very early in evolution, with evidence of a similar tooth-like structure in the forebears of modern molluscs from the Cambrian,[100] before the rise in atmospheric oxygen that occurred late in this geological period. This increase in oxygen that occurred during the Cambrian would have created a scenario where Fe bioavailability in water was decreased due the formation of insoluble Fe oxides and hydroxides. Given the high Fe demands required for radula synthesis,[64] this may have necessitated mechanisms allowing storage of Fe, and other transition metals, in body tissues.[101] It is also possible that Fe hyperaccumulation plays an important role in the adhesion of bivalve molluscs to substrates. Catechol moieties have a strong affinity for Fe, and will form crosslinks that add cohesion and strength to mussel adhesion plaques.[102]

Fe is also hyperaccumulated in some ascidians. This is a group of primitive filter-feeding chordates, also known as sea squirts, on the basis of their effective siphons, or as tunicates, owing to the tough outer mantle surfaces resembling a tunic. Of the three main suborders of ascidians, Fe accumulation is largely restricted to the Stolidobranchia. Although the highest reported concentration of Fe in a hyperaccumulating ascidian occurs in the tunic (Table 1), for most species the blood cells are the main Fe reservoirs, similar to the pattern observed for V (see *Vanadium*).[66] Intriguingly, the stolidobranchs are the ascidian suborder that display relatively low concentrations of V. This suggests that Fe accumulation is "compensating" for V accumulation (or vice versa), insinuating that Fe and V have similar, albeit unknown, functions in this group. This concept is supported by a number of circumstantial lines of evidence. For example, seasonal fluctuations of Fe and V burdens are similar in *Ciona intestinalis*,[103] while some of the entities associated with V accumulation may also bind Fe (e.g. tunichrome, VBP-129).[104,105] However, there are also novel biomolecules in ascidians with specific putative roles in Fe handling. Ferrascidin, for example, is a small peptide isolated from ascidians considered to have a strong Fe-binding capacity.[106] To date, however, Fe-binding by ferreascidin has not been shown *in vivo*, and the protein is thought to exist in a different cellular compartment to the metal,[107] suggesting that ferreascidin is not involved directly in Fe sequestration in blood cells.

There are reports that some aquatic insect larvae hyperaccumulate Fe.[108,109] However, this is likely a function of the metal adhering to the body wall.[26] Similarly, the livers of sperm whales (only one of three individuals),[110] dolphins and some penguin species,[111] have all been reported to meet the 1 000 mg kg^{-1} hyperaccumulation threshold for Fe. However, verifying whether this accumulation is facultative (e.g. associated with Fe roles as a co-factor, component of hemoglobin), or a consequence of consuming food with high Fe burdens, is difficult for these migratory predators.

Titanium (Ti)

Titanium is not an obvious target for hyperaccumulation. For example, to date there are no confirmed roles for Ti in biology,[112] and the ambient concentrations of Ti in seawater are very low (~4-200 pM).[113] In natural waters Ti is most stable in its tetravalent form (Ti^{IV}), where it is most often associated with the particulate fraction. This association is in the form of adhesion to, or incorporation into, inorganic colloids, a phenomenon of particular importance in estuarine settings.[114] Consequently, marine filter feeders that ingest particulate matter are likely to be exposed to Ti through the diet.

The only group in which Ti hyperaccumulation has been observed are the ascidians. Specifically, Levine showed that *Eudistoma ritteri* accumulated whole body Ti concentrations in excess of 1 500 mg kg^{-1}.[115] It is worthwhile noting that follow-up studies reported lower concentrations (150 mg kg^{-1}), and attributed these differences to developmental stage and/or environmental factors.[17] In another ascidian, *Ascidia dispar*, compartment-specific Ti accumulation was examined, with the blood cells shown to accumulate Ti to 1 552 mg kg^{-1}, and the tunic accumulating 126 mg Ti kg^{-1} (Table 1).[67] In general, Ti accumulation in the tunicates is less widespread than for V (see *Vanadium* below), but this could be a function of the relatively fewer studies that have attempted Ti measurement. The only other group that displays significant Ti accumulation, albeit well below the threshold for hyperaccumulation, are the sabellid polychaetes. The only characterised example is that of the feather duster tube worm, *Eudistylia vancouveri*, which has Ti tissue burdens of 72 mg kg^{-1}.[116]

Based on chemical properties, it has been suggested that the biological roles of Ti accumulation in ascidians are similar to those of V,[115] which still remain enigmatic. However, a number of specific biological functions for Ti in hyperaccumulating ascidians have been suggested. These include acting as a sunscreen protecting the animal from ultraviolet (UV) light damage, a role in anti-microbial defence (through the generation of UV-activated reactive oxygen species), wound repair, and as a protective structural component of the tunic.[112] All of these hypotheses await experimental testing.

Vanadium (V)

Vanadium is the second most common trace metal in seawater, occurring at concentrations in the order of 30 nM.[50] Although V can exist in a number of oxidation states in natural waters, pentavalent V (V^{V}) dominates in oxic seawater, occurring primarily as HVO_4^{2-} and $H_2VO_4^{-}$.[117] At high concentrations V can exert toxic effects on biological systems by acting as an analogue of phosphate, and through generation of oxidative stress.[118] However, it does have some important biological functions. For example, V acts as a metal co-factor for bacterial haloperoxidases and nitrogenases, while its anti-diabetic properties have been exploited for the development of human medicines.[50]

The remarkable capacity of ascidians to hyperaccumulate V has been recognized for more than a century.[119] The best example of V accumulation is found in *Ascidia gemmata*, which displays blood V concentrations in excess of 17 000 mg kg^{-1} (Table 1),[68] representing a bioaccumulation factor (ratio of tissue concentration to seawater concentration) of more than 10^7. Patterns of metal hyperaccumulation in ascidians are phylogenetically aligned, with the sub-order Phlebobranchia accumulating the highest concentrations of V, followed by the Aplousobranchia and Stolidobranchia suborders, the latter of which generally accumulates little V and instead hyperaccumulates Fe (see *Iron*).[120] There are also differences in the oxidation states of accumulated V in these species, with trivalent V (V^{III}) the primary storage form in cells of phlebobranchs, and tetravalent V (V^{IV}) the main form in aplousobranchs.[120] The highest concentrations of V (often greater than 10 000 mg kg^{-1}) are found in the vacuoles of specific blood cells called signet ring cells (also termed vanadocytes).[121] The protective tunic may also contain high V concentration, although these are usually an order of magnitude lower than those found in vanadocytes.[122] Intestinal and branchial sac tissues, which likely represent uptake pathways, also display elevated V (~100 mg kg^{-1}).[123]

Interestingly, V is transferred to gametes,[124] and body V concentrations peak in reproductive season,[103] suggestive of a role in development akin to that suggested for Zn in squirrelfish (see *Zinc*).

The only other group that comes close to matching the V hyperaccumulating capacity of the ascidians, are the polychaetes, in which hyperaccumulation has been noted in a small number of species.[69,125,126] In polychaetes the branchial crowns display the highest concentrations of V (up to 10 500 mg kg^{-1} in *Perkinsiana littoralis* Table 1).[69] Concentrations in the trunk of these species are significantly lower (~50 to 900 mg kg^{-1}),[69,125] albeit still high relative to species that do not accumulate V. In fact, for most animals tissue levels of V are below the limits of detection for most analytical approaches.

The mechanisms by which V is accumulated in ascidians has been the subject of significant investigation (Figure 3).[123] Pentavalent V, the dominant oxidation in state in natural waters, shares some physicochemical properties with phosphate, and passage through epithelial phosphate transporters is considered the likely pathway of V uptake.[127] Vanadium accumulates to high concentrations in epithelial cells of the intestine and branchial sacs, and exits these cells into the blood through an unknown mechanism, thought to be passive owing to the favourable electrochemical gradient.[123] The exact oxidation state of V (i.e. tetravalent or pentavalent) at this stage in assimilation is unknown. In the plasma, V is bound by specific high affinity proteins (e.g. vanadinP, VBP-129), capable of sequestering 6 to 13 V atoms per peptide.[105,128] These proteins shuttle V to the cell membrane of vanadocytes, where uptake is likely achieved by phosphate transporters and/or other unknown transporters.[123] In the blood cell cytoplasm, V is present in a tetravalent form, with reduction from the pentavalent form facilitated by vanabins, specific V-binding proteins which act as both chaperones and reductants.[129] Reductive power is provided by NADPH from the pentose phosphate pathway, which is upregulated in V-accumulating ascidians.[130] Tetravalent V is then transported into the vanadocyte vacuole, likely via an ascidian homologue of the divalent metal transporter , DMT-1.[123] This transport is coupled to proton efflux, powered by the actions of a proton pump which can reduce vacuole pH levels to less than 2.[131] The low pH of the vacuoles is also essential for the maintenance of vacuolar V in a reduced state, with reduction achieved by the actions of an as yet uncharacterised agent. In addition to high proton concentration, the vanadocyte vacuole is also highly enriched in sulfate,[132] thus the V is essentially bathed in a strong sulfuric acid solution. The exact chemical species of V storage forms, pH levels, and sulfate concentrations, are all highly variable, even within the same cell.[133] Less is known regarding V handling in polychaetes. However, it has been shown that antibodies raised against ascidian V-binding proteins cross-react with polychaete tissues,[134] and that storage of V also occurs in a sulfuric acid-rich environment.[135]

The biological function of V hyperaccumulation in ascidians and polychaetes remains elusive. Based partly on the localization of V in the blood of ascidians and the respiratory tissues of polychaetes, early studies suggested that V may play a role in oxygen acquisition and transport, as a component of a respiratory pigment.[135,136] However, studies have failed to show reversible oxygen binding in the blood of ascidians, largely excluding this as an explanation for V hyperaccumulation.[137] In fact, rather than a facilitator of aerobic metabolism, it has been hypothesized that high tissue V burden may in fact promote hypoxia tolerance.[138] This is a hypothesis that has yet to be tested, but it has been noted that anaerobic capacity does not appear to correlate with V accumulation in ascidians,[139] suggesting that this may not be the explanation behind V hyperaccumulation.

Ascidians are also characterised by the presence of tunichromes, small oligopeptides with a number of putative roles, and a high metal-binding affinity.[104] One proposed function of tunichrome is as a structural component of the protective tunic. Consistent with the observation that V can be incorporated into the tunic, it has been suggested that metal-tunichrome complexes might provide a particular strong outer barrier, and thus serve an important protective role.[104] This may be facilitated by catechol crosslinking, as suggested for Fe in mussel adhesion plaques (see *Iron*).

Another common proposition is that the accumulation of V is an anti-predator mechanism. There is some evidence supporting this concept for polychaetes. For example, if offered the V-rich branchial crowns of the polychaete *Perkinsiana littoralis*, rock cod (*Trematomus berancchii*) will not feed, but they will consume offered trunk tissues containing much lower V concentrations.[69] A similar finding has been observed for the ascidian *Phallusia nigra*.[122] A wrasse (*Thalassoma bifasciatum*) offered fresh preparations of whole tunic, internal tissues, and blood, all containing high concentrations of V rejected these food items. However, this deterrence disappeared when the wrasse was offered these same tissues that had been previously frozen. Analysis showed that the acid component of the ascidian tissues, which was neutralized by freezing, and not the V component, was responsible for this effect.[122] Similar studies to specifically link V to an anti-predatory effect have not been performed for polychaetes. Odate and Pawlik also showed that putative anti-microbial properties of V hyperaccumulation were acid-dependent.[122] Previous work had associated V with the lack of fouling epibionts in some ascidians,[140] with the suggestion that anti-microbial effects relate to the capacity of V to generate reactive oxygen species through its interaction with tunichrome.[139] However, specific evidence that these effects are mediated by V is lacking.

Zinc (Zn)

Zinc is an essential trace element, necessary for a variety of processes including transcription, enzyme structure and activity, antioxidant defence, maintenance of membrane integrity, and cell signalling.[141] Although Zn is a relatively abundant element in the Earth's crust, its concentrations in the oceans are generally low, ranging from 50 pM to 9 nM.[83] In natural waters, Zn is divalent and exists as a variety of chemical species depending on pH, chloride ion and dissolved organic matter concentration.[142,143] In aquatic organisms absorption from dietary Zn is considered more important than that from waterborne Zn with respect to overall Zn uptake.[144-146]

Homeostasis of body Zn burden is critical for ensuring that toxic effects do not accrue.[147] However, in some molluscs the level at which tissue Zn is maintained qualifies as hyperaccumulation. For example, Miramand and Guary found mean concentrations of 1 450 mg Zn kg^{-1} in the digestive gland of the common octopus (*Octopus vulgaris*) collected along the coast of Monaco (Table 1).[70] This phenomenon is not geographically unique, as multiple additional studies have indicated similar Zn levels in the digestive gland of this widely-distributed species.[88,148] However, while the ability of octopus to accumulate high concentrations of Zn is commonly reported, the magnitude of the accumulation does vary. For example, concentrations of digestive gland Zn as high as 14 720 mg kg^{-1} have been described,[88] although the reasons for differences in the extent of accumulation are not understood. Zinc hyperaccumulation in cephalopods is not restricted to octopus, but has also been observed in squid and cuttlefish.[87,149]

The localization of Zn to the digestive gland in cephalopods is made possible by the ability of the Zn-binding protein metallothionein (MT) and MT-like proteins to be expressed at high levels in this organ.[150] These sulfur-rich proteins play a number of important roles in normal biological processes by virtue of their ability to bind metals, such as Zn, that are required as enzyme cofactors. The ability to chelate Zn, coupled with their inducibility, also allows MT to effectively reduce the bioreactivity of Zn and minimize toxic effects associated with its accumulation.[151] These proteins are the major intracellular regulators of Zn metabolism, and work in association with dedicated Zn transporters of the ZnT/CDF (Slc30) and ZIP (Slc39) protein families.[152] The role of MT and Zn transporters in cephalopod Zn hyperaccumulation has yet to be specifically studied, but this general scheme for control of cellular Zn homeostasis appears to be highly conserved, from prokaryotes to mammals.[152]

The exact purpose of Zn hyperaccumulation in cephalopods is not known. However, it is known that Zn has important roles in ameliorating oxidative stress. Therefore, one possibility is that Zn hyperaccumulation in the molluscan digestive gland could facilitate antioxidant defense, particularly in response to increased metabolism during warmer periods of the year.[153] This may be especially relevant for organisms such as the common octopus, an active carnivore that exhibits rapid growth during a relatively short life span of 1 to 2 years.[154] This could be mediated by Zn induction of MT, with this protein known to function as an effective scavenger of reactive oxygen species.[155,156] Zinc is also a cofactor of copper-zinc superoxide dismutase (SOD), an enzyme that performs an important role in antioxidant defense by converting reactive superoxide radicals to oxygen or hydrogen peroxide. Indeed, elevated Zn levels in the common octopus have been shown to be positively correlated to SOD activity,[157] with the highest levels in the digestive gland for both Zn and SOD activity occurring in summer.

Bivalve molluscs are also well documented to be hyperaccumulators of Zn. The New Zealand oyster, *Ostrea sinuata*, contains Zn concentrations in the soft portions of the whole body that exceed 1 000 mg kg^{-1}, with the highest levels existing in the mantle (4 760 mg kg^{-1})[71] (Table 1). In fact, Zn hyperaccumulation appears to be especially prominent in oysters. Carpene et al. found that some species accumulated Zn (>1 000 mg kg^{-1}) to concentrations three orders of magnitude higher than other bivalves in the same environment, suggesting that this accumulation is biologically rather than environmentally driven.[62] As with cephalopods the expression of MT and MT-like proteins in bivalves increases as tissue concentrations of Zn increase,[89] suggesting that mechanisms of uptake and sequestration are likely conserved among molluscs. The subcellular compartmentalization of Zn into insoluble fractions such as Zn-phosphate granules is also an important means for handling Zn load in bivalves.[158]

As for cephalopods, a biological role for hyperaccumulated Zn in bivalves has not been determined. Oysters and other bivalves have the ability to filter very large volumes of water to satisfy their nutritional and respiratory requirements, and as such increased uptake of Zn (and other metals such as Cu; see above) could occur as a by-product. There is also circumstantial evidence that Zn hyperaccumulation is associated with reproduction. Seasonal fluctuations in trace metal bioaccumulation in bivalves has been observed,[159] with peaks occurring before the reproduction period. More studies examining the role of Zn hyperaccumulation in reproduction in molluscs are warranted.

Facultative hyperaccumulators of trace elements are most often marine invertebrates. One notable vertebrate exception to this is the squirrelfish family of teleost fish (Holocentridae), where females can accumulate Zn in the liver at concentrations up to 500 times higher (2 630 mg kg^{-1}) than those observed in livers of other vertebrates, including male squirrelfish (Table 1).[72] The Zn accumulation observed in female squirrelfish is independent of diet and occurs in the absence of elevated Zn levels in the environment.[160] The difference in accumulation between males and females is partly a consequence of an enhanced capacity of female squirrelfish to absorb Zn across the intestinal epithelium relative to males, with this acquired Zn then being preferentially accumulated in the liver.[161] As with molluscs, Zn uptake and sequestration is likely achieved by the roles of MT and Zn transporters (Figure 4). It has been shown, for example, that hepatic Zn concentration is closely correlated to hepatic MT, which binds upwards of 70% of the Zn present in the liver.[160] Ongoing research is seeking to examine the characteristics of Zn transporters in this system. However, it is known that hepatic Zn transport in squirrelfish is regulated by 17β-estradiol.[162] This observation is consistent with the presence of multiple estrogen response elements in the promotor region, and the estrogen responsiveness, of the gene for the human Zn importer ZIP6.[163,164] Because this gene is highly conserved through evolution, and present in fish,[165] it is a strong candidate for estrogen-regulated Zn uptake in squirrelfish.

Owing to the clear sex differences in hepatic tissue burden, a reproductive role has been suggested for Zn hyperaccumulation in squirrelfish. During sexual maturation in females a marked increase in hepatic Zn accumulation occurs.[166] Studies of the reproductive cycle of female squirrelfish indicate a systemic shuttling of sequestered Zn from the liver to the ovaries prior to spawning (Figure 4).[167] At least a portion of this hepato-ovarian Zn transfer occurs via the bloodstream where Zn is bound to the hepatically-produced yolk protein vitellogenin (VTG).[167] This redistribution can result in ovarian Zn levels as high as 215 mg kg^{-1}.[167] The exact mechanism by which Zn is taken into the ovaries is not known. One possibility is that there is incorporation of VTG-bound Zn into the oocytes. Alternatively, ovarian Zn uptake may be mediated by ZIP9, which was recently discovered as an androgen-gated Zn importer on the granulosa cells of Atlantic croaker (*Micropogonias undulatus*) ovarian follicles.[168]

A number of roles related to reproduction have been identified for Zn. For example, Zn is required for female gamete development and fertilization in mammals, and sufficient Zn levels are necessary for early mitotic divisions in the mammalian preimplantation embryo.[169, 170] This does not, however, appear to be specific to mammals, as Zn content also increases during oocyte development in zebrafish for example, and the epithelial-mesenchymal transition that occurs during gastrulation is dependent on the presence of the ZIP6 Zn transporter.[171, 172] Likewise it appears the intent of loading Zn into the squirrelfish ovary is to make this Zn available to the developing oocyte. Indeed, the average Zn concentration in eggs produced by captive-bred squirrelfish is 1 668 mg kg^{-1}, upwards of two orders of magnitude greater than that observed in the eggs of other studied teleosts.[162]

Several hypotheses exist regarding the possible function of this hyperaccumulation of Zn in squirrelfish eggs. Elevated levels of Zn (≥ 1 000 mg kg^{-1}) have been shown to be necessary for viability of the squirrelfish egg,[162] thus Zn is proposed to enhance the chances of successful hatching. High concentrations of Zn may also be required by the larvae, for example in the proper development and function of the eye.[173] The tapetum lucidum, a reflective layer of the eye underlying the retina, acts to enhance vision in low light conditions such as those experienced by the nocturnal squirrelfish, and is known to be a Zn-rich tissue.[173] It is noteworthy that the eye is large in larval squirrelfish relative to total body size.

Conclusions and perspectives

The current review covers a wide range of trace elements with often distinct physicochemical properties. Despite this, a number of common themes were discerned.

Studies of metal hyperaccumulation would be aided by measurements of elemental concentrations in environmental media (sediment/soil, water, diet), allowing researchers to identify scenarios of facultative *versus* obligate accumulation. Special care should also be taken to ensure that tissue handling protocols are robust, to eliminate the confounding effects of metal-rich gut contents and metals adhering to body surfaces and mucus.

It is also remarkable that we understand so little regarding the functions of metal hyperaccumulation in these systems. There is a significant need for further research that investigates behavioral consequences of hyperaccumulation (i.e., predator and microbial defence), and the roles of these elements in biology (e.g., oxidative defence and reproduction). However, it is also possible that in many hyperaccumulating species this phenomenon is an ancient evolutionary adaptation to cope with reduced metal bioavailability in a period of Earth's history. This may complicate efforts to link accumulation to function.

In several groups, hyperaccumulation is not element-specific. Some ascidians, molluscs, and polychaetes are able to accumulate multiple metals, although this accumulation may be mutually exclusive. This does, however, indicate that the mechanism of accumulation in these species may be relatively promiscuous. Importantly, there is also evidence that the pathways by which uptake and accumulation occur are conserved, both within accumulators and relative to non-accumulators. This suggests that hyperaccumulating animals may function as model organisms, offering insight into metal handling, thus elucidating processes involved in human disease and wildlife toxicology.

Conflicts of interest

The authors have no conflicts of interest to declare.

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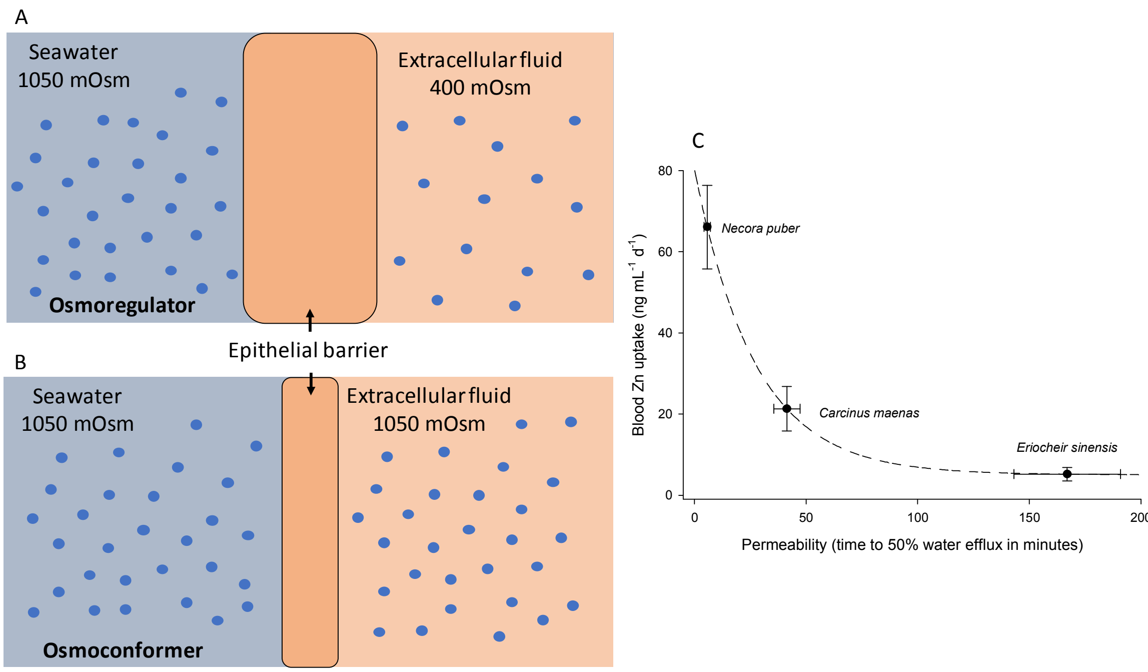
Table 1: Selected examples of trace element hyperaccumulation in animals.

Trace element	Species	Description	Tissue	Concentration mg kg^{-1} (mM)	Reference
As	*Tharyx marioni*	Cirratulid polychaete	Palp	13 048 (174)†	59
	Callopistria floridensis	Insect (moth caterpillar)	Whole body	1 462 (20)	60
Cu	*Loligo opalescens*	Squid (mollusc)	Digestive gland	8 370 (131)	61
	Ostrea edulis	Oyster (mollusc)	Mantle	1 000* (16)[a]	62
	Morone americana	Teleost fish	Liver	1 020* (16)	63
Fe	*Plaxiphora albida*	Chiton (mollusc)	Radula	98 087 (1756)[b]	64
	Nacella concinna	Limpet (mollusc)	Viscera	11 372 (204)[c]	65
	Molgula manhattensis	Ascidian	Tunic	7 588 (136)	66
Ti	*Ascidia aspar*	Ascidian	Blood cell	1 552 (30)	67
V	*Ascidia gemmata*	Ascidian	Blood	17 677 (347)*†	68
	Perkinsiana littoralis	Polychaete fan worm	Branchial crown	10 461 (205)	69
Zn	*Octopus vulgaris*	Octopus (mollusc)	Digestive gland	1 450 (22)	70
	Ostrea sinuata	Oyster (mollusc)	Mantle	4 760 (73)	71
	Holocentrus rufus	Teleost fish	Liver	2 631 (40)*	72

Values are reported as mean values of dry weight concentrations (except: † which indicates maximal or single measured concentration and * which indicates wet weight). a = estimated value from manuscript figure; b = element in biomineralized form; c = value may include radula.

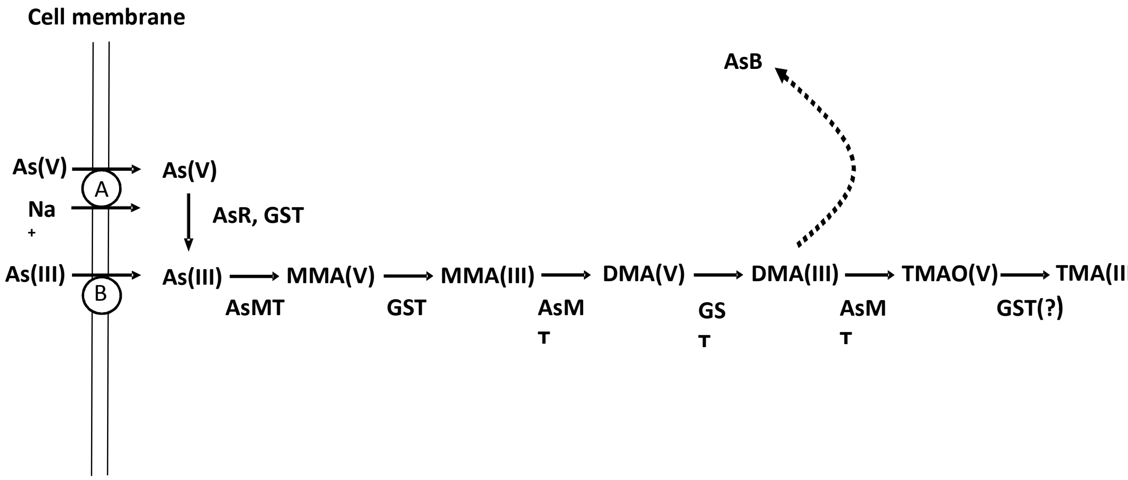
Figures

Figure 1: Diagrammatic representation of barrier importance in a marine osmoregulatory (A) or marine osmoconformer (B), and a demonstration of the relationship between epithelial permeability and Zn uptake in marine crabs in 100% seawater (data from ref. 31).



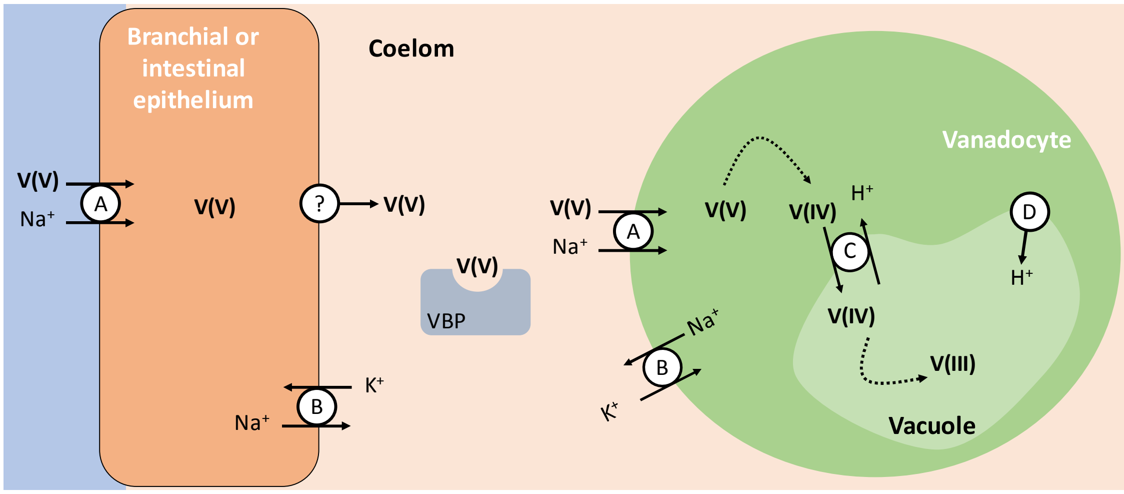
mOsm = milliosmoles per kilogram

Figure 2: Inorganic As uptake and biomethylation pathway.



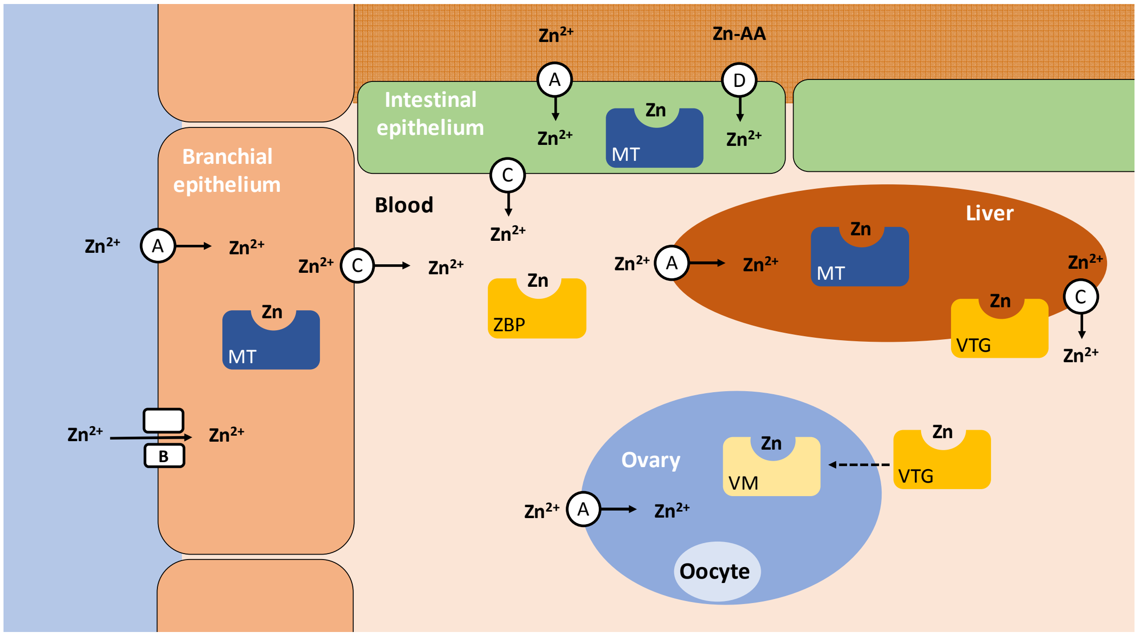
A = phosphate transporter; B = aquaglyceroporin and/or hexose permease; As(V) = arsenate; As(III) = arsenite; MMA(V) = monomethylarsonic acid; MMA(III) = monomethylarsonous acid; DMA(V) = dimethylarsonic acid; DMA(III) = dimethylarsonous acid; TMAO(V) = trimethyl arsine oxide; TMA(III) = trimethyl arsine; AsB = arsenobetaine; AsR = arsenate reductase; GST = glutathione S-transferase; AsMT = arsenite methyltransferase; dotted arrow represents putative biotransformation steps.

Figure 3: Overview of V uptake, transport and storage in ascidians (after ref. 122).

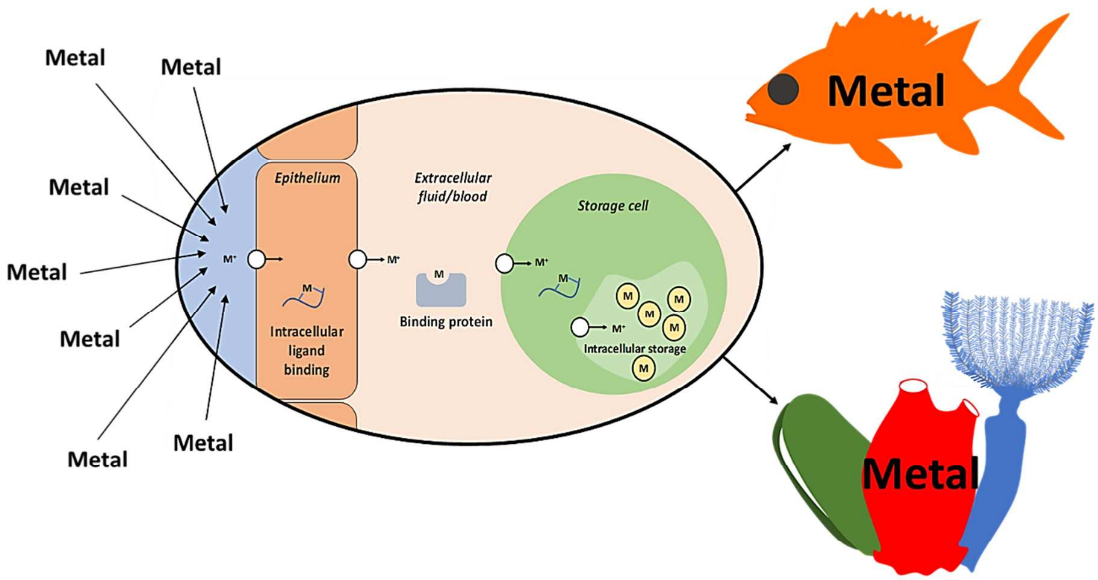


A = phosphate transporter; B = sodium/potassium ATPase; C = divalent metal transporter-1; D = proton ATPase; VBP = vanadium binding protein (e.g. vanadinP of VBP129); ? = unknown transporter; dotted arrows represent reduction steps.

Figure 4: Overview of zinc uptake and systemic zinc transport in female squirrelfish.



A = ZIP transporter; B = calcium channel; C = ZnT transporter; D = amino acid transporter; MT = metallothionein; ZBP = plasma Zn-binding proteins (e.g. albumin); VTG = vitellogenin; VM = vitellogenin metabolite



Facultative trace element hyperaccumulation in animals is reviewed, examining mechanisms of uptake and accumulation, and biological roles.