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

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**From sea squirts to squirreelfish: facultative trace element
hyperaccumulation in animals**

E. David Thompson^{1,*}, Christer Hogstrand², Chris N. Glover^{3,4}

¹ Department of Biological Sciences, Northern Kentucky University, USA

² Faculty of Life Sciences and Medicine, King’s College London, UK

³ Faculty of Science and Technology and Athabasca River Basin Research Institute,
Athabasca University, Canada

⁴ Department of Biological Sciences, University of Alberta, Canada

** Corresponding author at:
SC 245 Nunn Dr
Highland Heights, KY 41099
Email: thompsonel@nku.edu*

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.

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3 40 **Significance to metallomics**
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7 42 This review examines examples of hyperaccumulation in animals, the mechanisms by
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9 43 which this is achieved, the biological roles that have been proposed for this phenomenon, and
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11 44 identifies knowledge gaps requiring further research. The hyperaccumulation of trace metals
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13 45 such as arsenic, copper, iron, titanium, vanadium and zinc in animal models can offer
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15 46 significant insight into human metal handling disorders and the risks associated with
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17 47 environmental metal contamination.
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49 Introduction

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

71 Hyperaccumulation: defined and refined

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73 In the current review, we define hyperaccumulators as species that concentrate
74 elements to levels greater than 1 000 mg kg⁻¹, on either a whole animal or tissue basis. To
75 place this threshold in context, Luoma and Rainbow conducted a literature survey of
76 bioaccumulation in aquatic organisms, including those from contaminated ecosystems, and
77 found that 88% of trace element concentrations fell between 0.1 and 100 mg kg⁻¹.¹⁴ It is
78 important to highlight that our definition of hyperaccumulation is simplified relative to that
79 used by previous authors,¹¹ in that it uses the same threshold for all trace elements, rather
80 than making element-specific distinction. Consistent with some definitions of
81 hyperaccumulation in the plant literature,¹⁵ we largely exclude from discussion those animals
82 in which experimental exposures can result in increased tissue element burdens, and also
83 those animals exposed naturally to extreme environmental contamination scenarios.
84 Consequently, we focus on facultative hyperaccumulators. These are animals that
85 strategically concentrate elements, without evidence of a toxicological impact, and in spite of
86 relatively low environmental concentrations. It is notable that for many of these species the
87 functional role of hyperaccumulation has yet to be discerned.

88 In some studies hyperaccumulation can be a consequence of how animals or tissues
89 are handled for analysis. For example, failure to flush or depurate gut contents can result in
90 artificially elevated tissue burdens by measuring gastrointestinal sediments or prey items that
91 may have elevated metal contents, but which are not accumulated.¹⁶ This will be particularly
92 important where there is an unrecognized environmental enrichment of elements (e.g.
93 contamination and/or metal-enriched geology). An example of this effect is in studies of Fe
94 accumulation in field-collected ascidians, where it was noted that body burdens reduced
95 significantly after animals were held in clean seawater for several days following collection.¹⁷
96 This was attributed to the flushing of Fe-containing particulate matter originating from the
97 point of collection, from the body cavity. The importance of this artifact is underlined by the

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3 98 drop in Fe body burden, which took the animals from a hyperaccumulator status (as high as
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5 99 2600 mg kg⁻¹), to values under this threshold (as low as 900 mg kg⁻¹).¹⁷ A similar effect is
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7 100 likely to explain many of the observations of hyperaccumulation in the sponges.¹⁸ Here, the
8
9 101 confounding factor is not only the presence of abiotic factors such as sediments associated
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11 102 with sponge tissues, but also the accumulation of trace elements associated with symbionts.
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13 103 For example, the observation of elevated molybdenum (Mo) concentrations in the tissue of
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15 104 the sponge *Halichondria phakellioides*, was attributed to a bacterial symbiont, and not the
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17 105 sponge itself.¹⁹ Similarly, elevated arsenic (As) concentrations in giant clams have been
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19 106 attributed to uptake of the element by commensal algae through mimicry of phosphate in low
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21 107 phosphate waters.^{20,21}

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24 108 A related phenomenon influencing hyperaccumulation designation is the
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26 109 consideration of adsorbed (i.e. adhering to the tissue/animal surface) metal, as accumulated
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28 110 burden. For example, a common biological response to metal exposure in aquatic animals is
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30 111 the secretion of mucus.^{22,23} In a laboratory study examining waterborne aluminium (Al)
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32 112 accumulation in freshwater crayfish, mean gill Al concentrations in excess of 1 200 mg kg⁻¹
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34 113 were reported. However, the vast majority of branchial Al was complexed to mucus, and not
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36 114 actually accumulated inside the animal.²⁴ Similarly, the carapace of crustaceans,²⁵ and the
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38 115 cuticle of insects,²⁶ can bind elements such as nickel (Ni) and Fe at levels close to, or greater
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40 116 than, 1 000 mg kg⁻¹. This implies hyperaccumulation even though the elements associated
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42 117 with these tissues are not, technically, absorbed (i.e. taken into the animal).

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45 118 Another issue in collating hyperaccumulation data is inter-individual variation, and
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47 119 the reporting of burden values as a mean. Many authors have noted that tissue accumulation
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49 120 can vary significantly, even between animals of the same species collected at the same time.¹⁷
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51 121 The reasons for variations in individual burdens are not always known, but include factors
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53 122 such as fed state, sex, developmental stage/age, and/or reproductive state.^{17,27,28} The
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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

1
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3 148 ionic and osmotic conformer, has been shown to accumulate Fe and Ni across its skin
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5 149 surface.^{33,34} This may, therefore, be a contributing factor explaining the over-representation
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7 150 of marine osmoconformers among hyperaccumulating animals.
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11 152 **Trace element uptake and the importance of chemical speciation**

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15 154 The key first step necessary for trace element hyperaccumulation is uptake across an
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17 155 epithelial surface. The bioavailability of a given element is dependent upon a number of
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19 156 chemical and biological factors that influence the permeability of the epithelium.¹⁰ Chemical
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21 157 factors include element concentration, chemistry of the medium (water or diet), metal
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23 158 speciation, and size fraction of any particulate element. Biologically, uptake will be
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25 159 determined by fundamental characteristics of the transporting surface, including the cell types
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27 160 present, and biological modification of the organism-environment interface (e.g. mucus, acid-
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29 161 base fluxes), with these themselves varying as a function of life history, developmental stage,
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31 162 fed state and environmental characteristics such as salinity, temperature, and dissolved
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33 163 oxygen.^{34,35}

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35 164 As noted above, trace elements are absorbed via one of three epithelial surfaces in
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37 165 aquatic animals: the epidermis, gill or gut. While these surfaces all possess distinct chemical
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39 166 and biological properties, the fundamental pathways of uptake are conserved. Waterborne
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41 167 trace elements are almost entirely absorbed as the free metal ion, through specific membrane
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43 168 transport pathways.¹ Consequently, water chemistries that favor the formation of anionic
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45 169 trace element complexes decrease bioavailability by reducing free metal ion availability and
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47 170 thus access to the transporter.¹⁰ The transporters that comprise these pathways may be
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49 171 dedicated to the translocation of specific nutrient metals, or may be promiscuous and absorb a
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51 172 number of substrates (e.g. divalent metal transporter; DMT-1).¹ It is worth noting that these
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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

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3 198 reported for metals in the digestive system of molluscs.³⁶ However, there is little evidence for
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5 199 this being an important pathway of metal uptake in the transport epithelia of other
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7 200 hyperaccumulating groups such as polychaetes and ascidians.
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11 202 **Value of studies in hyperaccumulating animals**

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15 204 The study of hyperaccumulating animals has significant utility from both fundamental
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17 205 and applied perspectives. Investigating basic biological function is greatly facilitated by
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19 206 animal models that exhibit extreme phenotypes, an approach in alignment with Krogh's
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21 207 Principle ("For a large number of problems there will be some animal of choice or a few such
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23 208 animals on which it can be most conveniently studied").⁴³ To this end the study of
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25 209 hyperaccumulating animals can offer significant insight into mechanisms of trace element
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27 210 absorption and intracellular handling. There is particular value in studying the mechanisms
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29 211 by which these species can withstand levels of trace metals that would be highly toxic to
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31 212 other species. Hyperaccumulating animals could also find utility as model systems for human
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33 213 conditions associated with metal accumulation, such as Wilson's disease, hemochromatosis,
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35 214 and the myriad of metal accumulation disorders in neuropathological disease.¹³ This utility is
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37 215 conferred by metal transport pathways and cellular handling mechanisms that are largely
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39 216 conserved between humans and animals.¹
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43 217 The use of metal hyperaccumulating animals in the remediation of contaminated
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45 218 environments has been proposed. Two groups of organisms have particular promise in this
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47 219 regard. When cultivated in contaminated waters, bivalve molluscs such as pearl oysters can
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49 220 accumulate trace metals to levels that exceed the 1 000 mg kg⁻¹ threshold.¹¹ The second group
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51 221 are sponges, which have been shown to hyperaccumulate Fe.^{18,44} These animals are of
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53 222 specific interest in that they bring added value as remediators, through the cultivation of
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223 pearls and the extraction of novel bioactive materials, respectively.¹¹ A related concept is the
224 use of hyperaccumulators to extract precious metals. Species that can concentrate valuable
225 elements in tissues from a dilute source, have the potential to make metal extraction
226 economically viable. For practical reasons, bacterial and plant species,⁴⁵ and/or animal waste
227 products (e.g. chicken feathers),⁴⁶ are advantageous over the use of animals in these
228 approaches. However, biomimetic technologies based on animal models are being explored.
229 For example, a compound modelled on tunichrome, a key entity associated with vanadium
230 (V) accumulation in ascidians (see *Vanadium* below), has recently been shown to effectively
231 remove gold (Au) from test effluents.⁴⁷

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

240
241 **Trace elements in facultative hyperaccumulators**

242
243 *Arsenic (As)*

244 Arsenic is a naturally-occurring metalloid element that can be found worldwide at low
245 concentrations in surface water (~20 nM).⁵¹ However, As concentrations can be enriched
246 through anthropogenic processes such as Cu refining, herbicide production, and wood
247 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 $2\,000\text{ mg kg}^{-1}$, with the palps being especially high in As (up to $13\,000\text{ mg kg}^{-1}$)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 $1\,000\text{ mg kg}^{-1}$ 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

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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

298 mg kg^{-1} .^{79,80} In this case there is evidence suggesting that this accumulation protects the
299 insect against predation.⁸¹ This indicates that, in at least some situations, trace element
300 hyperaccumulation in insects that feed on hyperaccumulating plants has a strategic benefit,
301 whether or not that accumulation is strictly facultative in nature.

302

303 *Copper (Cu)*

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

318 There are several groups of aquatic biota that have species which hyperaccumulate
319 Cu. In fish, two known examples have been characterized. In the striped bass (*Morone*
320 *americana*)⁶³ and the mullet (*Mugil cephalus*),⁸⁶ some individuals display hepatic Cu levels
321 as high as 2 440 and 1 936 mg kg^{-1} , respectively, although only for the former species were
322 mean tissue Cu levels greater than 1 000 mg kg^{-1} (1 020 mg kg^{-1} ; Table 1). A high variation

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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. Carpenne 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

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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 (Fe₃O₄), 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.

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420 *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.

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449 *Vanadium (V)*

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

458 The remarkable capacity of ascidians to hyperaccumulate V has been recognized for
459 more than a century.¹¹⁹ The best example of V accumulation is found in *Ascidia gemmata*,
460 which displays blood V concentrations in excess of 17 000 mg kg⁻¹ (Table 1),⁶⁸ representing a
461 bioaccumulation factor (ratio of tissue concentration to seawater concentration) of more than
462 10⁷. Patterns of metal hyperaccumulation in ascidians are phylogenetically aligned, with the
463 sub-order Phlebobranchia accumulating the highest concentrations of V, followed by the
464 Aplousobranchia and Stolidobranchia suborders, the latter of which generally accumulates
465 little V and instead hyperaccumulates Fe (see *Iron*).¹²⁰ There are also differences in the
466 oxidation states of accumulated V in these species, with trivalent V (V^{III}) the primary storage
467 form in cells of phlebobranchs, and tetravalent V (V^{IV}) the main form in aplousobranchs.¹²⁰
468 The highest concentrations of V (often greater than 10 000 mg kg⁻¹) are found in the vacuoles
469 of specific blood cells called signet ring cells (also termed vanadocytes).¹²¹ The protective
470 tunic may also contain high V concentration, although these are usually an order of
471 magnitude lower than those found in vanadocytes.¹²² Intestinal and branchial sac tissues,
472 which likely represent uptake pathways, also display elevated V (~100 mg kg⁻¹).¹²³

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3 473 Interestingly, V is transferred to gametes,¹²⁴ and body V concentrations peak in reproductive
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5 474 season,¹⁰³ suggestive of a role in development akin to that suggested for Zn in squirrelfish
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7 475 (see *Zinc*).

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9 476 The only other group that comes close to matching the V hyperaccumulating capacity
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11 477 of the ascidians, are the polychaetes, in which hyperaccumulation has been noted in a small
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13 478 number of species.^{69,125,126} In polychaetes the branchial crowns display the highest
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15 479 concentrations of V (up to 10 500 mg kg⁻¹ in *Perkinsiana littoralis* Table 1).⁶⁹ Concentrations
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17 480 in the trunk of these species are significantly lower (~50 to 900 mg kg⁻¹),^{69,125} albeit still high
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19 481 relative to species that do not accumulate V. In fact, for most animals tissue levels of V are
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21 482 below the limits of detection for most analytical approaches.

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23 483 The mechanisms by which V is accumulated in ascidians has been the subject of
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25 484 significant investigation (Figure 3).¹²³ Pentavalent V, the dominant oxidation in state in
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27 485 natural waters, shares some physicochemical properties with phosphate, and passage through
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29 486 epithelial phosphate transporters is considered the likely pathway of V uptake.¹²⁷ Vanadium
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31 487 accumulates to high concentrations in epithelial cells of the intestine and branchial sacs, and
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33 488 exits these cells into the blood through an unknown mechanism, thought to be passive owing
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35 489 to the favourable electrochemical gradient.¹²³ The exact oxidation state of V (i.e. tetravalent
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37 490 or pentavalent) at this stage in assimilation is unknown. In the plasma, V is bound by specific
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39 491 high affinity proteins (e.g. vanadinP, VBP-129), capable of sequestering 6 to 13 V atoms per
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41 492 peptide.^{105,128} These proteins shuttle V to the cell membrane of vanadocytes, where uptake is
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43 493 likely achieved by phosphate transporters and/or other unknown transporters.¹²³ In the blood
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45 494 cell cytoplasm, V is present in a tetravalent form, with reduction from the pentavalent form
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47 495 facilitated by vanabins, specific V-binding proteins which act as both chaperones and
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49 496 reductants.¹²⁹ Reductive power is provided by NADPH from the pentose phosphate pathway,
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51 497 which is upregulated in V-accumulating ascidians.¹³⁰ Tetravalent V is then transported into
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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-

523 tunichrome complexes might provide a particular strong outer barrier, and thus serve an
524 important protective role.¹⁰⁴ This may be facilitated by catechol crosslinking, as suggested
525 for Fe in mussel adhesion plaques (see *Iron*).

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

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544 *Zinc (Zn)*

545 Zinc is an essential trace element, necessary for a variety of processes including
546 transcription, enzyme structure and activity, antioxidant defence, maintenance of membrane
547 integrity, and cell signalling.¹⁴¹ Although Zn is a relatively abundant element in the Earth's

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3 548 crust, its concentrations in the oceans are generally low, ranging from 50 pM to 9 nM.⁸³ In
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5 549 natural waters, Zn is divalent and exists as a variety of chemical species depending on pH,
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7 550 chloride ion and dissolved organic matter concentration.^{142,143} In aquatic organisms
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9 551 absorption from dietary Zn is considered more important than that from waterborne Zn with
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11 552 respect to overall Zn uptake.¹⁴⁴⁻¹⁴⁶
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13 553 Homeostasis of body Zn burden is critical for ensuring that toxic effects do not
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15 554 accrue.¹⁴⁷ However, in some molluscs the level at which tissue Zn is maintained qualifies as
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17 555 hyperaccumulation. For example, Miramand and Guary found mean concentrations of 1 450
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19 556 mg Zn kg⁻¹ in the digestive gland of the common octopus (*Octopus vulgaris*) collected along
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21 557 the coast of Monaco (Table 1).⁷⁰ This phenomenon is not geographically unique, as multiple
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23 558 additional studies have indicated similar Zn levels in the digestive gland of this widely-
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25 559 distributed species.^{88,148} However, while the ability of octopus to accumulate high
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27 560 concentrations of Zn is commonly reported, the magnitude of the accumulation does vary.
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29 561 For example, concentrations of digestive gland Zn as high as 14 720 mg kg⁻¹ have been
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31 562 described,⁸⁸ although the reasons for differences in the extent of accumulation are not
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33 563 understood. Zinc hyperaccumulation in cephalopods is not restricted to octopus, but has also
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35 564 been observed in squid and cuttlefish.^{87,149}
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39 565 The localization of Zn to the digestive gland in cephalopods is made possible by the
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41 566 ability of the Zn-binding protein metallothionein (MT) and MT-like proteins to be expressed
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43 567 at high levels in this organ.¹⁵⁰ These sulfur-rich proteins play a number of important roles in
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45 568 normal biological processes by virtue of their ability to bind metals, such as Zn, that are
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47 569 required as enzyme cofactors. The ability to chelate Zn, coupled with their inducibility, also
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49 570 allows MT to effectively reduce the bioreactivity of Zn and minimize toxic effects associated
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51 571 with its accumulation.¹⁵¹ These proteins are the major intracellular regulators of Zn
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53 572 metabolism, and work in association with dedicated Zn transporters of the ZnT/CDF (Slc30)
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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. Carpenne 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

598 among molluscs. The subcellular compartmentalization of Zn into insoluble fractions such as
599 Zn-phosphate granules is also an important means for handling Zn load in bivalves.¹⁵⁸

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

608 Facultative hyperaccumulators of trace elements are most often marine invertebrates.
609 One notable vertebrate exception to this is the squirrelfish family of teleost fish
610 (Holocentridae), where females can accumulate Zn in the liver at concentrations up to 500
611 times higher ($2\ 630\ \text{mg kg}^{-1}$) than those observed in livers of other vertebrates, including
612 male squirrelfish (Table 1).⁷² The Zn accumulation observed in female squirrelfish is
613 independent of diet and occurs in the absence of elevated Zn levels in the environment.¹⁶⁰
614 The difference in accumulation between males and females is partly a consequence of an
615 enhanced capacity of female squirrelfish to absorb Zn across the intestinal epithelium relative
616 to males, with this acquired Zn then being preferentially accumulated in the liver.¹⁶¹ As with
617 molluscs, Zn uptake and sequestration is likely achieved by the roles of MT and Zn
618 transporters (Figure 4). It has been shown, for example, that hepatic Zn concentration is
619 closely correlated to hepatic MT, which binds upwards of 70% of the Zn present in the
620 liver.¹⁶⁰ Ongoing research is seeking to examine the characteristics of Zn transporters in this
621 system. However, it is known that hepatic Zn transport in squirrelfish is regulated by 17β -
622 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 ($\geq 1\,000\text{ mg kg}^{-1}$) 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.

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

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682 **Conflicts of interest**

683
684 The authors have no conflicts of interest to declare.

685
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690
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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	<i>Tharyx marioni</i>	Cirratulid polychaete	Palp	13 048 (174) [†]	59
	<i>Callopietria floridensis</i>	Insect (moth caterpillar)	Whole body	1 462 (20)	60
Cu	<i>Loligo opalescens</i>	Squid (mollusc)	Digestive gland	8 370 (131)	61
	<i>Ostrea edulis</i>	Oyster (mollusc)	Mantle	1 000* (16) ^a	62
	<i>Morone americana</i>	Teleost fish	Liver	1 020* (16)	63
Fe	<i>Plaxiphora albida</i>	Chiton (mollusc)	Radula	98 087 (1756) ^b	64
	<i>Nacella concinna</i>	Limpet (mollusc)	Viscera	11 372 (204) ^c	65
	<i>Molgula manhattensis</i>	Ascidian	Tunic	7 588 (136)	66
Ti	<i>Ascidia aspar</i>	Ascidian	Blood cell	1 552 (30)	67
V	<i>Ascidia gemmata</i>	Ascidian	Blood	17 677 (347)* [†]	68
	<i>Perkinsiana littoralis</i>	Polychaete fan worm	Branchial crown	10 461 (205)	69
Zn	<i>Octopus vulgaris</i>	Octopus (mollusc)	Digestive gland	1 450 (22)	70
	<i>Ostrea sinuata</i>	Oyster (mollusc)	Mantle	4 760 (73)	71
	<i>Holocentrus rufus</i>	Teleost fish	Liver	2 631 (40)*	72

1151

1152 Values are reported as mean values of dry weight concentrations (except: [†] which indicates maximal or single measured concentration and *
1153 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.