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Trace Metals

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In this chapter an overview of sampling and analytical techniques for the marine trace metals (and their stable isotope ratios) is given, focusing largely on the six bio-essential transition metals (iron, manganese, copper, nickel, zinc and cobalt). The aim of this chapter is to introduce the reader to the breadth of techniques and methods currently available to study the biogeochemical cycles of trace metals and their isotopes in the ocean. We note that we do not cover all existing and historical techniques as some are no longer used, some remain immature for trace metal studies, and some are just emerging or are still being developed. A more detailed focus on the methods used by the authors is also provided. We anticipate the continuing development and refinement of methods; as with any expanding and developing scientific field, novel strategies and techniques continuously come and go. For further background reading on marine trace metal distribution and key biogeochemical processes in the ocean, the reader is referred throughout the chapter to appropriate overviews, articles and textbooks available online, including the freely available GEOTRACES electronic atlas and data products, as well as the GEOTRACES 'cookbook'.

Keywords:Marine trace metal biogeochemistry · Marine trace metal sampling · Marine trace metal sample handling · Marine trace metal analysis · Marine metal stable isotope analysis · Marine trace metal speciation analysis

3.1 Introduction

3.1.1 Trace Metals in the Ocean

Seawater comprises all naturally occurring chemical elements including metals (Bruland et al. 2014). Metals are often defined to include the 40 transition metal elements (periodic table: d-block; 31 natural and 9 artificial metal elements), the rare earth elements (lanthanides and actinides, both f-block), the s-block elements except hydrogen (H) and helium (He) (e.g. lithium (Li), magnesium (Mg)) and some elements from the p-block (aluminium (Al), gallium (Ga), indium (In), tin (Sn), thallium (Th), lead (Pb) and bismuth (Bi)). Most metals occur at trace concentrations in the ocean, hence the common terminology 'trace metal'. Six of the transition metals are essential in small quantities for all living organisms both on land and in the sea, needed for maintaining metabolic processes such as carbon uptake, photosynthesis and nitrogen fixation (de Baar et al. 2018; Goldhaber 2003; Sunda 2012; Vraspir and Butler 2009). Listed in order of their typical abundance in the living cells of marine unicellular biota, the bio-essential metals are iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), nickel (Ni) and cobalt (Co) (Bruland et al. 1991; de Baar et al. 2018; Morel et al. 2014). Some biota also appear to utilize cadmium (Cd) under some conditions to substitute for Zn and Co in enzymes such as carbonic anhydrase (Lane and Morel 2000; Lane et al. 2005; Price and Morel 1990). Other trace metals have no known biological function (e.g. Al; (Adams and Chapman

2007) or are entirely toxic (e.g. mercury (Hg) and arsenic (As)) (Hunter 2008; Tercier Waeber et al. 2012). However, it should be recognized that all ‘essential’ elements can be toxic at high concentrations (Shah 2021; Tercier Waeber et al. 2012), and there can be a narrow optimum concentration range, as, for example, seen in the ocean for Cu (e.g. Bruland et al. 1991). In the ocean, the naturally low concentrations of trace metals mean that most are beneficial or quite harmless for biota, but elevated concentrations of some metals due to, for example, anthropogenic environmental pollution can result in localized negative effects (Shah 2021; Tercier Waeber et al. 2012). For instance, the transition metals chromium (Cr), Pb, Ni, Cu, Zn, silver (Ag) and Cd are common industrial by-products and can enter the aquatic system via untreated waste waters, accumulate in sediments and become toxic for benthic organisms (Shah 2021). Fortunately, in many nations most waste waters are nowadays treated to remove pollutants, but in other nations the purification treatments of waste waters have yet to begin.

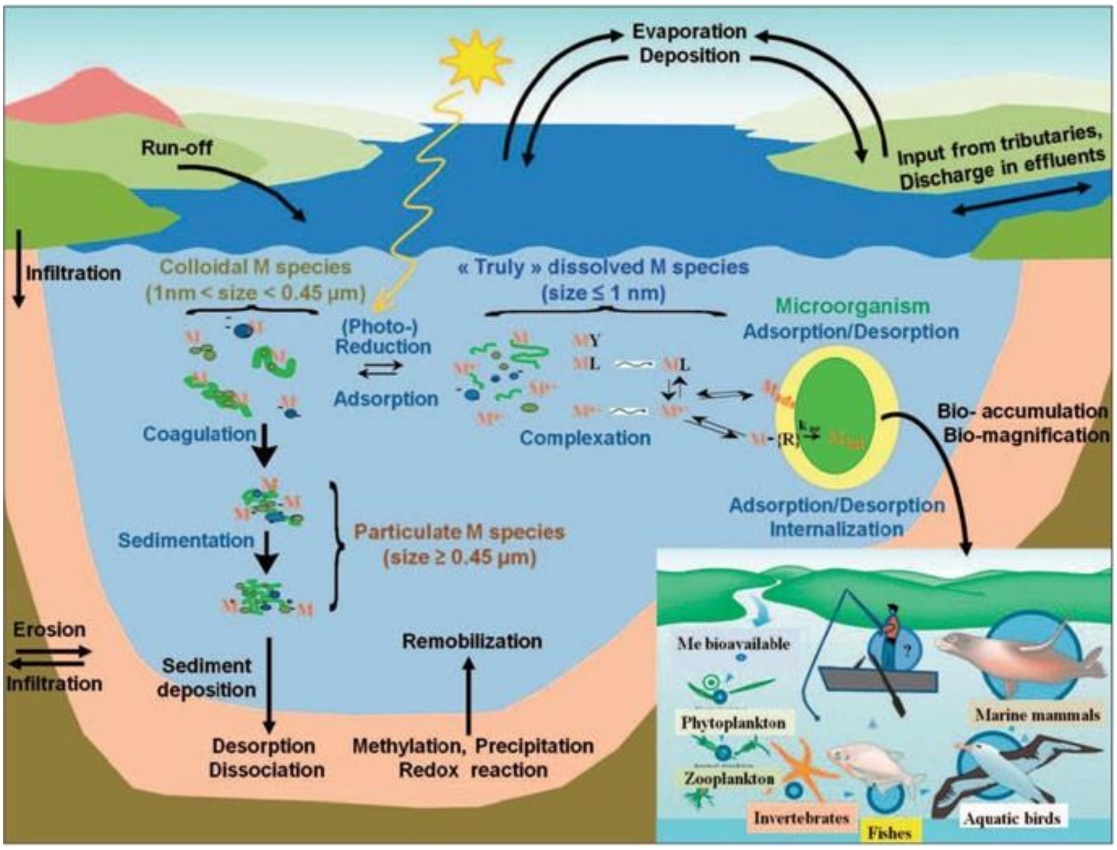

Fig. 3.1 Simplified schematic of trace metal (M) cycles in the marine environment. The conceptual representation includes metal sources, sinks and internal biogeochemical and physical processes that drive metal cycling and fate (Tercier Waeber et al. 2012). Note that the size definition of particulate, dissolved and colloidal trace metals used in the schematic is one of many operationally defined definitions, dependent on the filter sizes used. M^+, free metal ions; ML, organic metal complexes (M complexed with various organic ligands (L)); MY, inorganic metal complexes

The growth of phytoplankton, the organisms which form the base of the marine food web, depends on the availability of both sunlight and nutrients in the upper water column. Nutrients include carbon and the ‘macro’-nutrients such as nitrogen, phosphorus and silicate but also include so called ‘micro’-nutrients, i.e. the six transition metals mentioned above, of which Fe (Fig. 3.1) is arguably the most important (Bruland et al. 2014). A scarcity of one or several of these trace metals in surface waters can limit overall primary productivity and change the community structure and functioning of marine ecosystems. Micronutrient limitation is especially acute in some remote ocean environments, commonly referred to as High Nutrient, Low Chlorophyll (HNLC) regions, where upwelling of deep water has provided ample macronutrients for phytoplankton, but a paucity of micronutrients such as Fe means that macronutrients cannot be fully utilized, and as a result growth is lower than one may expect (Moore et al. 2013). However, the role ofmicronutrients such as Fe is not limited simply to remote HNLC regions, as Fe has also been shown to limit or co-limit phytoplankton growth elsewhere in the ocean (e.g. Achterberg et al. 2013; Le Moigne et al. 2014; Mills et al. 2004; Moore et al. 2009; Moore et al. 2006; Nielsdóttir et al. 2009; Ryan-Keogh et al. 2013).

The importance of Fe for influencing marine ecosystem productivity and global carbon cycling, in particular, has long been recognized. Varying Fe input into the ocean over time is even thought to have played an important role in driving the dramatic and regular sawtooth shifts in atmospheric carbon dioxide during the recent glacial-interglacial cycles of our planet (Martin et al. 1990; Yamamoto et al. 2019). The first reliable experimental evidence for Fe limitation was by Fe addition experiments (bioassays) in surface water samples from the sub-Arctic North Pacific Ocean in August 1987 (Martin and Gordon 1988). One year later, in austral summer 1988, Fe limitation was demonstrated in bioassays in the Southern Ocean (Buma et al. 1991; de Baar et al. 1990), and a few years later the first in situ Fe fertilization experiment was done (Martin et al. 1994). Ever since these experiments, the investigation of the bio-limiting role of Fe has become one of the major research topics in ocean plankton ecology. Nevertheless, due to the very stringent requirements to rule out inadvertent Fe contamination (see Sect. 3.2), Fe limitation remains a challenging line of plankton research.

Ocean productivity and the biogeochemical cycles of the ocean are also shaped by the availability of other trace metals, co-limitation by two or more factors (e.g. light and Fe) and/or variability in nutrient requirements between species and environmental conditions (e.g. Arrigo 2005; Buma et al. 1991; de Baar et al. 1990; Morel et al. 2014; Saito et al. 2008). For instance, Fe together with other bio-essential trace metals (Mn, Co, Ni, Cu, Zn) can influence the taxonomic composition of key ecological communities with wide reaching influences on overall marine primary productivity (Boyd et al. 2017; Hutchins and Boyd 2016; Moore et al. 2013; Twining and Baines 2013).

Besides being essential micronutrients, trace metals and other minor elements can serve as useful tracers of human activity (e.g. radioactive tracers) or of the physical, geological and chemical processes that shape biogeochemical cycles in the oceanic water column and in the sediment on the sea floor below (Anderson 2020). Notably, isotope ratios of trace metals can provide information of internal oceanic processes (biological, scavenging and redox cycling) and external sources and sinks of metals to/from the ocean (Conway et al. 2021). Briefly, biochemical and geochemical reactions can lead to small, but measurable, mass-dependent fractionation of the isotope ratio of a certain element (e.g. Fe; Dauphas et al. 2017). Each fractionation process can lead to distinctly different isotope ratio signatures that can be used to ‘fingerprint’ processes, sources and sinks of certain elements in the ocean interior. Accordingly, the use of isotope ratios of trace metals such as Fe, Zn, Cd, Ni, Cu and Cr has exploded in the last decade as powerful tracers of oceanic trace metal cycling, fluxes and transport (Conway et al. 2021). In addition to modern water column measurements, trace metals or trace metal isotope ratios preserved in geologic archives also provide valuable information about the past ocean. For a recent comprehensive summary of the state of the field for using trace metals and their isotopes as proxies for past ocean productivity and other processes, see Horner et al. (2021).

3.1.2 Trace Metal Concentrations and Distributions

Concentrations of dissolved trace metals in seawater range from sub fmol kg^{-1} at the lower end to 10 μmol kg^{-1} at the upper end (μ (micro) ¼ 10^{-6}, n (nano) ¼ 10^{-9}, p (pico) ¼ 10^{-12}, and f (femto) ¼ 10^{-15}), where the concentration of 10 μmol kg^{-1} is an arbitrary upper value typically used to define the separation of trace elements from the major elements in seawater (Bruland et al. 2014; de Baar et al. 2018). Concentrations in seawater are often reported in units of mol kg^{-1} (e.g. nmol kg^{-1}), or mol L^{-1} (e.g. nmol L^{-1} which is the same as nM), where mol kg^{-1} units are generally recommended. Reporting in mol per mass units is recommended because mass, unlike volume, is unaffected by temperature- and/or pressure-derived changes in the density of a sample that can occur during or after collection. Volume can be affected by reasonably large changes in pressure and/or temperature between sample collection depth and later laboratory analysis; as such, the reported values in mol L^{-1} may differ (slightly) between the environmental conditions at which the sample was collected and those of analysis. Nevertheless, when analyses (see Sect. 3.5) are based on volume rather than mass, it can be argued the results should be reported in the units they were obtained in (e.g. L^{-1} in case of volume-based measurements) with the required information for any subsequent conversion (e.g. to nmol kg^{-1}) in the metadata (see Sect. 3.6) as a variety of different units is possible and use often depends on traditions within (sub-)disciplines.

Seawater contains a complex 'soup' of truly dissolved metal ion species, organic-complexed metals and small particles, the sum of which, for ease, is commonly referred to as 'dissolved'. Dissolved metals are thus typically operationally defined as any metal species that has passed through a filter, usually 0.4 or 0.2 μm in pore size (Fig. 3.1), with the latter becoming the common standard in recent decades (Cutter et al. 2017). Smaller filtration sizes and techniques (i.e. ultra-filtration; see Sect. 3.4.2 for more information) are sometimes also employed to look at concentrations of different fractions of the 'dissolved' pool of trace metals in seawater (e.g. Fitzsimmons et al. 2015b; Homoky et al. 2021), for example, colloids (~0.02–0.4 μm in size; see Fig. 3.1).

The distributions of the different trace metals in the ocean are each controlled by a different combination of biological, chemical and physical processes that lead both to different spatial and temporal patterns for each metal and to distinct relationships between the distributions of different trace and major elements (e.g. Bruland et al. 2014; de Baar et al. 2018). Ultimately, trace metals are added to the ocean from external boundary 'sources' such as rivers, margin and deep-sea sediments, wind-blown dust from continents (especially deserts), anthropogenic pollution and from venting from submarine hydrothermal activity (Anderson et al. 2014; de Baar et al. 2018). Similarly, trace metals are removed from the ocean by so-called sinks, which also occur at ocean boundaries and thus are often at or near the same locations as sources (e.g. marine sediments, hydrothermal vent deposits) (Bruland et al. 2014; de Baar et al. 2018). Within the ocean, active uptake by biota and passive particle scavenging act as internal sinks of trace metals. Trace metals associated with particulate matter can also be released back into the dissolved phase via bacteria- induced degradation, grazing or abiotic dissolution mechanisms, either in shallow surface waters or deeper in the water column or by respiration processes in the underlying sediment (e.g. Anderson et al. 2014). As such, particles can also act as internal ocean sources for trace metals. Generally, for most trace metals, their 'final' sink from the ocean is permanent burial in marine sediments (e.g. Bruland et al. 2014; de Baar et al. 2018).

The combination of specific sources, sinks and internal cycling that is unique to each trace metal then merges with the general physical ocean circulation, resulting in characteristic oceanographic dissolved distributions of trace metals that reflect their intrinsic biogeochemical behaviour and chemistry (Aparicio-González et al. 2012; Bruland et al. 2014; de Baar et al. 2018). Historically, dissolved trace metals have been broadly grouped into several definitions: (1) conservative, trace metals with a relatively narrow concentration range that varies in concert with salinity; (2) nutrient type, trace metals that are taken up by phytoplankton in the surface ocean and are regenerated with depth, leading to depleted surface concentrations and elevated deep concentrations; (3) scavenged, trace metals with strong particle interactions that are removed from the ocean; or (4) hybrid, trace metals which do not fit into a single distribution type (Aparicio-González et al. 2012; Bruland et al. 2014). In fact, as more becomes known, most dissolved trace metals exhibit aspects of some or all of these distributions.

3.1.3 Pioneering Marine Trace Metal Biogeochemistry

The overall very low concentrations of (dissolved and particulate) trace metals in the ocean meant that insight into their distributions and roles in the ocean was hindered until clean sampling and analytical techniques started to become available in the 1970s (see Sect. 3.2, Box 3.1) (Bruland and Lohan 2003; Protti 2001). More recently, in 2006, the international GEOTRACES programme that aims to identify the processes and quantify the fluxes that control the distributions of key trace elements and isotopes in the ocean has made major leaps in assessing the concentration, distribution and biogeochemical cycling of Fe and other trace metals in the global ocean (Anderson 2020; Henderson et al. 2018). A key focus point of GEOTRACES is the inter-comparability of methods and data quality between the

~36 countries involved (GEOTRACES-Group 2006), making sure that sampling efforts and measurements by different nations and laboratories give comparable results that can be combined into data products (Mawji et al. 2015). Prior to the inception of the GEOTRACES programme in the early 2000s, data was often not inter-comparable, owing to the difficulty of trace metal sampling without contamination and analysis using instruments with suitable enough resolution to quantify low trace metal concentrations (Johnson et al. 2007). Indeed, such challenges had meant that before the initiation of the

GEOTRACES field programme, dissolved Fe had been measured at only 25 locations worldwide down to a depth of 2000 m depth (Anderson et al. 2014; Anderson 2020; GEOTRACES-Group 2006). Currently, hundreds of full-depth profiles of various trace metals, including Fe, can be found in the GEOTRACES inventory database, and this number is quickly expanding (Schlitzer et al. 2018; Anderson 2020). For example, GEOTRACES has now facilitated ocean transects of Fe in all ocean basins, illuminating both the external sources/sinks and internal cycling of Fe and building on previous understanding (e.g. Abadie et al. 2017; Conway and John 2014b; Ellwood et al. 2018; Fitzsimmons et al. 2015b; Klunder et al. 2012; Klunder et al. 2011; Moffett and German 2020; Nishioka et al. 2020; Rijkenberg et al. 2014; Saito et al. 2013; Tagliabue et al. 2017; Tonnard et al. 2020). However, despite this explosive increase in oceanic trace metal data and a number of decades of high-quality insights, scientists are still working to understand the details of the processes and factors that drive the distributions of many trace metals in the ocean, as well as the interactions between micronutrients and marine microbes that drive marine productivity and marine carbon sequestration. Consequently, it is still very challenging for scientists to make accurate predictions about the role of trace metals in influencing climate change in the past, present and future ocean.

Box 3.1: Technical Advances and Trace Metal Clean Techniques

The importance of trace metal clean techniques together with technical advances in analytical methods and instrumentation for trace metal chemistry is very nicely illustrated by the ‘lure of gold story’ of the mid-nineteenth century (Pilson 2012). The text below retells the story that was published by Pilson (2012).

Gold gained much attention in 1872 when it was announced that the waters of the English Channel contained 65 mg of gold in each ton of water (Sonstadt 1872). At the time, the value of gold in one ton of water was only about 6.5 cents, much lower than today’s value. However, despite this, as well as a later assessment by Svante Arrhenius (~1900) that reported only 6 mg per ton in the English Channel (the equivalent of 0.6 cents per ton (Jensen et al. 2020; Riley et al. 1965)), societal interest in extracting gold in seawater persisted. The vastness of the ocean opened up the possibility of immense wealth even at the lowest estimates of 6 mg of gold per ton of water. Indeed, it was thought that if extraction of gold from the ocean was possible, such an endeavour would generate enough wealth to make every living person on Earth a millionaire twice over. Thus, in the first four decades of the twentieth century, patent after patent was issued at patent and trademark offices around the world for gold extraction methods and techniques from seawater. Fritz Haber, a Nobel prize laureate, was one of the many researchers that jumped onto the bandwagon of developing oceanic gold extraction methods. His interest was sparked by the promise to help pay off the German World War I debt. However, with his newly developed procedure, Haber found gold concentrations of only 0.004 mg per ton seawater (20 picomoles per kg). Back then, this was only worth about 0.004 cents per ton. This finding crushed any hopes and prospects of ever extracting gold from the sea economically. The Dow Chemical Company further illustrated the uneconomic nature during the 1950s when processing nearshore seawater to extract magnesium and bromine and also investigating the extraction of gold as a side project. The company processed 15 tons of seawater, but extracted only 0.09 mg of gold with an estimated value of 0.01 cent. This value stood in stark contrast to the exorbitant cost of ~ $50,000 that the Dow Chemical had spent on the extraction process. A more recent study quantified a concentration of about 50–200 femtomoles gold per kg seawater, a level two orders of magnitude less than was accepted decades earlier (Falkner and Edmond 1990). In fact, Falkner and Edmond (1990) deduced that the relatively 'large' amount of gold extracted by Dow Chemical was probably caused by 'unclean' reagents and containers that provided the bulk of the gold collected. Using modern values, we can now estimate that the entire world's ocean contains 'only' 14,000 tons of gold, five times less than the world gold holdings in central banks in early 2021 (69,400 tons) as published by the World Gold Council.

3.1.4 Future Challenges in Marine Trace Metal Biogeochemistry

Currently, the global marine Fe cycle, and those of other trace metals, is undergoing major changes because of ocean acidification, stratification, warming, deoxygenation, anthropogenic pollution and land use change, amongst other factors (Hutchins and Boyd 2016; Tagliabue et al. 2017). These changes raise questions about how future change will affect marine ecosystems, marine primary productivity and carbon uptake by the ocean, underlining the importance of studying the biogeochemical cycle of bio-essential metals such as Fe in the past and present ocean. Here, global ocean biogeochemical models come into play, since they are important tools that aid in our understanding of the impacts of future change and test hypotheses regarding biogeochemical processes (Tagliabue et al. 2016). The current generation of models do a reasonable job when it comes to macronutrients such as phosphate, but as of yet, they do a much poorer job of reproducing oceanic micronutrient distributions and as such vary widely in their predictions, especially for Fe (Tagliabue et al. 2016). The poorer predictive power of models for Fe in the ocean than for the macronutrients results largely from (i) the complex biogeochemistry of Fe, (ii) the short residence time of Fe and (iii) the insufficiently constrained sinks and sources (Tagliabue et al. 2016; Tagliabue et al. 2017). This uncertainty in the marine Fe cycle, given its known importance for global carbon cycling, raises large challenges for climate and earth system modelers and thus creates hurdles for civil society and stakeholders to evaluate and implement appropriate climate change actions and policy. The distributions of trace metals such as Ni, Zn and Cd are perhaps easier to incorporate into models because their behaviour is more predictable when considering the ocean from a three-dimensional perspective in which both ocean circulation and the biological pump play an important role (e.g. Middag et al. 2020; Tagliabue et al. 2016; Vance et al. 2017; Weber et al. 2018). However, the interaction of these other bio-essential trace metals and their effect on marine ecosystem functionality and biogeochemical fate are only just emerging, which undoubtedly will reveal currently unknown roles or feedback loops in the future. With more interdisciplinary science, more data on spatial and temporal variability and more ocean observations using new technologies and methods, trace metal scientists will most probably gain more insight into the fate and behaviour of trace metals and their isotopes in the ocean. Such knowledge will be essential for providing a holistic understanding of the processes that are essential to achieve a safe, sustainable, clean, healthy and predictable ocean, as is the goal of the UN Decade of Ocean Science for Sustainable Development.

3.2 Trace Metal Clean Procedures

In the early 1970s, technical advances in analytical chemistry and instrumentation with high sensitivities and low detection limits (Bruland et al. 2014; Protti 2001) paved the way for marine chemists to improve their understanding of concentrations, distributions, speciation (i.e. the chemical form of an element) and associated biogeochemical behaviours of trace metals in the marine environment (Bruland and Lohan 2003). However, the extremely low concentrations of many trace metals in natural seawater – commonly in the nanomolar range and often lower than the detection limits of common analytical techniques – together with the omnipresence of such metals in the terrestrial environment (Bruland and Lohan 2003; EPA 1996; Protti 2001; Richter 2003; Sander et al. 2009) complicated contamination-free analysis of trace metal samples both on ships and in the laboratory. Consequently, along with the development of powerful analytical techniques came the recognition and appreciation of the importance of using rigorous trace metal clean techniques in all stages of sampling and laboratory work (sample collection, storage, handling, treatment and analysis) to avoid the inadvertent introduction of contamination (Bruland et al. 1979, 2003; Cutter et al. 2017; EPA 1996; Sander et al. 2009).

The concept of trace metal clean laboratories for environmental analyses (Patterson et al. 1976) was pioneered by Patterson after he recognized that measurements of environmental Pb concentrations were often too high because of the often inadvertent introduction of Pb to the samples during sample collection, handling and storage (Patterson 1965). This realization resulted in Patterson's development of rigorous trace metal clean protocols for the elemental analyses of environmental samples in the early 1970s (EPA 1996; Patterson et al. 1976). This introduction of trace metal clean protocols led to the first oceanographically reliable and consistent trace metal data in the late 1970s with the first dissolved Cd and Zn data published between 1976 and 1978, respectively (Boyle et al. 1976; Bruland et al. 1978; Martin et al. 1976). Concurrently, the Geochemical Ocean Sections Studies (GEOSECS) programme (1972–1978) was conducting global surveys designed to investigate the three-dimensional distributions of chemical, isotopic and radiochemical tracers in the ocean (Chester 1990) (see Box 3.2). However, the GEOSECS sampling system did not permit uncontaminated samples to be collected for certain easily contaminated trace metals such as Zn, Fe and Pb

(Bruland and Franks 1983; Pilson 2012). Thus, the first reliable vertical profiles of dissolved Fe were only published in 1981 (Landing and Bruland 1981). By the 1980s, thanks to analytical advances in trace metal chemistry and the use of trace metal clean techniques, two notions were adopted: (i) seawater concentrations of many trace elements are a factor of 10–1000 lower than previously believed (e.g. compare Brewer 1975 and Bruland and Franks 1983), and (ii) trace metals have well-defined distributions in the world’s ocean that relate to physical, chemical and biological features of the water column (Chester 1990; Pilson 2012), i.e. trace metal distributions are ‘oceanographically consistent’. For instance, in the 1920s, Fe in seawater was said to be 1–25 µmol L^{-1} followed by values of 20 nmol L^{-1} –5.3 µmol L^{-1}in the 1930s and values of 60 nmol L^{-1} –0.1 µmol L^{-1} in the 1950s (de Baar 1994 and references therein), whereas today, values of <2 nmol L^{-1} in surface waters are the consensus (e.g. Anderson 2020; Schlitzer et al. 2018 and references therein).

Box 3.2: Evolution of Trace Metal Clean Procedures

Preventing water samples from becoming contaminated during sampling and analytical processing constitutes one of the greatest difficulties encountered in marine trace metal analysis. Therefore, it is imperative that extreme care is taken to avoid contamination when collecting and analysing samples for trace metals. Before the 1970s, marine chemists did not commonly follow rigorous trace metal clean procedures, did not have clean materials and equipment, and did not pay enough attention to sample handling in order to avoid the inadvertent introduction of contamination (Chester 1990). However, even with the gradual introduction of trace metal clean protocols from the late 1970s onwards and with care being taken during sample collection and analysis, samples taken before the GEOTRACES programme, i.e. before 2006, were scarce and sometimes still contaminated and inconsistent with oceanographic features.

For example, the GEOSECS programme was the first major ocean programme to generate geochemical ocean sections in all three major ocean basins in the years 1972–1978. While focusing on the ocean carbon cycle, samples were also collected for several other tracers, that is, various isotopes and trace elements. At the time, the GEOSECS vertical oceanic profiles of Fe and Ni (orange profiles below) were deemed to be major breakthroughs and were considered to be some of the very first reliable vertical profile datasets (Fig. 3.2), with values much lower than previously thought (e.g. Bruland 1983). However, later work has shown the profiles for these elements overestimated concentrations and were too variable (see below), likely due to contamination and the limits of techniques available.

However, GEOSECS was the role model for GEOTRACES that started its ocean sections campaign between 2007 and 2008 with GEOTRACES expeditions that were part of the International Polar Year. The GEOTRACES vertical profiles shown here (blue profiles; Fig. 3.2) were collected in 2011 and 2017, some 35 years after the also shown GEOSECS data. These improved profiles (lower, more accurate concentrations, which are also oceanographically consistent) illustrate the significant advances made in both the collection of samples and measurement of trace metal concentrations since GEOSECS.

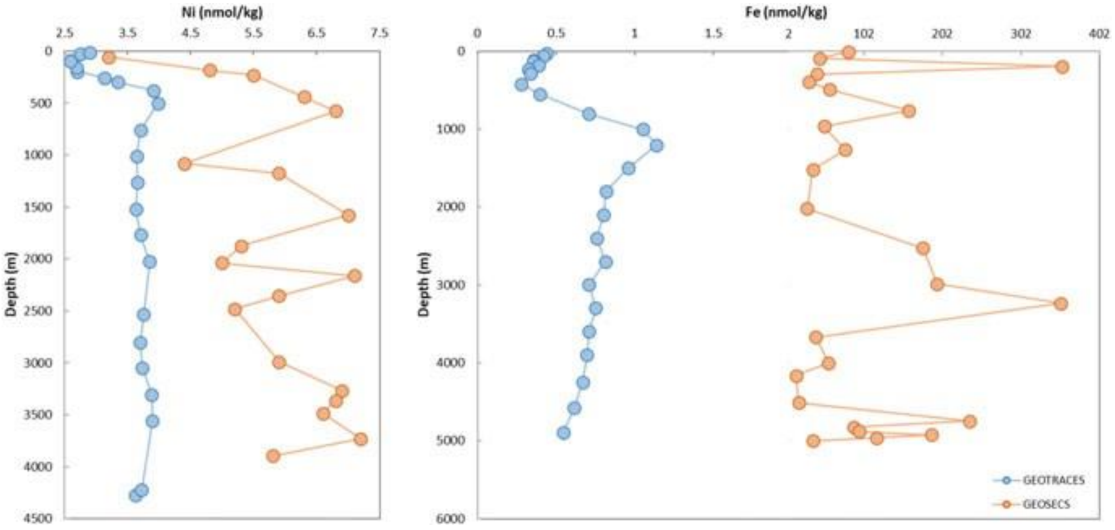

Fig. 3.2 Comparison of Ni and Fe profiles collected and measured during GEOSECS in the 1970s and GEOTRACES in 2011 and 2017. Displayed GEOSECS and GEOTRACES stations were located in the same ocean basins (Ni and Fe stations are 118 km and 137 km apart, respectively). These data show how endeavours such as the GEOTRACES programme added much to the ability of marine chemists to collect reliable and oceanographically consistent trace metal data. GEOSECS data for Ni (Station 3; North Atlantic) and Fe (Sargasso Sea) are from Sclater et al. (1976) and Brewer et al. (1972), respectively. GEOTRACES data for Ni are from GA02 station 5 (North Atlantic; Middag et al. 2020) and data for Fe are from GA03 station 8 (North Atlantic; Schlitzer et al. 2018; data originates from various investigators). Please note the change in scale for the x-axis (Fe concentration) for the Fe profiles

While apparently ‘clean’ to the regular laboratory scientist, the exceptionally low levels of trace metals in seawater mean that many common laboratory procedures or equipment (e.g. glassware) have the potential to contaminate samples beyond the point where the ‘true’ trace metal concentrations can be established. There are numerous pathways by which samples may become contaminated, and potential sources include metallic or metal-containing labware or gloves (e.g. talc gloves that contain high levels of Zn), containers, sampling equipment (improperly cleaned and stored), chemicals, reagents, reagent water and atmospheric inputs (EPA 1997). Additionally, human contact (hair, dead skin, exhalation) and dust particles in a laboratory can be a significant source of trace metal contamination to samples (EPA 1997). In the following sections, we describe the procedures required to make ultraclean trace metal measurements in the most pristine marine environments in order to produce reliable, accurate and reproducible trace metal data. The section below, however, only gives a general overview of trace metal clean concepts and principles, and various laboratories have developed slightly different procedures depending on their research objective and traditions. For more details on trace metal clean techniques, the reader is also referred to the GEOTRACES Cookbook (Cutter et al. 2017).

3.2.1 Trace Metal Clean Environment

A clean laboratory atmosphere, in which the contact of the sample with particles in the air and other surfaces is minimized, is one of the key features of trace metal clean practices. Such a clean atmosphere is ideally provided by a clean laboratory, which consists of one or more rooms that are kept under positive pressure by drawing air through a series of filters that effectively remove particles, including ultrafine particles (<0.5 µm), from the atmosphere (Sander et al. 2009). To be classed as a trace metal clean working space, the working space must comply with the class 100 standard by the Federal Standard 209 or the equivalent ISO 5 class standard by ISO-14644-1 (Fig. 3.3a) (Cutter et al. 2017; Goldberg 1996). Cleanrooms or working spaces that fall under these two standards implement high-efficiency particulate air (HEPA) filter systems to obtain a permissible density of less than 3500 (<0.5 µm) dust particles per m^3 of air (Tovar-Sánchez 2012). Ambient outdoor air in a typical urban area contains 35,000,000 particles (> 0.5 µm) per m^3 of air, and ordinary activities performed by people generate millions of particles every minute (Goldberg 1996; Nehme 2020). For instance, the rate of particle emission during normal human speech ranges from 1 to 50 particles per second, which equates to 60,000–3,000,000 particles per m^3 (Asadi et al. 2019). If full clean room facilities are not available or needed, a clean working atmosphere should be provided via ISO class 5 or class 100 laminar flow benches, or a non-metal glove box fed by particle-free air or nitrogen (EPA 1997). Care must be taken to avoid metallic components such as screws to mount screens of laminar flow benches, as they will cause contamination (Sander et al. 2009).

Onboard ships, when samples are collected and prepared for storage or onboard analysis, full clean room facilities are not usually available, even though a shipboard environment is more prone to cause contamination (Fe ship, metallic structure, Zn anodes, paint, engine exhaust, waste water, etc.) than a land-based facility (Gillain et al. 1982; Sander et al. 2009; Tovar-Sánchez 2012). When permanent clean room facilities are not available on a ship, converted shipping containers commonly act as designated mobile clean rooms on research expeditions. Such a mobile laboratory is fitted with a HEPA air filtration system and laminar flow benches to comply with clean room standards (Fig. 3.3b). If such a mobile laboratory is unavailable, a standard shipboard laboratory can be converted into a temporary clean room, commonly referred to as a ‘bubble’. A bubble consists of a polyvinylchloride (PVC) plumbing tubing structure (or similar material) covered by plastic film that is also used for lining the walls and benches (Fig. 3.3c). To accord with clean room regulations, clean air is usually provided via HEPA filtered air that also keeps the working environment inside the bubble over-pressured (Sander et al. 2009).

Fig. 3.3 Photographs of examples of trace metal clean facilities. Photo of a land-based ISO-6 clean room

laboratory with ISO-5 laminar flow hoods (a) (photograph credit: Tim Conway, University of Florida, USA), the NIOZ trace metal clean container (b) (photograph credit: Loes Gerringa, NIOZ, Netherlands) and a trace metal clean working 'bubble' on board a research vessel (c) (photograph credit: Gert van Dijken, Stanford University, USA)

3.2.2 Trace Metal Clean Practices

Apart from the atmosphere, the second most likely source of contamination for samples comes from the human investigator (see Box 3.3). To minimize and avoid this source of contamination, a strict trace metal clean working procedure must be followed during all phases of sampling and laboratory work. It is recommended that protective clothing is worn in all laboratory operations since humans are the main contamination risk in cleanrooms, particularly through the shedding of particles from personal clothing, exacerbated by movement (Goldberg 1996). A study with test subjects that wore cotton tracksuits vs cleanroom uniforms in an ISO 5 cleanroom showed that test subjects wearing tracksuits shed on average 34,955,780 particles (>0.5 µm) per minute while walking, while test subjects in full cleanroom attire shed on average only 106,328 particles (>0.5 µm) per minute while walking (Cleanroom-Technology 2011). The clean room uniform commonly comprises a clean room coverall, disposable plastic gloves (powder-free), a hair net, dedicated plastic shoes or plastic foot covers and eye protection (e.g. EPA 1997; EPA 1996; Sander et al. 2009; Tovar-Sánchez 2012). Often, two pairs of gloves are used by the human analyst, one for ultraclean handling and one for clean (i.e. dirtier) handling. If it is even suspected that gloves have become contaminated, work must be halted, the contaminated gloves removed and a new pair of clean gloves put on (EPA 1997; EPA 1996). In addition, all surfaces that equipment, samples, reagents and standards come into contact with are potential sources of contamination, and thus all equipment and work surfaces should be wiped with a lint-free cloth prior to use, or at least on a regular basis, to remove dust. All apparatuses and laboratory equipment used for trace metal work must be non-metallic, and glass materials and coloured plastics should be avoided (EPA 1997; EPA 1996; Tovar-Sánchez 2012). When not being used, laboratory equipment should be covered with clean plastic wrap, stored in a clean bench or plastic box or bagged in clean polyethylene bags (colourless zip-type bags are recommended) (EPA 1997; Tovar-Sánchez 2012).

Box 3.3: Trace Metal Clean Practices

The key requirement for reliable and contamination-free trace metal data is compliance with trace metal clean practices throughout the entire process, from equipment preparation all the way through to sample collection and eventual analysis (Sander et al. 2009). Two of the most important factors in avoiding and minimizing sample contamination are (1) an awareness of potential sources of contamination including the position of the investigator's arms and hands relative to the airflow, open samples and reagents and the flow and direction of the investigators breathing and (2) strict attention to work being done (EPA 1996; EPA 1997). Therefore, it is imperative that trace metal clean procedures are carried out by well-trained and experienced personnel (EPA 1997; Tovar-Sánchez 2012).

3.2.3 Trace Metal Clean Sample Bottles

Appropriate container material for sample storage is also key in trace metal chemistry, notably when considering the general long contact times (days, weeks, months or years) between seawater sample and container wall. Two opposing aspects are important here: (1) sample contamination by different kinds of container materials and (2) trace metal losses by surface adsorption to the container wall (Gillain et al. 1982). Both processes are dependent on the surface to volume ratio and the sample bottle material (Jensen et al. 2020). Generally, samples for dissolved trace metal analysis are acidified for storage to avoid 'wall adsorption' of metals and thus undermeasurement of the 'true' concentration of a metal of interest in the sample (Cutter et al. 2017; EPA 1997). Both fluoropolymers (specifically fluorinated ethylene propylene (FEP), perfluoroalkoxy alkane (PFA) or polytetrafluorethylene (PTFE)) and low-density polyethylene (LDPE) bottles are used for acidified sample storage owing to their low intrinsic trace metal composition and low levels of metal adsorption (Cutter et al. 2017; Noble et al. 2020). High-density polyethylene (HPDE) bottles have been shown to contain organometallic trialkyl aluminium (Al) compounds and are thus deemed unsuitable for dissolved Al analysis Cutter et al. 2017, but can be used for most other metals. Generally, LDPE bottles are recommended for sample storage of samples reserved for dissolved and particulate trace metal analysis as well as for speciation and isotope analysis. Fluoropolymer bottles are chemically and thermally more resistant compared to LDPE bottles and are also deemed ‘cleaner’ due to their commonly lower metal blanks (e.g. Gasparon 1998; Noble et al. 2020 and references therein). However, the high cost and environmental impact of fluoropolymer may be a limiting factor for the use of this material.

3.2.4 Trace Metal Cleaning Procedures for Sample Bottles

Trace metal analysis results can easily become inaccurate if sample bottles are contaminated. To remove potential sources of contamination in sample bottles, all sample bottles should be thoroughly cleaned, both to remove dust and any metals that could exchange with the sample during storage. This cleaning goes beyond usual cleaning of labware and often involves soaking the equipment in soap or acidic solutions in order to remove organics and/or leach metals from the plastic itself (Apte et al. 2002; Cutter et al. 2017; Sander et al. 2009). The procedure used to prepare bottles for seawater samples is typically different between each laboratory and individually assessed for suitability. Differences arise because groups implement methods based on historical experience or differences in intended sampling objective or metal of interest (Apte et al. 2002). Today, however, most laboratories use similar methods which have been standardized by advice from the international GEOTRACES programme and the accumulated experience of the community since the 1970s. For example, the minimum effective cleaning procedure recommended by GEOTRACES for analysing sub-nanomolar levels of most trace metals in seawater involves soaking of the bottle in alkaline detergent to remove organic residues (grease and fat), soaking in diluted hydrochloric or nitric acid to mobilize and desorb solid phase and/or adsorbed contaminants from the bottle wall, followed by exhaustive rinsing with ultra-high purity water (UHPW) (Cutter et al. 2017). The cleaned bottles are then double bagged using at least two (resealable) polyethylene bags and stored until use either empty or filled with dilute high purity acid (Apte et al. 2002; Cutter et al. 2017; Sander et al. 2009); however, it should be noted that bottles cleaned in such a way might not be useable for some speciation studies (see Sect. 3.5.3). Bottles should be handled at later stages using clean gloves, and the final ‘clean’ steps should be carried out in a dedicated clean working space. Obviously, all work involving acids should be carried out safely in well-vented areas with the correct personal safety precautions (Apte et al. 2002).

3.2.5 Trace Metal Clean Reagents

Systematic contamination of samples may often also be caused by using chemical reagents or water of insufficient purity during processing and analysis (Bowie and Lohan 2009; Sander et al. 2009). This type of contamination is usually indicated by the systematic measurement of unexpectedly high metal concentrations or by high procedural ‘blanks’, which in the latter case is the amount of metal involuntarily added to a low-metal or ultrapure water sample during processing and analysis (using the same analytical steps as for the actual samples; see Sect. 3.6 for more information on procedural blanks; Bowie and Lohan 2009; Sander et al. 2009). Thus, all chemicals and reagents used for the analysis of trace metals must be of high purity, typically denoted as ‘ultrapure’ grade, which are relatively expensive (or for some reagents simply not available). As such, many laboratories utilize chemical or physical procedures to reduce trace metal impurities by removing metals from lowergrade reagents (Bowie and Lohan 2009). Reagent cleaning methods are common practice in many trace metal laboratories, for example, using clean sub-boiling distillation methods to obtain purified acids or reagents or isopiestic distillation for purifying ammonia (e.g. Sander et al. 2009 and references therein). Dilutions of reagents for trace metal methods or rinsing of clean equipment must be carried out using ultra-high purity water (UHPW), produced from deionized water by commercially available filtration systems, and defined with a resistivity of >18.18 MQ-cm. Similar UHPW may also be prepared by sub-boiling distillation of deionized water. To verify the purity of reagents, reagent blanks and/or process blanks should be determined regularly (see Sect. 3.6). Furthermore, to avoid contamination of clean reagents, reagent preparation, handling and manipulation must be performed under rigorous trace metal clean conditions (see Sect. 3.2.1 and 3.2.2), and reagents must be stored and dispensed into acid-cleaned fluoropolymer or LDPE bottles (see Sect. 3.2.3) (EPA 1997). Equipment such as pipette tips or measuring cylinders that may be used for dispensing reagents must also be checked and/or acid cleaned or acid rinsed prior to use to prevent metal contamination of the samples.

3.3 Trace Metal Clean Sample Collection

Marine trace metal chemists still depend on the collection of water samples for trace metal analysis because, unlike for some physical and other chemical oceanographic parameters, instruments to make in situ measurements for trace metals are either not yet mature or not readily available (Capodaglio et al. 2001; Grand et al. 2019). The sections below will focus on the collection of samples from both the shallow and the deep oceanic water column for trace metal analysis. We note that aerosol dust sampling and sediment-water interface sampling procedures for trace metal analysis also exist; however, these methods fall outside the scope of this chapter and are not discussed here.

The three main difficulties a marine trace metal chemist is confronted with at sea during sample collection are (Gillain et al. 1982):

1. *Representative samples* – obtaining a sample that accurately represents, both in time and space, the conditions of the water chemistry of the system targeted for the study is challenging. This concern is of primary importance for a relevant description of the system of interest (Wilde and Radtke 1998). Where the trace metal or parameter of interest is expected to vary dramatically in space or time (e.g. coastal settings), high spatial- and/or temporal-resolution sampling is needed to account for this variability. For metals with conservative (i.e. invariant, or salinity related) distributions, fewer sampling points may be needed. Obtaining representative samples also includes a need to store or

process (e.g. filtration) samples accordingly to avoid artefacts from storage (e.g. bottle adsorption or contamination levels). For samples that are sensitive to rapid chemical alterations (e.g. Fe^{2+} oxidation), special time-sensitive precautions may need to be deployed in the field.

2. *Minimizing contamination* – the main sources of contamination in the field are the sampling platform, the personnel and the sampling device. For open ocean environments, where large research vessels are required, the vessel must be considered as the main source of contamination in surface waters. Generally, research ships, owing to their Fe structure and other metal features such as propellers, Zn anodes, paint and engine exhausts, act as large sources of trace metals to the immediate environment (Gillain et al. 1982; Tovar-Sánchez 2012). Thus, care must be taken when collecting surface samples or when deploying sampling devices, for example, by considering the ships' draught and wind as well as current direction relative to sources of contamination (Gillain et al. 1982; Tovar-Sánchez 2012). Where possible, such as in coastal studies, surface samples may be collected from small non-metallic boats.
3. *Sampler choice* – reliable devices and techniques to minimize and eliminate contamination during surface water and deepwater sampling have been developed by the community over several decades (Tovar-Sánchez 2012) and are now standardized and intercompared by programmes such as GEOTRACES (e.g. Cutter and Bruland 2012; Middag et al. 2015a). The volume of sample needed, the sampling depth and the element/s of interest are key factors when choosing a sampling device. Although the choice of appropriate samplers for collecting trace metal samples is fundamental, adequate cleaning treatments (i.e. flushing the sampler with low trace metal seawater multiple times before use, and conditioning of the device), verifying cleanliness and correct handling of the devices are also vital (Capodaglio et al. 2001).

Commonly used trace metal clean samplers and analytical techniques are detailed in the following sections. The description of samplers is supposed to guide the reader through available and mature low-cost and high-priced sampling devices that are currently in use for trace metal sample collection by the scientific community. At present, the trace metal clean rosette (see Sect. 3.3.1.1) is the workhorse of the trace metal community and the backbone for the collection of large datasets within the GEOTRACES programme (see De Baar et al. (2008) for a brief history of GEOTRACES sampling systems). This chapter also introduces low-cost devices that provide lower-resolution solutions, facilitating relatively low-cost trace metal observations and allowing the filling of current gaps in data coverage in environments not accessible with a rosette system. The low-cost systems can produce equally high-quality data if trace metal clean procedures are followed (see Sect. 3.2), but as for all systems, rigorous intercomparison of results and crossvalidation of protocols are recommended (Cutter et al. 2017).

3.3.1 Dissolved Trace Metal Sampling

3.3.1.1 Depth Profile Sampling

When sampling below the surface (~ > 10 m), the choice of the sampling device is made based on the study objective, analytical requirements, characteristics of the system and the available capabilities. General approaches used for deepwater and vertical profile sampling are akin to those used by surface seawater sampling activities (see Sect. 3.3.1.2) and commonly include pump set-ups and/or discrete bottle sampling. Care should be taken not to touch the bottom with the deployed water sampler, as disturbed sediments and associated metals could contaminate the sample or damage the sampler. The deepest sampling depth is commonly 5–10 m above the bottom. Further, it has to be noted that instrument deployments during strong currents can result in an error (underestimation) of the deployment depth (Turk 2001), if not checked by pressure sensors attached to the instrument itself. Additionally, if the deployment platform is drifting, care has to be taken that the bottom depth remains deep enough to avoid running the instrument aground.

Discrete Bottle Sampler Systems

Bottle samplers allow marine chemists to obtain discrete samples from specific water depths, both in shallow and deep waters (e.g. Cunliffe and Wurl 2014; Cutter et al. 2017; Van Dorn 1956). This section covers the use of bottle samplers in deep waters (for use in surface waters, see Sect. 3.3.1.2). Bottle samplers can be obtained in different sizes and generally consist of a cylinder or 'bottle' with stoppers at each end (Cunliffe and Wurl 2014) that can be closed at a desired depth, either manually using a messenger or electronically.

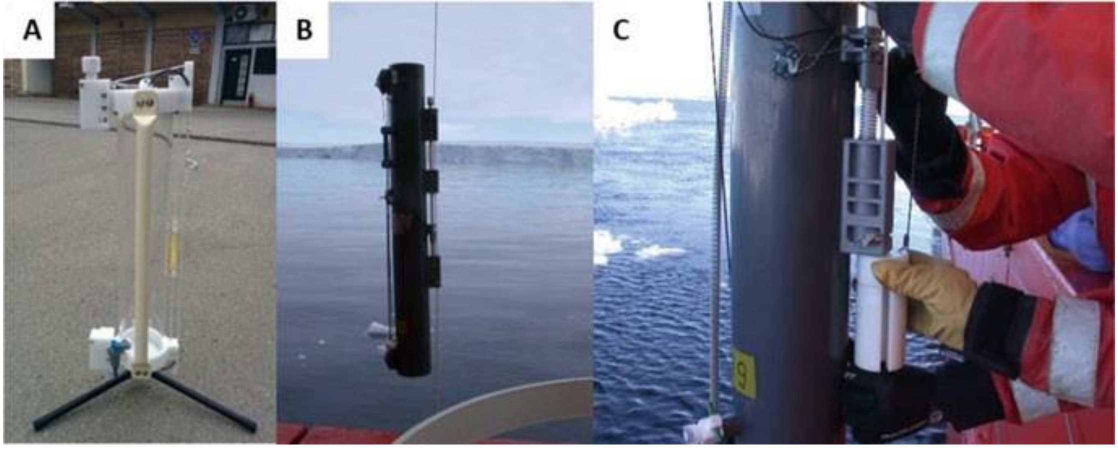

Fig. 3.4 Photographs of a custom-built acrylic bottle sampler (a) (photograph credit: Dario Omanović, Rudjer Boskovic Institute, Croatia) and a commercially available GO-FLO sampling bottle attached to a Kevlar cable (b), or with messenger (c) (photograph credit: Gert van Dijken, Stanford University, USA)

The electronic version is preferred in deep waters. To minimize contamination, trace metal clean bottle samplers are often made of transparent acrylic or fluoropolymer-lined opaque PVC, and their interior is totally free from metal parts (Fig. 3.4; see Box 3.4; Cunliffe and Wurl 2014). The earliest version of a (non-trace metal clean) bottle sampler is commonly referred to as Van Dorn sampler, but since the establishment of the GEOTRACES programme, external spring bottles such as internally fluoropolymer-coated ~10–12 L Niskin-X and GO-FLO bottles are most commonly used (Fig. 3.4), obtained from General Oceanics (see Cutter and Bruland (2012) for more details), or Ocean Test Equipment (slightly different samplers). Different laboratories have also constructed custom-designed bottle samplers based on requirements, with an example being the Royal Netherlands Institute for Sea Research (NIOZ) Titan system which makes use of custom-designed ‘Pristine’ bottles (see Rijkenberg et al. (2015)) (Fig. 3.5d).

Bottle samplers can be individually or serially attached directly to a non-metallic cable (e.g. Kevlar) to allow contamination-free sampling or mounted on a carousel (Fig. 3.5), which is often referred to colloquially as a CTD rosette (because of sensors measuring conductivity, temperature and depth), or just a rosette, to allow marine chemists to obtain discrete water samples from various depths (Bruland et al. 1979; Cunliffe and Wurl 2014; Van Dorn 1956). The latter is commonly used for depth profiling during GEOTRACES expeditions. However, regular rosette systems, like regular bottle samplers, are too contaminating to collect pristine water samples for trace metal analysis. Modifications are thus required. Such modifications typically take the form of coating the regular rosette frame with epoxy or replacing it with titanium and removing any sacrificial metal anodes. Such ‘trace metal clean’ rosettes are then loaded with trace metal bottle samplers. One of the first such trace metal clean rosette-based systems for collecting trace metal samples was designed by the Trace Metal/Plankton Group at Moss Landing Marine Laboratories, first used by Murray et al. (1992) and described by Sanderson et al. (1995).

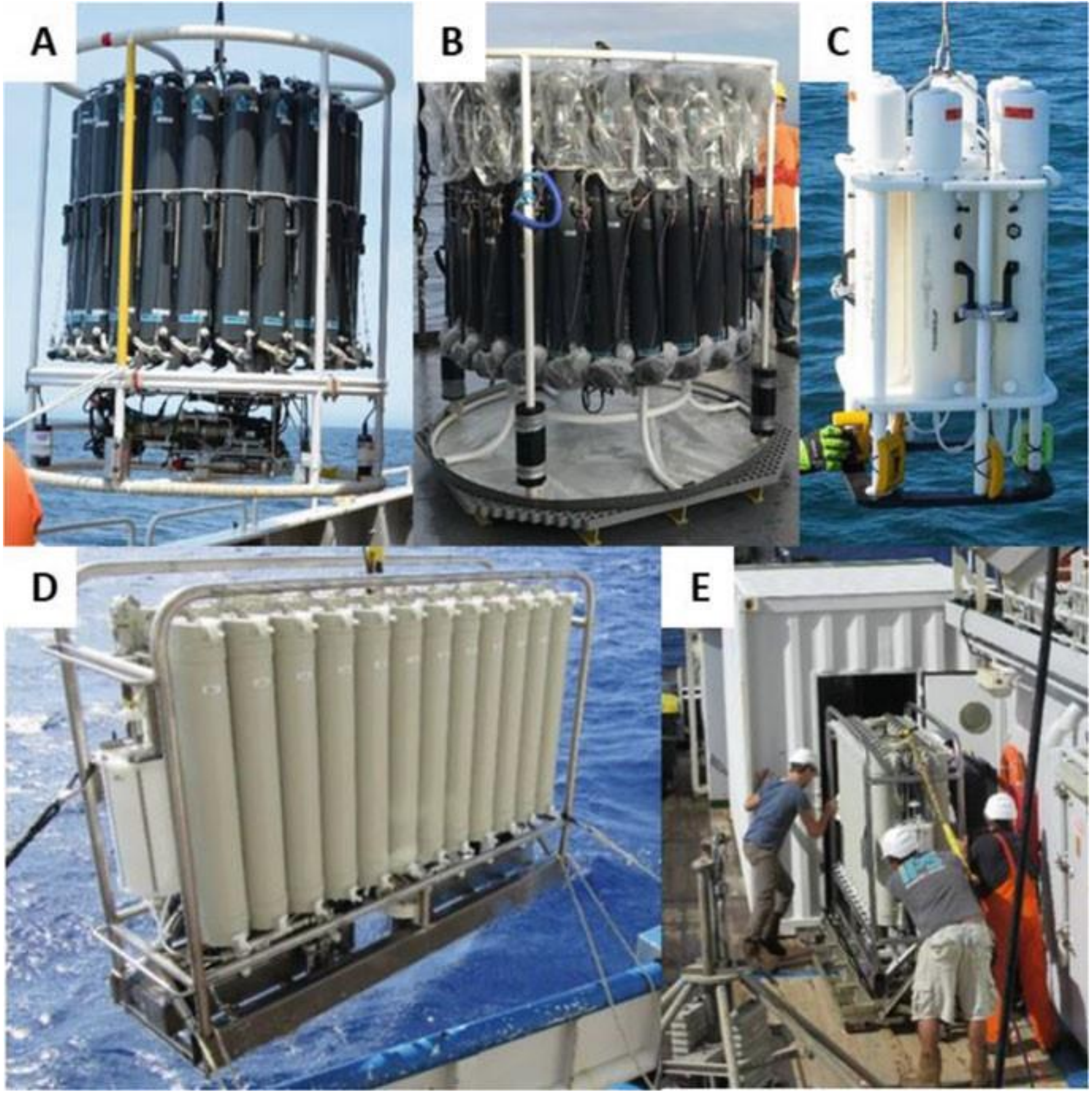

Fig. 3.5 Photographs of various trace metal clean rosette samplers. Photographs of the Japanese Niskin-X sampler system (a) (photograph credit: Taejin Kim, Pukyong National University, South Korea), a commercially available CTD rosette system from General Oceanics with GO-FLO bottles (b) (photograph

credit: Antonio Tovar Sánchez, ICMAN (CSIC), Spain), a modified small CTD system (c) (photograph credit: Antonio Tovar Sánchez, ICMAN (CSIC), Spain) and the custom-built NIOZ sampling system (d, e) (with its lightproof samplers rather than the original PVDF samplers; Rijkenberg et al. 2015) that can be transferred to a custom-designed clean laboratory container for processing (photograph credit: Loes Gerringa, NIOZ, Netherlands)

Today, 'trace metal clean' rosettes are commercially available for ocean sampling (e.g. Seabird Scientific or General Oceanics) and are widely used for clean water sampling. Currently, a number of different varieties of trace metal multiple bottler samplers in various size ranges are in use, such as the GO-FLO trace metal rosette used by the US GEOTRACES programme (Cutter and Bruland 2012), the NIOZ Titan system (De Baar et al. 2008) and the Japanese Niskin-X sampler system (Obata et al. 2017) (Fig. 3.5).

Similar to the individual bottle deployment, a rosette arrangement allows samples to be taken at different water depths with a vertical resolution of ~5 m (Strady et al. 2008). One of the major advantages of a rosette sampler, however, is the possibility for simultaneous collection of multiple samples at one depth. This simultaneous collection is especially useful when large volumes of water are needed for experimental work such as culturing/incubation studies or when studying elements and isotopes with inherently low seawater concentrations (e.g. radium (Ra)). Further advantages of a rosette system are: (i) faster deployment compared to the deployment of multiple bottle samplers on a cable, (ii) higher-resolution sampling capability since more bottles can be used during a single deployment and (iii) higher reliability in relation to messengers (see below) that sometimes fail to trip bottles at the desired depth that is often estimated rather than measured, in contrast to a rosette system (Sanderson et al. 1995).

When deploying individual bottles on a Kevlar cable, a (plastic covered) weight should be attached to the bottom of the cable, several meters below the last sampler, to keep the cable taut. Prior to deployment, the bottles should be attached to the cable and armed (closure system ready for use). Each sampler, except the one closest to the bottom, will be equipped with a plastic-coated metal weight or 'weighted messenger' attached to the Kevlar cable via a lanyard. When the bottles are at their desired depth, the first messenger will be dropped down the cable by the investigator which closes the first sampler by tripping the spring-loaded valve (closure system) (Cunliffe and Wurl 2014). This mechanism also causes the next messenger to drop, closing the subsequent samplers in rapid succession. Enough time has to be allowed for the messengers to trip each sampler—which can take up to 1 h in 6000 m water depth – before winching the cable to the surface (Measures et al. 2008). By touching the cable with one hand, it is possible to feel a strong 'thump' on the cable as each messenger triggers the subsequent sampler. Upon recovery, plastic gloves can be placed over the spigots of bottles before the bottles are transferred to a clean room for sample collection and filtration via the sampling valves/spigots (Cutter and Bruland 2012). It is critical to note and record if there are any leaks from the samplers or any open samplers upon retrieval, since leakages and open bottles may affect the integrity of the sample and/or may result in contamination. When using bottle samplers to collect shallow waters (<100 m), it is also critical to be aware of, and to avoid, sources of surface contamination, for example, the wake or plume of trace metals associated with a research ship. Thus, discrete bottle sampler systems (either individual or on a rosette) are usually not used to sample water shallower than 10 m for trace metals.

Some of the individual bottle samplers and rosette systems can be deployed with bottle samplers in the open position, while others can be deployed with bottle samplers in the closed position – since they open themselves automatically at a fixed depth (usually ~10 m) to avoid contamination of the sampler by the surface microlayer (SML) which is particularly rich in trace metals (Caroli et al. 2001). During descent, the open bottle samplers are flushed. Commonly, individual samplers on a cable are closed prior to ascent, while rosette systems close samplers during the ascent. Rosette systems either close at pre-programmed depths (using a pressure sensor) or are triggered electronically via the conducting cable at the desired depth (Fitzsimmons and Boyle 2012; Measures et al. 2008). The bottles can either be closed on the fly (usually at winch speeds of 0.3 m/s), so the bottles are always moving into clean water that has not been in contact with the rosette frame (to avoid possible contamination of the water via the frame), or after 1–2 min after reaching the desired depth to allow the temperature and salinity readings of the CTD sensor to equilibrate. The latter is commonly done for titanium systems that pose minimum risk of contamination due to the absence of sacrificial anodes and other contaminating metal components.

As with regular rosette systems, trace metal rosettes are also commonly equipped with various sensors (e.g. for oxygen, fluorometer and transmissometer) including conductivity, temperature and pressure (CTD) sensors providing real-time readouts during deployment if deployed via a conducting hydro wire. After recovery, the rosette is secured, plastic covers are often immediately placed on top of the bottle samplers, and plastic gloves are placed over the spigots. Typically, bottle samplers are then removed individually from the rosette frame and carried into a dedicated clean laboratory/bubble, where they are secured to a purpose-built rack for sub-sampling (Cutter and Bruland 2012). An alternative approach is to use a custom-built option like the NIOZ Titan system (Fig. 3.5), where the bottle samplers remain on a custom-built titanium frame, and the whole frame is transferred to a custom-designed clean laboratory container for processing, without needing to remove individual bottles (Rijkenberg et al. 2015). The NIOZ Titan system has been proven to be effective and clean (Middag et al. 2015a) and has some other advantages over commercial systems. For example, the Titan system was motivated by problems with GO-FLO bottles, specifically that their closure system is notoriously fickle in cold waters (Measures et al. 2008). To address this issue, the Titan system houses 24 polypropylene (lightproof) or PVDF samplers (23 L), so-called pristine samplers, with butterfly valves that close the bottles hydraulically. A drawback of this system is its size and weight, limiting deployment from smaller ships and requiring a strong winch and cable.

Box 3.4 Cleaning Bottle Samplers

There is some discussion about whether cleaning of water samplers mounted on a CTD rosette (i.e. GO-FLO and trace metal Niskin bottles) is needed or desirable before and between system deployments. If these bottles are cleaned, acid concentrations should be kept low (0.1 M HCl is recommended in the GEOTRACES 'Cookbook'; Cutter et al. 2017), and no acid should contact the outside of the bottle, the nylon components in particular.

Other discrete, bottle-based sampling systems that can be used for depth profiles are the MITESS (moored in situ trace element serial sampler; Bell et al. 2002) or an autonomous underwater vehicle (AUV) sampler. Although the former was designed for moorings (see below), the system can also be deployed on a hydro wire to collect vertical trace metal profiles in a so-called VANE mode (Fitzsimmons and Boyle 2012). In the VANE mode, the MITESS is loaded into a weathervane-type PVC and polycarbonate structure that orients the sampler upstream of the potentially contaminating hydro wire (Fitzsimmons and Boyle 2012). The system then autonomously opens and closes a pre-cleaned sampling bottle at a desired depth. Trace metal samplers based on AUV systems are currently being developed and tested for trace metal clean sampling of mid-waters and deep waters in areas that are not easily accessible with research vessels, that is, areas near or under ice shelves, sea ice, and icebergs.

Pumping System on CTD Rosette

A so-called pump CTD system enables water sampling with higher volume (effectively unlimited volume) and higher resolution (vertical resolution of 1 m) relative to the typical bottle sampler rosette (Strady et al. 2008). This configuration allows for the detection of small vertical structures in trace metal distribution across interfaces (e.g. the nutricline or the redoxcline) or the halocline (Strady et al. 2008). The CTD pump system combines a rosette and a pump system, i.e. a peristaltic pump, and was developed in 2001 in collaboration between IOW (Institut für die Ostseeforschung, Warnemünde) and MPI (Max Planck Institute for Microbiology, Bremen). The system consists of a submersible CTD rosette with fluoropolymer-coated Niskin bottles, an acoustic Doppler current profiler (ADCP), a pump probe and a digital flow meter for the water stream. The flow rate of the system at ~300 m can be up to 2.9 L min^{-1} (Strady et al. 2008), and the water is pumped directly through a nylon hose to a clean laboratory on board ship for sub-sampling. However, the use of a pump typically limits the application of the system to a depth down to around 350 m (Strady et al. 2008).

Moored in Situ Serial Samplers

Moored in situ trace metal samplers have been developed for time series sampling to resolve temporal and seasonal variabilities in trace metal concentrations in various marine environments (Bell et al. 2002). Such in situ samplers collect and preserve samples for later laboratory analysis. Moored in situ samplers can be very useful (Bell et al. 2002), especially for established monthly time series stations such as at the Southern Ocean Time Series (SOTS; Trull et al. 2010), Bermuda Atlantic Time Series Study (BATS; Michaels and Knap 1996) and the Hawaiian Ocean Time Series (HOT; Karl and Lukas 1996). Currently, there are various moored samplers available that are suitable for trace metal work, with the most notable examples being MITESS (Bell et al. 2002) and ACE (autonomous clean environmental sampler; Fig. 3.6c, d; van der Merwe et al. 2019). Other systems such as PRISM (portable remote in situ metal; Mueller et al. 2018) and ANEMONE (advanced natural environmental monitoring equipment; Okamura et al. 2013) are also available, and others will likely be developed. Both the MITESS and the ACE samplers are selfpowered and can be deployed for 6–12 months at various depths on standard deep- sea moorings.

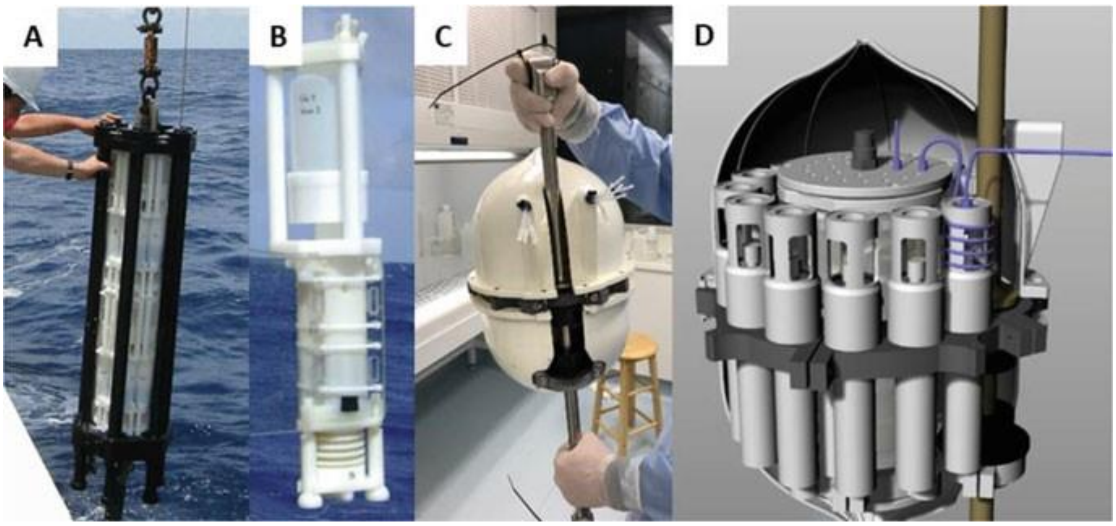

Fig. 3.6 Photographs of the MITESS (a) (photograph credit: Edward Boyle, Massachusetts Insitute of Technology, USA), the MITESS module in the VANE configuration (b) (photograph credit: Jessica Fitzsimmons, Texas A & M University, USA) and the ACE sampler (c) and module (d) (photograph credits: Pier van der Merwe, University of Tasmania, Australia)

A comparative advantage of the moored samplers compared to the commonly used rosette sampler is that the deployment itself requires no trace metal expertise, since the entire sampler is prepared in a clean room and no additional handling is necessary (Bell et al. 2002; van der Merwe et al. 2019).

The MITESS collects unfiltered 500 mL samples at any depth by opening and closing a sample bottle lid at a predefined depth. The time-controlled bottles are originally filled with high purity dilute acid that is replaced by denser seawater during sampling via passive density-driven flow (Bell et al. 2002). These samples are preserved over the deployment time of several months at pH 2.5 by the diffusion of high purity acid out of a diffusion chamber inside of the bottle. The sampler itself is made entirely out of ultra-high molecular weight polyethylene (UHMW) and can hold up to 12 bottles that are individually controlled by independent modules so that the failure of a single unit does not affect the entire set of the time series samples (Bell et al. 2002). The MITESS is programmed by wireless communication, and the electronic board retains a record of the timing of bottle opening and closure (Bell et al. 2002). A comparison of Fe data of GO-FLO and MITESS on the GEOTRACES IC2 expedition in 2009 did not show any differences between the two sampling systems, indicating that these samplers can be used interchangeably to collect trace metal samples, either on moorings or for discrete samples in the 'VANE' mode (Fitzsimmons and Boyle 2012). Potential issues with MITESS, however, are that the seawater is not filtered prior to being acidified (meaning that some portion of ocean particulate material will be dissolved during deployment) and that biofouling may occur, since the sample intake is not physically removed from the sampler body.

In contrast to MITESS, the ACE sampler collects filtered (0.2 µm, polyethersulfone filter membrane) samples into 65 mL fluoropolymer containers. The time-controlled sampler works by drawing seawater through up to 12 individual intake tubes via acid-washed filters into the UHP filled sample bottles using individually programmable micro-peristaltic pumps (density displacement mechanisms; van der Merwe et al. 2019). The intake tubes are maintained in an upstream position relative to the device to minimize contamination during sampling (van der Merwe et al. 2019). A key advantage of this system is that samples are filtered to remove particles, and intake pots are made of PFA which together with their small surface area reduces biofouling and thus potential sampling artefacts and contamination (van der Merwe et al. 2019). However, a disadvantage of the ACE system is the fact that the filtered samples can only be acidified back in the laboratory after recovery of the sampler which might result in low-biased results due to wall absorption.

ROV-Based Discrete Samplers

Remotely operated vehicles (ROVs) with manipulator arms that operate on spatial resolutions on the single cm scale are a useful tool for sampling high trace metal environments such as under ice, near sediments or within fluids from hydrothermal vents and seeps along oceanic spreading centres, subduction zones and subsurface volcanoes. Multiple samplers such as the isobaric gas-tight sampler (IGT; Seewald et al. 2002), the titanium syringe sampler (Majors sampler; Von Damm et al. 1985) and the Kiel pumping system sampler (KIPS; Garbe-Schönberg 2006) are commonly in use to collect trace metal samples at a depth of up to 4000 m and can be easily attached to a ROV manipulator arm (Fig. 3.7).

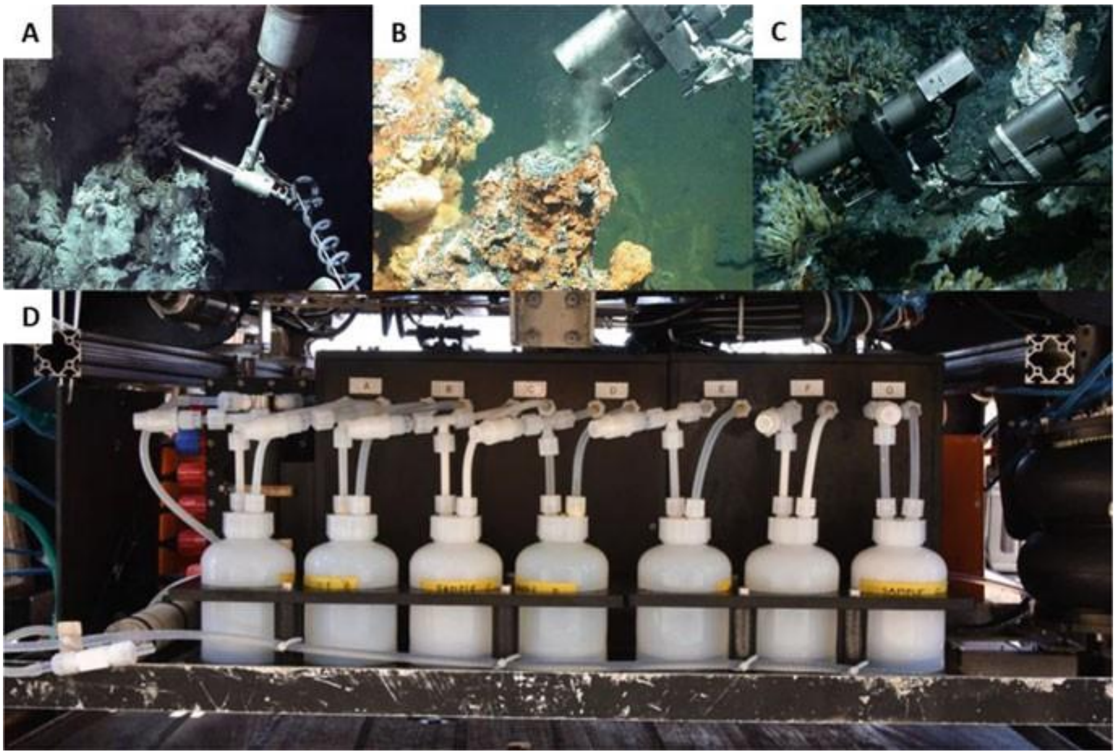

Fig. 3.7 Photographs of KIPS (a), Majors (b) and IGT (c) samplers attached on the manipulator arm of the ROV MARUM QUEST (photograph credit: MARUM – Centre for Marine Environmental Sciences, University of Bremen, Germany). Photo (d) shows the sample collection system of the KIPS device (photograph credit: Dieter Garbe-Schönberg, Christian-Albrechts-University Kiel, Germany)

These samplers are made of titanium or other inert materials, are acid and temperature resistant to withstand the hot and acidic conditions of hydrothermal working areas, are fully remotely controlled and are filled through a titanium nozzle or snorkel which can be directly inserted into the vent orifice or other localized trace metal sources. The Majors sampler can collect samples of up to 750 mL, but is non-gas-tight and designed to release pressure during ROV recovery. The IGT sampler can collect samples of 150 mL and is gas-tight up to 450 bar to prevent degassing of the sample during ascent, thereby avoiding precipitation and chemical alterations of the prevalent trace metals (Seewald et al. 2002). This sampler can thus be used to characterize major, trace, semi-volatile and volatile components (Seewald et al. 2002). The KIPS device was specifically designed for trace metal clean work (Garbe-Schönberg 2006). This device consists of a manipulator operated titanium nozzle with PFA (perfluoroalkoxy) tubing that leads to the PFA sampling flasks that are non-gastight. The latest version has up to seven PFA sampling flasks that are remotely controlled by motor-driven open-close valves (Garbe-Schönberg, pers. commun.). The PFA flasks have a volume of 750 mL, and in situ filtration and/or in situ fixation units can be added in-line. Large volume sample bags up to 10 L have also been successfully filled using the KIPS system. Various other sensors and probes (i.e. temperature, pH, oxygen) can be mounted on either of the samplers to record in situ parameters at the point of sampling, which can be transmitted directly to the ROV control room in real time. While such ROV-based samplers have proven their use in high trace metal environments, they are not commonly utilized in open ocean situations, due to the elevated level of background contamination compared to traditional bottle samplers.

3.3.1.2 Surface Sampling

There are three general approaches that can be used for surface water sampling (0–10 m): (1) pumping water to the surface from the depth of interest; (2) sampling by bottles lowered to an appropriate depth by line, sampling device or pole and then closed manually, automatically (pressure triggered) or by a signal from the surface; and (3) adsorbing the metals or compounds of interest on an appropriate material lowered to the desired depth (Capodaglio et al. 2001). Additionally, for sampling the microlayer at the sea surface, special approaches have been developed (see below). For trace metal clean sampling of surface waters that are not easily accessible, such as areas near ice shelves, sea ice and icebergs, drone sampling systems are currently being developed, spearheaded by the University of Tasmania, Australia.

Discrete Bottle Samplers

As described in Sect. 3.3.1.1, bottle samplers can be individually or serially attached to a Kevlar cable to allow marine chemists to obtain discrete water samples from various depth intervals, including near the surface if this can be done without contamination (Bruland et al. 1979; Cunliffe and Wurl 2014; Matamoros 2012; Van Dorn 1956). Individual bottle samplers can be deployed in two configurations, i.e. horizontal (type alpha) or vertical (type beta), depending on the study objectives (Cunliffe and Wurl 2014; Matamoros 2012). Type alpha samplers are ideal for sampling at the thermocline, narrow stratification layers or just above the bottom sediment (Cunliffe and Wurl 2014; Matamoros 2012). For more information on the bottle samplers, deployment and recovery, the reader is referred to Sect. 3.3.1.1.

Continuous Flow Samplers

Pumping systems, i.e. peristaltic pumps and diaphragm pumps (preferably an all-fluoropolymer inert type pump), with an extended inlet tube are frequently used by marine chemists to allow continuous and high-volume trace metal sampling (bulk sampling) of the near-surface water column (1–10 m) (Fig. 3.8) (Cunliffe and Wurl 2014; Tovar-Sánchez 2012). Prior to sample collection, it is recommended to condition the pre-cleaned tubing by pre-rinsing before collecting the unfiltered or filtered (in-line filtration) sample into an acid-cleaned sample container (Cunliffe and Wurl 2014), preferably in a clean space. To avoid contamination during sampling, the tubing should be extended ~3–4 m away from the sampling platform by attaching it to a plastic telescope bar or by deploying it via a boom or crane, which is usually the case for towed sampling devices as described next.

There are several versions of towed sampling devices (often referred to colloquially as 'tow-fish' or 'towed fish') that are deployed by marine trace metal chemists from a moving ship (Bowie and Lohan 2009; Cunliffe and Wurl 2014; Cutter et al. 2017; McDonnell et al. 2015; Tovar-Sánchez 2012). The simplest type consists of a subsurface torpedo-shaped heavy vehicle (Fig. 3.8). The water intake PTFE tube is attached to the nose of the towed fish, oriented into the oncoming water and connected to a PTFE diaphragm pump or a large peristaltic pump on board which supplies the sample water directly into the shipboard clean space (Cunliffe and Wurl 2014; Cutter et al. 2017; McDonnell et al. 2015; Vink et al. 2000). For underway surface sampling, commonly at relatively low speeds, the system is deployed from a boom or crane outside the bow wake of the ship to avoid sample contamination (Cunliffe and Wurl 2014; Cutter et al. 2017; McDonnell et al. 2015). Faster speeds are possible with this system if there is little or no swell and the towed fish remains outside of any breaking bow waves Cutter et al. 2017. Various sensors can be attached to the fish to provide accurate depth and temperature data. It is important to note, however, that most pumps are often not self-priming and may not be able to lift water to a height greater than 10 m (Cunliffe and Wurl 2014).

Fig. 3.8 Photographs of a peristaltic pump sampling system deployed via a rubber boat (a) (photo credit: Antonio Tovar Sánchez, ICMAN (CSIC), Spain) and a towed-fish sampling system deployed via a crane for continuous flow sampling of near-surface seawater (b, c) (photograph credit: Loes Gerringa, NIOZ, Netherlands)

Passive Samplers

Passive sampling techniques are based on the diffusion of a metal of interest from the seawater onto a collecting medium (the passive sampler), owing to Dickian molecular diffusion and a greater binding affinity of the metal of interest with the passive sampler relative to seawater (Knutsson 2013). The metals will concentrate on the passive sampler until a steady-state concentration gradient from seawater to the passive sampler is reached (Knutsson 2013; Zhang and Davison 1995). Passive sampling devices can be deployed for long periods of time (often days or months before saturation is reached) to provide long-term, time-weighted averages of the concentration of a metal in the water column or to accumulate sufficient concentration of a metal for analysis (Allan et al. 2008; Zhang and Davison 1995). Consequently, the use of passive samplers is beneficial in investigations where concentrations of metals are low and/or fluctuate widely (Allan et al. 2008). Passive samplers can provide a more representative picture of overall trace metal concentrations in a system of interest compared to active sampling techniques that commonly just sample one point in time (Allan et al. 2008; Davison and Zhang 1994; Zhang and Davison 1995). However, it is important to note that trace metal data from passive samplers do not equate to trace metal data from active samplers, since passive samplers exclusively sample the labile fraction of metals in situ, that is, the metal fraction that can easily diffuse through, and be adsorbed by, the passive sampler. This feature excludes various phases of the dissolved pool – for example, metals that are strongly bound to organic ligands – and thus passive sampler metal measurements are generally lower than dissolved metal concentrations. Therefore, passive samplers provide information on the supposedly 'bioavailable' metal fraction, i.e. the metal fraction that can be taken up by marine organisms, and consequently, passive samplers offer more toxicologically relevant data relative to active samplers (Allan et al. 2008) (see Box 3.5). While passive samples can be deployed in deeper waters on moorings, they are more commonly used in shallow waters in coastal areas.

The main passive samplers used for monitoring trace metals in marine waters are the diffusive gradient in thin film (DGT) device and the Chemcatcher (Fig. 3.9; Schintu et al. 2014). DGTs were developed by Zhang and Davison (1995) and consist of a small piston-like plastic device containing a Chelex 100 layer as a receiving phase overlaid with a well-defined diffusion layer of polyacrylamide hydrogel protected by a filter membrane (Fig. 3.9) (Allan et al. 2008; Schintu et al. 2014). The Chemcatcher comprises a fluoropolymer sampler body that retains a chelating disk as a receiving phase overlaid with a cellulose acetate diffusionlimiting membrane (Allan et al. 2008; Schintu et al. 2014). A comparison study of the DGT device and the Chemcatcher demonstrated that the two sampling devices provided similar information and were able to integrate concentrations reliably during their deployment period in surface waters (Allan et al. 2008). For field deployment, DGTs and Chemcatchers should be fixed between Perspex plates attached to a rope and a buoy to hold the devices in place (stationary) (Fig. 3.9). The time of deployment and retrieval must be recorded by the investigator to the nearest minute for later calculation of metal concentrations (Knutsson 2013). After recovery, samplers must be rinsed with UPHW water, preferably under clean conditions, and placed into two plastic bags for their transport in ice boxes back to the land-based laboratory. Gloves must be worn at all times when handling the passive sampling devices.

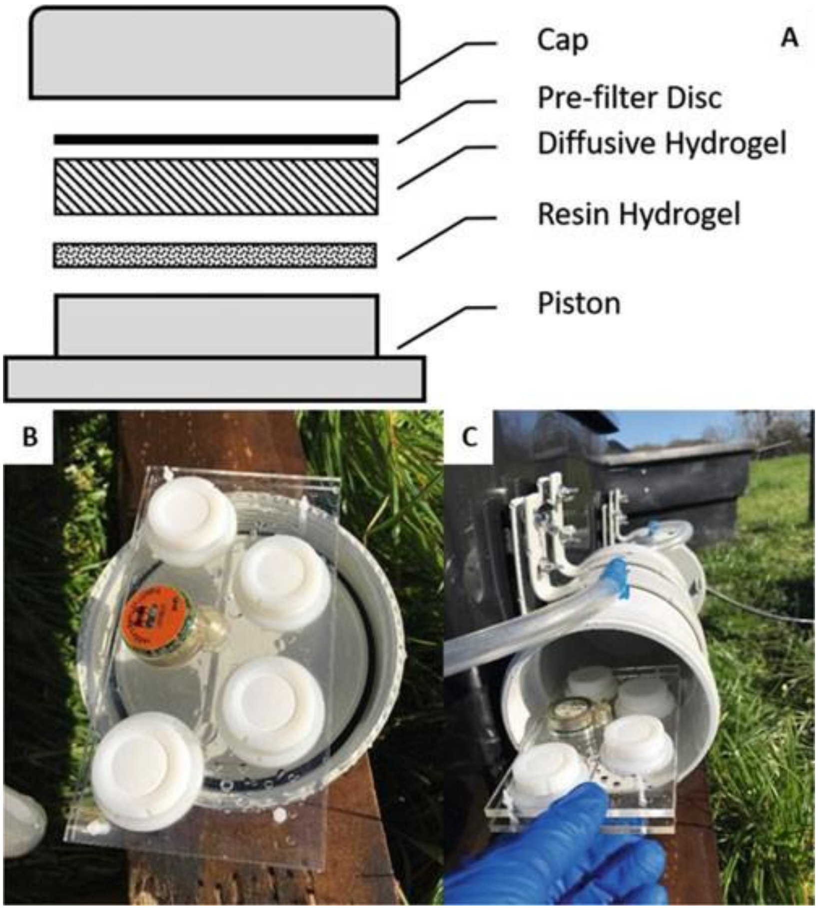

Fig. 3.9 Cross section of a functional DGT assembly (Figure courtesy of Billie Benedict, University of Otago, New Zealand) (a), and photograph of a diffusive gradient in thin film (DGT) passive sampler with DGTs and temperature data loggers mounted in acrylic plate holders for easy deployment and retrieval (b, c) (photograph credit: Amir Mohammadi, University of Waikato, New Zealand)

Box 3.5: Limitations of Passive Sampling Devices

Environmental factors can affect passive sampling including biofouling, presence of dissolved organic carbon (DOC), water turbulence and changes in temperature and salinity (Schintu et al. 2014). All these factors can alter the uptake rates of metals using the passive samplers and can thus create bias in field evaluations (Schintu et al. 2014). In particular, most of the environmental variables that can influence passive sampling measurements in the field are not fully accounted for in the laboratory-based calibration studies (i.e. quantification of the metal diffusion coefficient (D_e) through the diffusion layer of the sampler), which introduces uncertainty in the time-weighted averages of the metal concentration estimates.

Sea Surface Microlayer (SML) Sampler

Sampling devices that are commonly used to sample the sea surface microlayer (SML), defined as the top 1–1000 µm of the surface ocean, for trace metals include plate-, tube-, and screen-samplers (Fig. 3.10) (Cunliffe and Wurl 2014; Tovar- Sánchez et al. 2014; Tovar-Sánchez et al. 2020). These devices are usually deployed from shore or from a non-metallic boat. Two sampling materials are typically chosen for the samplers owing to their characteristic hydrophobicity, namely, borosilicate glass and plexiglass (plate- and tube-sampler). In the field, after preconditioning of the sampler (i.e. dipping it into surface water), the sampler is dipped into the ocean until most of the surface area is submerged and then withdrawn through the SML at a slow rate while wearing polyethylene gloves (Ebling and Landing 2015). After recovery, the sampler is held over a receiving bottle for the sample to drip off (Ebling and Landing 2015). The process is repeated until the desired volume of sample is acquired. To reduce contamination issues of the sample during sample handling (e.g. exposure to airborne particles), rotating glass drum samplers are gaining more and more attention (Fig. 3.10) (Cunliffe and Wurl 2014). The drum sampler can be towed over the water surface to sample the SML via capillary force, and the sample can then be collected into pre-cleaned containers (Cunliffe and Wurl 2014).

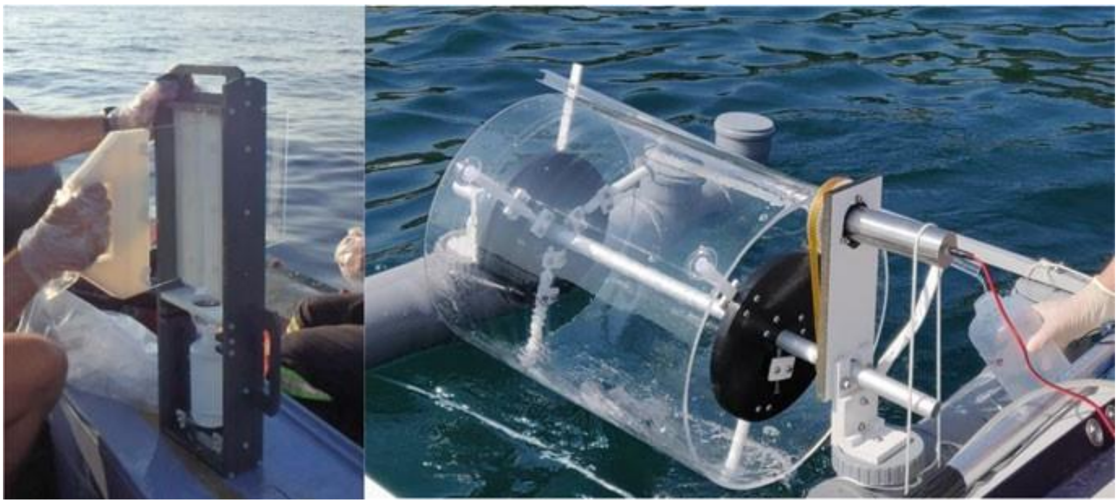

Fig. 3.10 Photographs of a sea surface microlayer (SML) plate sampler (left) (photo credit: Antonio Tovar Sánchez, ICMAN (CSIC), Spain), and a SML drum sampler prototype (right) (photograph credit: Dario Omanović, Rudjer Boskovic Institute, Croatia)

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Pole Sampler

One of the simplest methods to collect surface water samples is the manual collection of the water sample into pre-cleaned containers by submerging the sample bottle either directly from a non-metallic small boat or by using a non-metallic telescoping ‘pole’ (Fig. 3.11) (Tovar-Sánchez 2012). The bottle can be attached to the bottom of the pole with non-metallic clamps or secured on the pole via a plastic frame (Bowie and Lohan 2009; Turk 2001). The pole sampler can be deployed from shore or a non-metallic boat or even from a larger research vessel if conditions permit. The sampler should be deployed into the direction of the current to avoid sampling water that has been in contact with the sampling platform. Prior to collecting the sample, the sampling container should be conditioned (two or three times) with seawater below the SML. The pole is then submerged with the open bottle upside down, and at the desired depth, the system is turned to fill the bottle with ambient seawater. It is recommended that the investigator closes the bottle below the surface (wearing gloves) to avoid contamination from the SML (Cunliffe and Wurl 2014), which is particularly rich in trace metals (Capodaglio et al. 2001). To allow the collection of water samples at a specific depth, the bottle can be plugged with a non-contaminating silicone stopper attached to a line that the investigator pulls when the bottle is at the designated depth – the depth can be marked on the pole (Turk 2001). After recovery of the system, the sample bottles are immediately double bagged in polyethylene bags and processed in a clean room environment as soon as possible. When sampling for dissolved species, water is usually filtered as quickly as possible and then acidified with ultrapure reagents either shipboard or back on land (see Sect. 3.4.1).

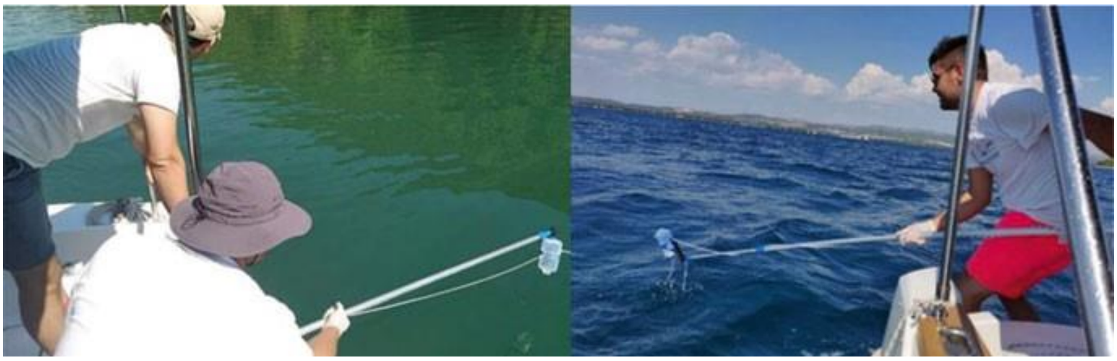

Fig. 3.11 Photographs of a pole sampler for collecting near-surface water samples directly from the coast or from an inflatable rubber boat (photograph credit: Dario Omanović, Rudjer Boskovic Institute, Croatia)

3.3.2 Particulate Trace Metal Sampling

Oceanic particles are an important, yet perhaps less quantified, part of the oceanic trace metal inventory. However, with advances in particle collection and analysis coming in recent years linked to large-scale field programmes such as GEOTRACES, this trace metal fraction is gaining more and more attention in the scientific community (Fig. 3.1) (e.g. McDonnell et al. 2015). Sampling for particulate metals can be done using shipboard filtration from trace metal bottle samplers, if their volume is sufficient to collect enough particles to measure the element of interest (see Sect. 3.3.2.1) (McDonnell et al. 2015; Planquette and Sherrell 2012) or by larger volume in situ filtration systems (see Sect. 3.3.2.2). GEOTRACES intercalibration efforts have shown that there is no systematic difference between particulate trace metals collected by direct bottle filtration and by in situ filtration, suggesting that these sampling strategies can be used interchangeably (Planquette and Sherrell 2012).

3.3.2.1 Bottle Sampler Collection

Once bottle samplers as used for dissolved metal sampling are back on deck, particles can be collected from the samplers, directly by pressurising the samplers to allow filtration (in-line filtration) or by filtering sub-samples from secondary containers after sub-sampling (off-line filtration) (Fig. 3.12). There are advantages of using bottle samplers for particle collection, namely, that the particulate metals collected can be related directly to dissolved metals measured from the exact same depth and that multiple sample depths can easily be collected from a single rosette cast.

Fig. 3.12 Photograph of a custom-built off-line particulate trace metal set-up (photograph credit: Mathijs van Manen, NIOZ, Netherlands). For the off-line particulate trace metal set-up, water samples were collected from the bottle samplers right after recovery into secondary containers to decrease between-cast turnaround time (Cutter et al. 2017)

The disadvantages are that only relatively small volumes can be filtered (10s of L) and thus there may be insufficient particulate material to measure some low-level trace metals of interest and that there is the possibility of particle loss by settling in the sampler prior to filtration. The latter requires that the investigator mixes samplers regularly and limits filtration time to 1–2 h (Cutter et al. 2017; McDonnell et al. 2015; Planquette and Sherrell 2012).

3.3.2.2 In Situ Filtration

In contrast to bottle sampler filtration, in situ filtration techniques allow the collection of very large volume (e.g. ~500 L; Twining et al. 2015b) size-fractionated samples of marine particulate matter from a single depth in the water column (Cutter et al. 2017), although multiple samplers can be deployed in sequence on a non-metallic cable to obtain a depth profile of particles in one single cast (McDonnell et al. 2015). Several titanium and stainless steel in situ systems are currently in use, including the ship-powered multiple unit large volume in situ filtration system (MULVFS, deployable to 1000 m depth; Bishop et al. 2012; Bishop et al. 1985), the battery-powered in situ McLane Research Laboratories Large Volume Water Transfer System (WTS-LV; referred to as ‘McLane pumps’; Fig. 3.13; deployable to 5500 m depth in water temperatures from 0 to 50 °C; Morrison et al. 2000) and the Challenger Oceanic Stand-Alone Pump System (SAPS; deployable to 6000 m depth; Fig. 3.13). Generally, multiple filters can be used for size fractionation in the samplers, and various filter types are available depending on the metal and the particle size of interest (McDonnell et al. 2015).

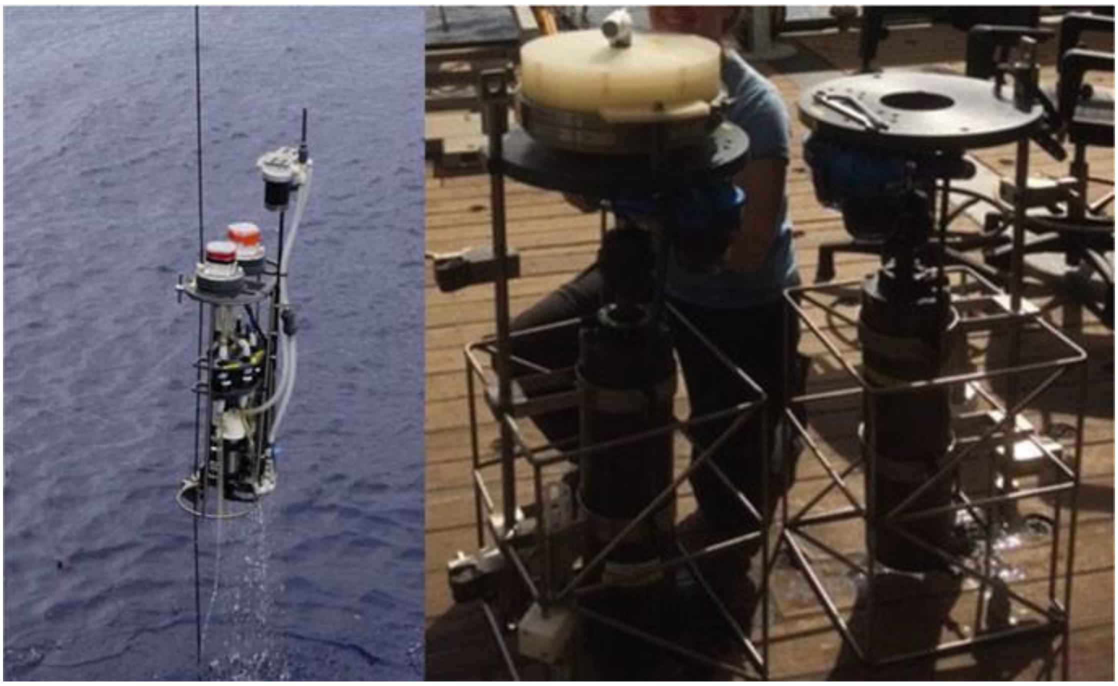

Fig. 3.13 Photographs of a McLane in situ pump (left) (photo credit: Alex Fox, Science Writer) and a Challenger Oceanic Stand-Alone Pump System (SAPS) (right) (photo credit: Maeve Lohan, University of Southampton, United Kingdom)

Depending on the system used, the target depth, the filter used and the prevalent particle concentration, large volumes of seawater can be filtered per cast with a pump speed of 1–50 L min^{-1} (Bishop et al. 2012; McDonnell et al. 2015). Conventionally, in situ pump systems are programmed to sample for several hours, but this obviously depends on the research objective and sample region.

Underway and towed sampling systems can also be used for the collection of particulate trace metals in surface waters via systems such as the towed fish using in-line filtration (Hales and Takahashi 2002; McDonnell et al. 2015). While these systems improve spatial and temporal resolutions in the upper water column as well as minimize the amount of ship time dedicated to sampling, particles might disintegrate or flocculate during collection (due to turbulent fluid environments in the tubing from the underway samplers to the ship) which inhibits quantitative assessments of size distributions (McDonnell et al. 2015).

3.4 Trace Metal Clean Sample Handling and Storage

Trace metal clean sample protocols have to be applied during all stages of sample handling and storage (see Sect. 3.2.2), especially during sample manipulation, e.g. acidification. Samples should be processed as quickly as possible after recovery of the sampling device to minimize loss of trace metals by absorption on samplers and/or bottles and avoid chemical alteration and/or speciation changes. The following sections illustrate required sample processing and handling steps at (near) ambient conditions.

3.4.1 Dissolved Trace Metal Samples

Large volume samples for operationally defined dissolved metals should be filtered through 0.2 µm cartridge filters such as Pall AcroPak capsules (Cutter et al. 2017). Investigators will typically choose a specific filter type and protocol tailored to their element of interest, choosing filters which have historically been shown to have low contamination. Different laboratories also follow different pre-cleaning protocols and use different filter brands, but a typical process is that the filters are cleaned with mild HCl, rinsed with UHPW and stored in UHPW before use (Cutter et al. 2017). Care must be taken to match the filter material and type with an appropriate cleaning method – for example, some filter types such as cellulose acetate filters should not undergo cleaning procedures besides rinsing with UHPW or sample media since they degrade under acidic conditions. Other materials may also not tolerate harsh acid cleaning.

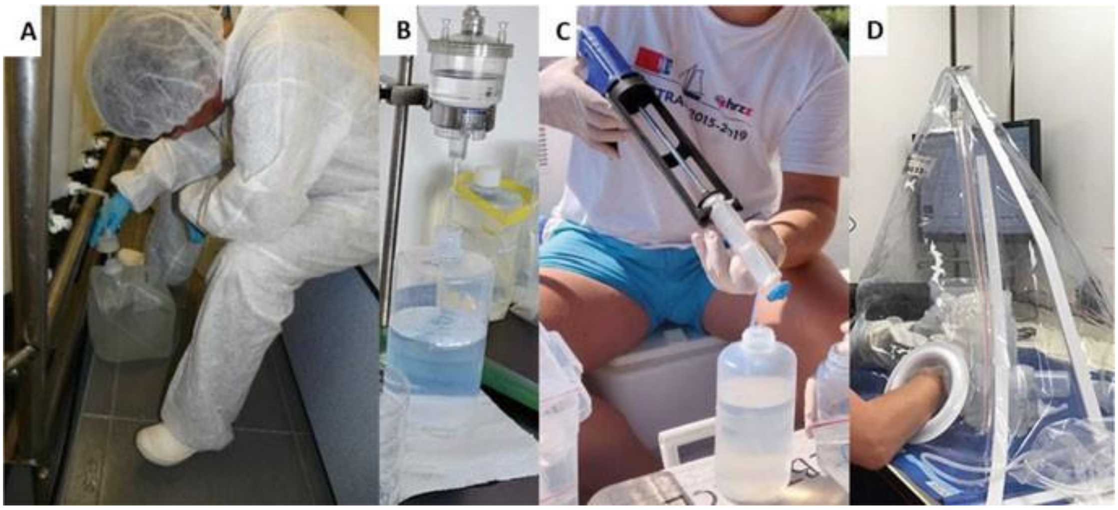

Fig. 3.14 Photographs of various filter set-ups for dissolved trace metals: in-line bottle filtration through Sartobran cartridge filters directly from the sampler (a) (photograph credit: Micha Rijkenberg, NIOZ, Netherlands), off-line filtration using an acid-cleaned vacuum filtration unit (b) (photograph credit: Dario Omanović, Rudjer Boskovic Institute, Croatia), off-line filtration through acid-cleaned plastic syringes (c) (photograph credit: Dario Omanović, Rudjer Boskovic Institute, Croatia) and syringe filtration within a ‘glove box’ under an inert gas atmosphere (d) (photograph credit: Andrea Koschinsky, Jacobs University Bremen, Germany)

Filtration of large volumes of water from bottle samplers through 0.2 µm cartridge filters is most efficient under positive pressure (filtered nitrogen (N_2) or compressed air) or vacuum (max. 0.5 bar) (Fig. 3.14a). However, care should be taken to avoid excessive pressure to prevent the risk of exploding bottles and/or the rupture or lysis of algal cells retained by the filter which may release intracellular metals into the sample (Apte et al. 2002; Cutter et al. 2017). Gravity filtration is not recommended for large volume samples over 0.2 µm filters owing to the slow flow rate which can lead to absorption or chemical alterations of the sample (Fig. 3.14) (Cutter et al. 2017). New filter capsules should be flushed, e.g. with ~0.5 L sample seawater prior to use and with ~0.2 L sample seawater in between different samples. One filter can be used for multiple depth profiles, preferably working from the surface to the deep, or filters can be dedicated to certain depth intervals, i.e. surface and deep ocean (Cutter et al. 2017). However, reusing of filters should be done with extreme care, especially if gradients are expected in the study region, for example, when going from particulate-rich samples around hydrothermal vents or near-sediment to surface samples. Filtration of small sample volumes can also be done using acid-cleaned plastic syringes with pre-cleaned filters, but this is typically too time-consuming for large samples (Fig. 3.14c). When sampling waters which are anoxic or from low-oxygen environments, once bottle samplers are brought to the surface, samples should be processed within a 'glove box' under an inert gas atmosphere (Fig. 3.14d). This approach ensures that the integrity of the sample is maintained, i.e. minimization of the ratio of oxidation and precipitation reactions which may change the phase (dissolved to particulate) and/or speciation of trace metals of interest (e.g. US-Geological-Survey 2006). However, it is important to note that a headspace of an inert gas such as N_2 has been shown to facilitate outgassing of CO_2 which can lead to changes in pH of the sample with potential consequences for dissolved metal concentrations and speciation (Fitzsimmons and Boyle 2012).

Prior to collection of a filtered seawater sample into an acid-cleaned sample collection bottle (see Sect. 3.2.4), it is recommended to condition and rinse the empty sample bottles (including the cap) at least three times with the filtered seawater sample, each of which is discarded to waste, before finally filling the bottle with the sample. Sample bottles for dissolved trace metal or isotope analysis should be filled to the bottle shoulder to ensure that bottles are filled to the same amount and thus acidified to a similar acid concentration later on (Cutter et al. 2017). Ideally, acidification of the sample to below pH 2 should be carried out as soon after filtration as possible (Cutter et al. 2017), in order to avoid wall adsorption that can take a long time to resolubilize (Jensen et al. 2020). Sometimes, however, shipboard acidification is not practical. In this case, filtered samples that are stored unacidified should be left for an appropriate time after acidification and before processing (typically several months), in order to resolubilize metals which have adsorbed to the container walls. The preferential method of sample acidification is to add a volume of concentrated ultraclean HCl to achieve a final concentration of either 0.012 or 0.024 M HCl in the sample, depending on the element of interest and the preference of the research group (Cutter et al. 2017). Use of HNO_3 for acidification is typically avoided because it complicates commercial transport of these samples (Cutter et al. 2017). Following acidification, sample bottles should be tightly closed and double bagged in resealable polyethylene bags for storage (preferably at room temperature and in the dark) until analysis. Labels should be put both on the sample bottle and the bag, so that samples can be kept organized.

For dissolved metal speciation (organic ligand) samples, filtered samples (0.2 µm) should be stored in acid-clean bottles, kept at natural pH (without acidification) and either stored in the fridge (+4 °C) or frozen (−4 °C or −20 °C) until voltammetric analysis in the home laboratory or measured 'fresh' directly on-board ship (Bruland et al. 2000; Buck et al. 2012; Pađan et al. 2020; Sander et al. 2005). In all cases, speciation samples must be stored in the dark in order to prevent photodegradation of the prevalent ligands, and it should be verified that the pre-cleaning procedure does not result in leaching of acid into the sample (i.e. a gentle acid cleaning procedure should be used). The most appropriate storage procedure of metal speciation samples, which avoids changes in speciation parameters pending analyses, is still a topic of discussion in the marine chemistry community (e.g. Buck et al. 2012).

3.4.2 Size-Fractionated Dissolved Trace Metal Samples

Current understanding of the cycling of metals is largely based on observations of the dissolved metal fraction, which is usually operationally defined as everything that passes through a filter with 200 nanometre pores (0.2 µm; see Fig. 3.1). However, such a sharp boundary does not reflect the continuum in which metals are actually present in seawater, which ranges from truly dissolved molecules (<0.02 um soluble fraction) to nanoparticles (<100 nm) via colloidal size(<200 or <400 nm) and even larger-size particles (Santschi 2018). Ultimately, all size cut-offs are arbitrary operational definitions, and at some point, the question whether something is a very small particle, or a relatively large molecule becomes a philosophical question. Perhaps the more interesting scientific consideration is, however, at what point molecular Brownian motion becomes dominant over gravitational settling (Honeyman and Santschi 1989; Wells and Goldberg 1992) or, in other words, whether a substance behaves like a particle or a dissolved substance. Such behaviour is of course a function of both particle size and density, as well as other factors such as temperature (e.g. Farley and Morel 1986) that are beyond the scope of the discussion in this chapter, but should occur somewhere around the transition from the colloidal to the particulate size class.

Metal size fractionation studies generally focus on the difference in size classes of particles and colloids before and after filtration, i.e. trace metal levels of particles in solution (the filtrate) and retained on the filter (the retentate) (Bergquist et al. 2007; Fitzsimmons and Boyle 2014b; Fitzsimmons et al. 2015a; Fitzsimmons et al. 2015b; Ussher et al. 2010). The most common practice used to classify trace metal concentrations beyond the dissolved and particulate fraction is referred to as ultrafiltration. Ultrafiltration (UF) is a pressure-driven filtration process that separates particulate matter from truly colloidal and soluble compounds using an ultrafine membrane media. For this, a 0.2 µm filtered seawater sample – with commonly used capsule filters that are not suitable for studying the material retained on the filter – undergoes another so-called UF step, often using either cross-flow filtration (CFF) or Anopore filter membranes. With this step, the soluble metal fraction can be obtained in the filtrate, and the difference between the dissolved (0.2 µm fraction) and soluble (UF fraction) gives the calculated colloidal metal fraction. The Anopore filter membranes have a pore size of 0.02 µm, whereas CFF filters are often defined by the cut-off size of molecules they let pass through, e.g. 10 kDa in case of a Millipore Pellicon XL (PLCGC) filter (Fitzsimmons and Boyle 2014a; Jensen et al. 2020), complicating direct comparison of size fractions between the two filtration techniques. There are advantages and disadvantages of both filtration techniques— for example, CFF is quicker for filtering larger volumes of seawater, but it is a more complex technique and requires more expensive equipment, more cleaning and more training. In contrast, Anopore filter units are cheaper and simpler to user, but much slower for filtering larger volumes. However, Anopore may be the better choice if only small volumes of sample (e.g. <150 mL) are required for analysis of the parameter of interest. For a more comprehensive discussion of the pros and cons of each filtration technique, when deciding which to use, we refer the reader to Fitzsimmons and Boyle (2014a).

3.4.3 Particulate Trace Metal Samples

Akin to the definition for dissolved metals, the ‘particulate’ trace metal phases are also operationally defined, based on particle size (see Sect. 3.3.2; Box 3.6). To collect particulate trace metals from bottle samplers, the use of pre-cleaned polycarbonate or fluoropolymer filters holders with polyethersulfone (PSE) or mixed cellulose ester filters of diameters between 25 and 47 mm is recommended (Cutter et al. 2017) (Fig. 3.15).

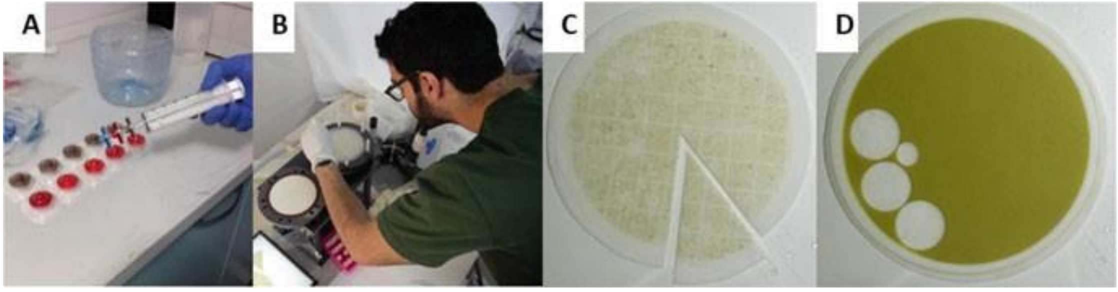

Fig. 3.15 Photographs of a stacked syringe filter system (a) (photograph credit: Dario Omanović, Rudjer Boskovic Institute, Croatia), and an in situ pump filter holder disassembled inside of a HEPA filtered clean air bubble (b) (photograph credit: Alex Fox, Science Writer, picturing Vinicius Amaral, University of California, USA). The filter holder shown in (b) can contain several filters for in situ size fractionation. Picture (c) shows a 51 μm polyester mesh filter and (d) shows a 0.8 μm polyethersulfone (PES) filter. Filters (c,d) can be sub-sampled for multi-investigator use (photograph credit: Daniel Ohnemus, Skidaway Institute of Oceanography, USA)

Depending on the type of analysis, it is usually advised to use the smallest filter diameter to maximise the particle loading per filter area and thus ensure a sufficient sample to filter blank ratio. The implemented filter pore size varies depending on the research objective, but 0.45 μm is currently the GEOTRACES standard for particulate trace metal sampling. If continuous size fractionation of the particulate trace metal pool is of interest, it is also possible to use different filter pore sizes with a single syringe in a stagged configuration (Fig. 3.15a).

For the actual filtration, the acid-cleaned filter holders with pre-cleaned filters should be connected to a pressurized bottle, container or pump outlet using acid- cleaned tubing (Fig. 3.14a). Trapped air in the filter holders should be cleared by unscrewing the holder to allow a small volume of water to flow around the filter, before the sample water can pass through the filter. The volume of water passing the filter must be recorded for later quantification (i.e. by collecting the water in a secondary container or measuring cylinder) and can vary drastically depending on the area of interest and associated particle loading in the water column. If the filter clogs, filtration should be stopped, and the filtrate volume should be recorded. To avoid filter rupture (pressure built up), the filtering rate should not exceed about one drop per second (Cutter et al. 2017). As stated by Cutter et al. (2017), filtration times >2 h should be avoided to prevent speciation changes and particles settling within the bottle. It is also important to seal and tighten the filter holder appropriately (i.e. avoid miscalculation of filtrate volume) and to ensure that the filter lies flat for successful filtration (i.e. trapping all particles on the filter). Leaking membrane filter holders should be identified and recorded since they can be a major source of contamination. Further, each filter holder should be marked with a unique number, so that samples can be kept organized.

To collect particulate trace metals from in situ pumps, various filter sizes (both membrane diameter and pore size) and plastic filter types can be used depending on the research objective and the preferred filter digestion method (McDonnell et al. 2015). GEOTRACES intercalibration expeditions have shown that cleaned polyethersulfone (PES) filters are a good choice for trace metal work (Bishop et al. 2012). Akin to the filtration step for the bottle samplers, multiple filter plates with different pore sizes can be paired for in-line size fractionation work (Fig. 3.15) with particles typically defined as suspended, slowly sinking or fast sinking (Riley et al. 2012) or relatively large- and small-size fractions based on used filter sizes (Lam et al. 2015). Most filter holders also contain a baffle system, i.e. a prefilter (plastic film or grid cover), sitting on top of the first filter to reduce turbulence, distribute particles evenly across the filter and minimize particle loss during pump retrieval (Bishop et al. 2012). An advantage of the in situ sampler is the possibility to distribute filter sub-samples to multiple investigators owing to the large filter holder size of many commercially available in situ pumps (~142 mm for standard McLane pumps and MULVFS) (Fig. 3.15c, d) (McDonnell et al. 2015).

Box 3.6: Ultrafiltration for Colloids and Particulates

It is also possible to study particulate trace metals collected on filters during ultrafiltration (UF; see Sect. 3.4.2). Commonly, particulate trace metals are studied on filters with pore sizes of 0.2 µm, 0.4 µm or larger; however, smaller fractions can be studied even though this process is more time-consuming and thus less practical. The longer processing time is problematic for maintaining the integrity of most trace metals (i.e. precipitation, chemical alteration, adsorption), especially for Fe given its tendency for wall absorption when samples are not acidified on time scales of hours (Fitzsimmons and Boyle 2012). Consequently, when relatively large ultrafiltered volumes are needed for analysis of the 'soluble' phase, preference is given to filters with a fast flow rate to avoid artefacts and alterations occurring during filtration (Jensen et al. 2020). However, none of those commonly used 'fast' filters allow assessment of retained material. For instance, cross-flow filtration (CFF) filters are not designed to capture the colloids that partly end up on the filter with the majority in the retentate. By contrast, Anopore filters may capture the colloid fraction, but are made electrochemically by the anodic oxidation of aluminium and contain particulate Fe inclusions; the latter makes these filters unsuitable for common chemical leaching and/or digestion techniques as well as microscopy techniques aimed at identifying and quantifying colloidal Fe (Fitzsimmons and Boyle 2014a). Thus, investigators interested in the colloidal fraction of trace metals need to implement UF with filters that are free of metals (low filter blank) such as polycarbonate (PC) filters or polyethersulfone (PES) filters (Cullen and Sherrell 1999). However, previous testing of these two filters resulted in slow flow rates, and thus these filters were deemed unsuitable for UF studies of trace metals (Fitzsimmons and Boyle 2014a). Other colloid separation techniques such as reverse osmosis-electrodialysis Koprivnjak et al. 2009) or flow field-flow fractionation techniques (Santschi 2018) do exist but have, to the best of our knowledge, so far, not been used to study colloidal trace metals in a contamination-free manner.

When filtration is complete, either on board or using an in situ pump (see Sects. 3.3.2.1 and 3.3.2.2), residual water in the filter holder has to be removed using syringes or vacuum to reduce the residual sea salt matrix for analytical simplicity (Cutter et al. 2017). Inside a laminar flow bench, filter holders should be disassembled, and filters should be removed using plastic acid-cleaned forceps before storing them individually in a clean labelled petri dish, tube or similar, at

−20 °C freezer to physically stabilize the sample. For larger filters, the primary filters may be cut up using a ceramic acid-leached blade scalpel to provide sub-samples. Photo documentation of the filter before and after sub-sampling can be of use to document filter heterogeneity Cutter et al. 2017. Once filters are removed, the filter holders should be rinsed with acid and UHPW before next use.

Prior to analysis of the particulate trace metals, the filters and/or the material on them needs to be digested (Sherrell and Boyle 1992). Various full or partial digestion techniques and protocols are available depending on the metal of interest, the filter used and whether the filter should stay intact or not. After digestion the digest can be (re-)diluted with a specific matrix solution prior to analysis (Sherrell and Boyle 1992). Rather than a full digestion of the particulate metal pool, researchers can also carry out partial digestions or 'leaches' with leachates of various strength to characterize a specific portion of the particulate trace metal pool, e.g. labile, refractory, bound to carbonates, bound to organic matter, etc. (Berger et al. 2008; Tessier et al. 1979). Sequential leaching techniques can also be used to characterize multiple fractions of the particulate trace metal pool. Although more time-consuming and costly, sequential leaching methods provide more detailed information about the origin and fate of trace metals in the study area (Tessier et al. 1979). Overall, many protocols exist for digestion and leaching methods that are not further detailed here but can be found elsewhere (e.g. Ohnemus et al. 2014; Rauschenberg and Twining 2015; Twining et al. 2015a).

3.5 Sample Processing and Analytical Techniques

Once a clean sample of seawater is collected, filtered, acidified (if appropriate for the element and analytical technique of interest) and stored, the next challenge is to analyse the sample for trace metal concentrations (see Sect. 3.5.1), isotopic composition (see Sect. 3.5.2) or speciation (see Sect. 3.5.3). This chapter has already discussed the challenges of collecting and processing contamination-free samples, and such procedures must be maintained throughout analysis to generate accurate results. However, contamination-free analysis is not trivial since most analyticalmethods aim to pre-concentrate the trace metals into a smaller size sample for analysis, and such an approach usually involves the use of multiple reagents, equipment and steps. Further, the challenges of pre-concentration and/or measuring the very low concentrations of trace metals and isotope ratios in seawater accurately are compounded by the sea-salt matrix, which contains very high concentrations of major ions such as Na^+, Ca^{2+} and Cl^- (at typical salinity these ions are present at 35 g kg^{-1}), all of which can interfere with the signal of interest.

As mentioned in Sect. 3.2, the advent of trace metal clean sampling and handling techniques in combination with advances in modern analytical chemistry and instrumentation was critical to obtain a first-order understanding of the concentration of trace metals in seawater, later followed by insights in both their speciation (see Sect. 3.5.3) and isotopic composition (see Sect. 3.5.2). Notably the availability of the graphite furnace as the sample introduction system to an atomic absorption spectrometer (GF-AAS) in the mid-1970s was pivotal, but this was superseded by flow injection techniques (FIA) which pre-concentrated the metal(s) of interest onto a column (Sohrin and Bruland 2011; Worsfold et al. 2014) and which have since largely been supplanted by the (even) more sensitive and powerful high-resolution (HR) inductively coupled plasma mass spectrometers (ICP-MS) (Sohrin and Bruland 2011). While flow injection methods are often still used shipboard, ICP-MS is the current standard for determination of trace metals and their isotopes (TEIs) in shore-based laboratories as it provides high sensitivity, high accuracy, low limits of detection (LOD), linear response to the analyte(s) over a wide dynamic range and high sample throughput and is a powerful tool for the simultaneous determination of multiple elements.

Here we describe ICP-MS techniques for trace metal analysis in Sect. 3.5.1.1, shipboard flow injection techniques (FIA) for trace metal analysis in Sect. 3.5.1.2., systems for in situ trace metal analysis in Sect. 3.5.1.3, multi-collector ICP-MS techniques for trace metal isotope analysis in Sect. 3.5.2 and voltammetry techniques for trace metal speciation in Sect. 3.5.3. Throughout, for further reading, we mainly refer the reader to synthesis review articles or textbook chapters as a starting point, rather than attempting to cite all of the available research on the topic.

3.5.1 Trace Metal Concentration Measurement Techniques

3.5.1.1 ICP-MS Techniques

Several varieties of ICP-MS exist (Olesik 2014), including high-resolution sector field (HR-SF) and quadrupole instruments that differ in how they separate analytes from each other and how they resolve interferences, which is beyond the scope of this chapter. A HR-SF-ICP-MS is typically required in laboratories analysing seawater samples, principally in order to resolve interferences from the argon (Ar) carrier gas typically used by ICP-MS instruments (Sohrin and Bruland 2011; Wuttig et al. 2019). In an ICP-MS, a sample is vaporized in the sample introduction system, and its elements are atomized and then ionized in an Ar plasma. The resulting ions enter the vacuum inside the instrument through two interface cones,i.e. the sampler cone and the skimmer cone, which focus and guide the ions into the mass spectrometer. The mass analyser separates ions according to their distinct mass to charge ratios (m/z) via magnetic and electrostatic fields before they reach and are measured at the detector. Variations in the magnetic and electrostatic fields allow detection of different ions based on their m/z ratio, where charge is usually +1 (or +2) in the plasma.

However, different elements can have ions with the same m/z ratio, and this must be carefully addressed. For example, Fe and Ni have isotopes at mass 58 (57.9332744 and 57.9353429 amu, respectively), and thus any signal measured at m/z 58 will have contributions from both $^{58}Fe^{+}$ and $^{58}Ni^{+}$. Such interferences are described as ‘isobaric’ and can be avoided by measuring another isotope of the element of interest or by doing a subtraction correction by measuring another isotope of the element without interference and applying a natural abundance ratio. Typically, isobaric interference peaks are too close together in m/z to be separated by HR-SF-ICP-MS, unlike many ‘polyatomic’ interferences (see below). A second potential isobaric interference type comes from doubly charged ions (e.g. $^{116}Sn^{++}$ also has a m/z of 58), but these are typically only formed at low levels in the plasma and as such only become a problem if that element is present at high concentrations in the sample.

The second potential type of interference on ICP-MS is known as a ‘polyatomic’ interference and arises from polyatomic molecules that are formed by the combination of two (or more) molecules in the plasma. For example, when measuring Fe, the most abundant isotope is ^{56}Fe, which does not have isobaric interferences. However, in the case of $^{56}Fe^{+}$, several polyatomic interferences cause problems, especially $^{40}Ar^{16}O^{+}$ from the carrier gas and $^{40}Ca^{16}O^{+}$ that can come from the seawater matrix. Another particularly problematic polyatomic interference arising from the seawater matrix is MoO^{+} which interferes with Cd measurements. Such interferences can cause issues for low-resolution instruments such as quadrupole ICP-MS, which may not be able to resolve the interference from the peak of interest. HR-SF-ICP-MS addresses many polyatomic interferences by using a higher ‘resolution’, which allows peaks that are close to each other to be separated (resolved). To obtain a higher resolution, narrower ‘slits’ are used that further constrict the ion beam and thus allow better separation of ions with very similar m/z. This separation achieves the goal of avoiding interferences, but comes at the expense of sensitivity (signal size) as less ions reach the detector. Alternatively, modern quadrupole ICP-MS instruments can be used, utilising ‘reaction cells’ as an alternative method to minimize polyatomic interferences for seawater applications (Jackson et al. 2018). In such instruments the m/z of the interference (or the target analyte) is changed in the reaction cell via a chemical reaction, allowing subsequent separation. Here we have focused on the more widespread use of HR-SF-ICP-MS.

Avoiding or correcting for interferences can be challenging, depending on the sample matrix and the relative concentrations of interferences and analytes of interest. Thus, the operation of a HR-SF-ICP-MS or any other types of ICP-MS for the determination of trace metals requires substantial expertise that varies with the application and objective of the research and the element of interest. Further discussion on this is beyond the scope of this chapter. In the next sections, we focus on matrix removal (salt matrix) and pre-concentration applications. These applications are required before most analytical techniques can be implemented, including ICP-MS. This section is then followed by some more detail on ICP-MS analysis techniques commonly used within the trace metal community.

Matrix Removal and Pre-Concentration Prior to ICP-MS Analysis

From a limit of detection perspective, the concentrations of most marine trace metals should be measurable directly using a HR-SF-ICP-MS without pre-concentration. However, the salt matrix prevents such direct injection of marine samples into the instrument as the high concentration of salts (Na^{+}, Ca^{2+}, Cl^{-}) leads to interferences and clogging of the cones and introduction system, resulting in substantial reduction and variations in signal sensitivity as well as inaccuracy. Thus, most ICP-MS seawater trace metal concentration techniques (as well as FIA; Section 3.5.1.2) and isotope ratio techniques (Section 3.5.2.2) involve a matrix removal step prior to analysis.

A matrix removal step typically also has the added benefit of constituting a significant pre-concentration step as analytes of interest are concentrated at the same time as the analyte is isolated from the (interfering) major ions in the matrix. Pre-concentration methods include co-precipitation with Mg, solvent extraction and/or solid-phase extraction (SPE) with chelating resins (Sohrin and Bruland 2011), where the latter is now the most commonly used technique in trace metal chemistry (Wuttig et al. 2019).

Typically, for SPE, a chelating ligand is immobilized by covalent bonds on a stationary resin phase packed in a column or sometimes added to the resin as beads (Lee et al. 2011b). A seawater sample solution of which the pH is adjusted to the right range, depending on the chelating resin and analyte of interest, is subsequently passed over the resin column where the analyte of interest forms a chelate with the resin's binding sites and is thus retained. Major matrix ions such as the sea salts (Ca, Na, K etc.) are usually not retained by the resin at certain pH, and hence the pH of the sample solution plays a key role during matrix removal methods using SPE. The retention efficiency (recovery) of the analyte of interest on the resin is also dependent on the seawater sample pH and thus should be observed carefully and adjusted if necessary using appropriate reagents (buffers or acids) (Sohrin and Bruland 2011). After retention, the analyte of interest is eluted from the resin with an appropriate acidic eluent. Typically, this is done with a much smaller volume than the original seawater sample, leading to a substantial pre-concentration factor (Fig. 3.16). A range of chelating resins exist and have been used historically by trace metal chemists, but notably Nobias PA-1 has become increasingly popular (Sohrin and Bruland 2011). This chelating resin is also used in the column material of the commercially available SeaFAST system (Elemental Scientific (ESI)), an automated pre-concentration system for undiluted seawater (Lagerström et al. 2013; Wuttig et al. 2019). This system is currently considered 'state of the art' and in use in many trace metal clean laboratories around the world, including the Royal Netherlands Institute for Sea Research (NIOZ; the Netherlands). The following section details some of the typical procedures, analysis steps and experiences gained by the NIOZ laboratory team using the SeaFAST system, as an example of a commonly used pre-concentration and salt removal method.

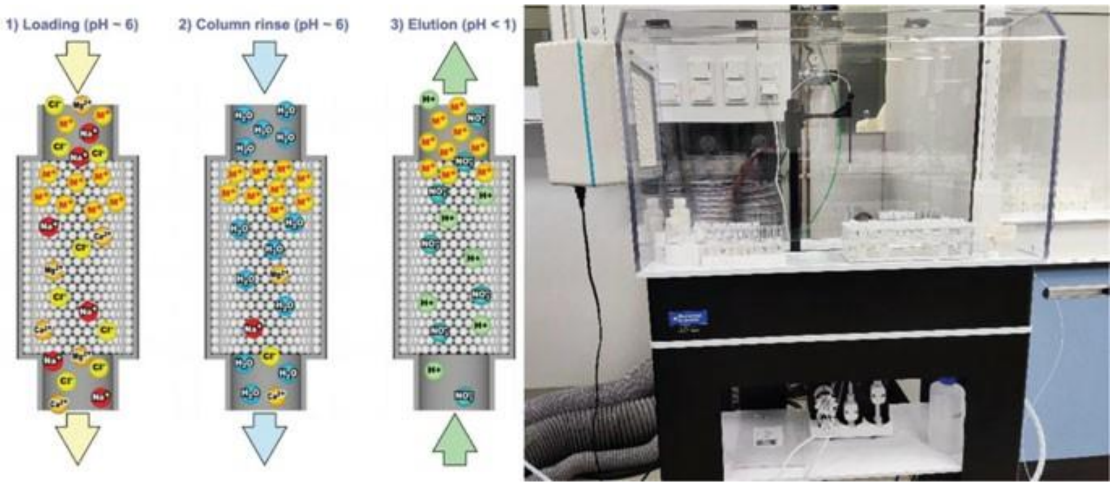

Fig. 3.16 Illustration of a solid-phase extraction (SPE) of metals on a Nobias Chelate-PA1 column in three steps (right) (figure courtesy of Elemental Scientific (ESI)), and a photograph of a SeaFAST (left) (photograph credit: Patrick Laan, NIOZ, Netherlands). SPE Extraction: (1) Loading: in this step seawater

at the right pH passes over the column, and analyte(s) of interest (M^+) are retained, whereas the matrix (salts) mostly passes through. (2) Rinsing: in this step the remaining seawater and most salts are rinsed from the column. (3) Elution: the analyte(s) of interest are eluted from the column with elution acid that can subsequently be analysed by ICP-MS

SeaFAST: Automated Extraction of Metals from Seawater

The SeaFAST system consists of an autosampler, a syringe pump module and valves that pre-concentrate metal(s) from acidified seawater samples onto the resin, and then either elute the analyte(s) directly into an ICP-MS system when using the in-line configuration (see Box 3.7) or collect the eluent in small vials for later analysis during the off-line mode (Lagerström et al. 2013). The latter mode is used in the NIOZ laboratory (Gerringa et al. 2020) and other trace metal clean facilities (e.g. Rapp et al. 2017; Wuttig et al. 2019) as it saves on instrument runtime and allows for better multi-resolution analysis on the ICP-MS (see Box 3.7). After off-line extraction (i.e. extracting the metals from the seawater matrix with the SeaFAST system), a whole batch of collected samples can be run right after each other using the ICP-MS with minimum idle time of the plasma, especially when using a high-throughput sample introduction system (e.g. the double loop MicroFAST MC (Elemental Scientific (ESI)) as in use in the NIOZ laboratory). Lagerström et al. (2013) describe the SeaFAST system in more details, but general steps regarding sample loading, pre-concentration, matrix removal and metal elution steps are briefly described below (Fig. 3.16).

Box 3.7: SeaFAST in-Line Versus off-Line Configuration

When using the SeaFAST in the in-line mode, the ICP-MS is running and idle, while the SeaFAST is still in the pre-concentration step, resulting in higher (and wasted) analysis costs. Additionally, the analytes are detected as an elution curve, meaning that the signal builds up to a maximum and decreases again in the shape of a peak. An ICP-MS generally does not measure analytes simultaneously but has to cycle through the analytes of interest which takes time especially if switching between resolution (low or high resolution to resolve interferences; see Sect. 3.5.1.1) is required, limiting the number of analytes that can be measured in an elution curve (i.e. after a given amount of time, the peak has passed so there is only a limited number of elements that can be measured in that time). Nevertheless, the in-line mode can be useful for method development of new analytes of interest, for example, testing when an analyte is eluted off the column with a given eluent. When using the SeaFAST off-line, the collected eluent is homogeneous in concentration, enabling analysis of as many analytes as the eluent volume allows, where obviously a greater elution volume leads to lower sensitivity as the analyte concentration is diluted.

During operation of the SeaFAST system, an autosampler probe moves into the sample and fills a sample loop (typically 10 mL) using an integrated vacuum pump. The sample loop is ‘overfilled’, i.e. the loop gets rinsed by the first ~2 mL of sample which goes to waste (i.e. 12 mL is taken up of which 10 ml stays in the loop). Subsequently, by syringe pump action, the 10 mL sample is pushed from the loop, buffered to the appropriate pH using an ammonium acetate buffer (see Box 3.8) and immediately passed over the Nobias PA-1 pre-concentration resin column. Prior to mixing, the buffer solution is passed over a 'clean-up' column with the same chelating resin as the pre-concentration column to minimize any trace metal contribution from the buffer solution. Depending on the needed pre-concentration factor, sequential 10 mL aliquots can be pre-concentrated over the column, where at NIOZ, 2 x 10 mL (20 mL) is commonly pre-concentrated for low-metal open ocean samples. After sample loading, UHPW is pushed over the pre-concentration column to remove the residual salt matrix remaining in the column. The ensuing elution of the chelated metals on the resin then occurs in the reverse direction (compared to sample loading) with elution by ultra-pure 1.5 M nitric acid into a clean sample vial using pressurized N_2 gas as a carrier. The volume (and strength) of elution acid can be varied depending on required ICP-MS analysis time and/or the desired pre-concentration factor. At NIOZ, a volume of 350 µL is typically used, resulting in a pre-concentration factor of ~57x (20 mL sample preconcentrated into 0.35 mL). A choice of sample vials can be used, including LDPE, PFA, polyvinylidene difluoride (PVDF) or polypropylene (PP), as long as they are rigorously cleaned before initial use and between different samples (see Sect. 3.2.3).

Box 3.8: Solid-Phase Extraction (SPE) and pH

At NIOZ, a pH of 5.8 ± 0.2 (obtained with a more dilute buffer than recommended by the manufacturer) has been determined optimal for the suite of routinely measured metals using the SeaFAST (Middag et al. 2015a), whereas a slightly higher pH and a more concentrated buffer are often used in other laboratories (Wuttig et al. 2019). There is a trade-off between pH and recovery, notably for Mn and Fe where a somewhat lower pH leads to better recovery for Fe, but below a pH of 5.5, recovery for Mn becomes non-quantitative (Middag et al. 2015a). Thus, it is important to check the pH of the seawater that passed the pre-concentration column regularly (easily done at the waste outlet of the SeaFAST) and monitor recovery in every extraction run. However, different laboratories report slightly different optimum pH, implying that the recovery of metals might vary between different set-ups depending on local laboratory conditions and practices.

The SeaFAST columns are cleaned after elution with 1.5 M nitric acid to eliminate carry-over (memory effects) of subsequent samples. Nevertheless, some carry-over can still occur – for example, Rapp et al. (2017) reported a carry-over of 0.5–1.3% for Fe and Ni, and Wuttig et al. (2019) also observed carry-over of <1% for some elements of interest. The carry-over effect can be minimized by pre-concentrating samples from presumably low to high initial trace metal concentrations or by processing of low-metal seawater and/or dummy samples (i.e. where the eluent is not collected but deposited in the waste of the system) in between samples.

At NIOZ, prior to pre-concentration using the SeaFAST system, an aliquot (30 mL) of each filtered and acidified seawater sample is pipetted into an acidcleaned FEP bottle, followed by addition of hydrogen peroxide (see Box 3.9) and an internal standard (see Box 3.10). In addition to major ions, seawater contains organic material, i.e. ligands, that can chelate metals (see Sect. 3.5.3 on speciation). Some of these organic ligands are destroyed by acidification after sampling, but others can persist in the sample, interfering with the extraction of metals from seawater via the chelating resins. In particular, Co and Cu chelated to dissolved organic ligands can pass through the pre-concentration column without binding to the resin (see Box 3.9; Fig. 3.17) (e.g. Biller and Bruland 2012; Lagerström et al. 2013; Middag et al. 2015a; Rapp et al. 2017; Wuttig et al. 2019). Using UV digestion after hydrogen peroxide addition (see Box 3.9) of samples prior to extraction destroys the organic ligands that chelate trace metals in solution which would otherwise outcompete the resin's functional groups (see Box 3.9).

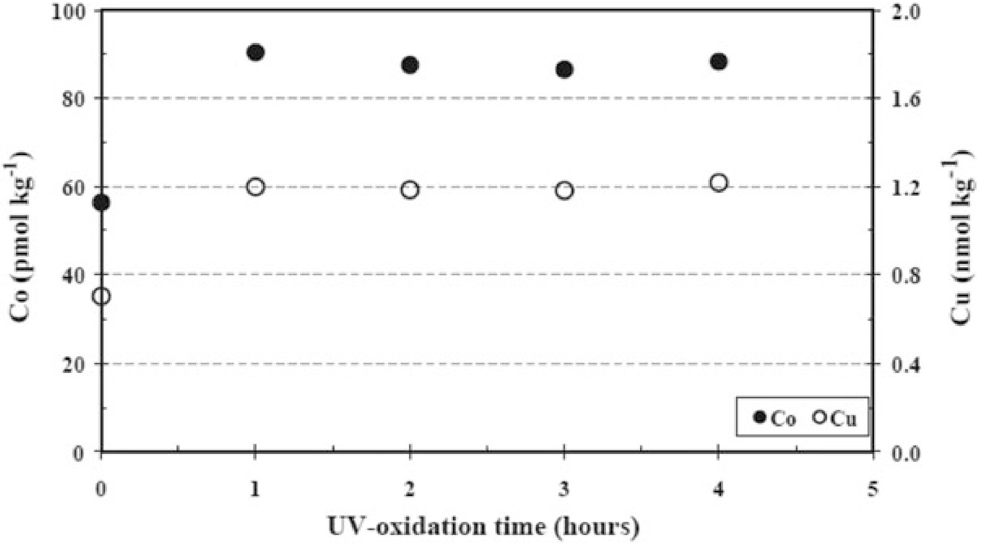

Fig. 3.17 Concentrations of Cu and Cd in acidified natural seawater, determined as a function of UV digestion time (Biller and Bruland 2012)

Box 3.9: UV Digestion

Rapp et al. (2017) and Wuttig et al. (2019) assessed the influence of quartz and FEP vessels on UV digestion efficiency and contamination. No difference between a FEP bottle and a quartz cuvette was observed with regard to the efficiency of UV digestion using either vessel material. However, contamination from quartz vessels was observed for Pb, Ti, Fe and Zn, whereas an increase in Ti was observed in the PTFE bottle during UV digestions (Rapp et al. 2017; Wuttig et al. 2019). These tests suggest that FEP bottles are suitable for UV digestion with the added advantage of being suitable for rigorous cleaning protocols due to their inert behaviour when subjected to hot acids, thus decreasing contamination from the digestion vessel. Moreover, FEP bottles can be placed directly into the SeaFAST autosampler after the digestion step, minimizing further sample handling.

At NIOZ, samples inside FEP bottles are irradiated in a custom-built UV box containing 4 TUV 15 W/G15 T8 fluorescent tubes for 4 h (Fig. 3.17) after addition of clean hydrogen peroxide (final concentration ~ 30 µM). The addition of hydrogen peroxide leads to the formation of reactive radicals during the UV irradiation, assisting in the breakdown of organic ligands. Using this procedure, an increase in concentration of 17% and 15–50% for Cu and Co, respectively, has been reported after UV digestion of samples (Wuttig et al. 2019). After irradiation, the samples are usually left for at least another 4 h to cool to room temperature and to let any leftover radicals react prior to the SeaFAST pre-concentration step.

A typical batch of samples processed at NIOZ, besides actual samples, consists of calibration standards (standard additions), reference samples and blanks (acidified UHPW). Natural seawater containing low concentrations of metals (e.g. North Atlantic surface water) is used as the matrix for standard additions, but if that is not available, low-metal seawater can be made by passing it over a chelating resin column. A calibration line is made by adding increasing amounts of in-house prepared multi-element stock standard solution (from high purity commercial standard solutions) with natural isotopic abundances to known volumes of the low-metal seawater (typically 30 mL). The highest added concentration depends on expected concentrations in the samples where the multi-element stock standard should be designed so that the highest standard addition is approximately 120% of the highest expected sample concentration. Usually, a calibration is prepared in duplicate where one set is extracted preceding the samples and one set extracted after the samples, in order to be able to account for any drift (changes in instrument sensitivity) or changes in recovery of the chelating resin over time.

An alternative approach for calibration is the use of the isotope dilution technique, which has been successfully used for seawater samples with SeaFAST extraction in the past (e.g. Lagerström et al. 2013; Rapp et al. 2017). This approach involves the addition of a known volume of a ‘spike’ made by dissolution of a highly purified single isotope of an element. For example, for Fe, where natural Fe abundance is 92% for ^{56}Fe and just 2% ^{57}Fe, a spike may be prepared that is 99% ^{57}Fe. This way, by adding a known amount of ^{57}Fe spike to a known amount of acidified seawater, prior to SeaFAST pre-concentration, and then measuring the ratio of ^{56}Fe/^{57}Fe in the pre-concentrated sample via ICP-MS, the original amount of natural sample Fe can be calculated (and then converted to a concentration using the volume or mass of the original sample). The benefit of the isotope dilution method is that ^{56}Fe/^{57}Fe ratios are not affected by recovery efficiency (i.e. efficiency of resin in retaining the element of interest), and so the sample concentration of Fe obtained is unaffected by incomplete recovery on the SeaFAST, variable recovery of different samples or changes in intensity of the signal on the ICP-MS during the analytical run. However, isotope dilution is not possible for monoisotopic elements, such as Mn and Co, and thus multiple elemental analysis requires a combination of isotope dilution and standard additions (if those elements are of interest). It also takes time and money to purchase and prepare spike solutions. Further, spike addition should be broadly matched to the expected concentration of the metal of interest (with some flexibility), and so it can be complicated to prepare a multi-element spike that is appropriate for a range of seawater samples (e.g. when Cd can vary from 0.00003 to 1 nmol kg^{-1} in a North Atlantic seawater depth profile, while Fe only varies from 0.1 to 0.6 nmol kg^{-1}; Schlitzer et al. 2018).

It is also worth noting that the methods used for trace metal isotopic analysis in Sect. 3.5.2 also often make use of isotope dilution techniques on larger volumes of seawater to generate concentration datasets. Ultimately, the choice between SeaFAST standard addition and SeaFAST isotope dilution (or other methods such as flow injection analysis) often depends on the capacity, capability and experience with one or the other method in a given laboratory, as both calibration methods have been shown to produce accurate, comparable and publishable results—and both have led to datasets included in GEOTRACES data products (see Sect. 3.6).

At NIOZ, besides calibration standards, a suite of in-house reference samples is processed in every SeaFAST extraction run (see also Sect. 3.5.1.4). These reference samples are usually (acidified) sub-samples of a large sample stored in 20 L batches in large acid-cleaned containers and are used to track the consistency within and between extraction runs. At NIOZ, North Atlantic deep water, North Atlantic surface water and column-cleaned low trace metal seawater (natural seawater passed over a large Nobias PA1 column prior to acidification) are typically used as in-house reference samples as they cover a wide range of trace metal concentrations. Triplicate samples of the in-house reference samples are extracted with the start and end calibration of each run and at least once in between the actual samples. In selected extraction runs, community consensus reference samples (CCRS) from the GEOTRACES programme (see https://www.geotraces.org/standards-and-refer- ence-materials/) are also run to verify the in-house reference samples to these CCRS. The CCRS were collected by trained experts at various global locations into large volume seawater containers and then sub-sampled into smaller LDPE bottles for distribution within the trace metal community. For example, the SAFe samples were collected from the SAFe station in the North Pacific and were made available to investigators by Ken Bruland at UC Santa Cruz (Johnson et al. 2007). The CCRS have been traditionally used (and accuracy and precision reported) by investigators measuring trace metal concentrations. However, most of these samples are now exhausted at the source, with laboratories using up what stocks exist. Thus, given the scarcity of the CCRS, these are usually measured only once or twice at NIOZ for all extraction runs for a specific expedition or project.

Besides the calibrations and reference samples, sample ‘blanks’ are also processed in each extraction run to quantify any contamination introduced via reagents, sample handling and instrumental processing. At NIOZ, acidified UHPW is used as such a ‘blank’. The metal measured in the blank is then subtracted from the measured sample concentration to account for any added contamination. It is important to verify the cleanliness and performance of the SeaFAST system prior to extracting ‘real’ samples for a project by running and analysing blanks and in-house reference samples to ensure satisfactory performance of the SeaFAST, i.e. good recovery and low blanks (ideally at least a factor 2 lower than the lowest observed concentrations). Analytical considerations regarding measurement precision, accuracy, blanks and data evaluation are further detailed in Sects. 3.5.1.4 and 3.6.

ICP-MS Analysis Following Extraction

When a full set of samples with calibration lines, reference samples and blanks has been extracted with the SeaFAST, the extracts can be measured on the ICP-MS. It is also worth mentioning that prior to the SeaFAST being available, trace metals were concentrated for ICP-MS using resin beads, Mg co-precipitation or in-house-built chelating resin column systems (e.g. Biller and Bruland 2012; Lee et al. 2011a; Sohrin and Bruland 2011; Wu 2007). In all cases, the methods end up with a small volume of acid that contains the pre-concentrated trace metals of interest ready for analysis by ICP-MS.

After tuning of the relevant ICP-MS instrument (at NIOZ, samples are analysed using a Thermo Fisher Element HR SF-ICP-MS; Fig. 3.18) and verifying the background signals of the analytes of interest are low and stable, the analytical run can start. Especially on instruments that are also used for other samples, the background concentrations of contamination-sensitive elements like Fe, Zn or Pb may gradually decrease with time till a stable background level is reached. It should be noted that tuning the instrument for optimal performance (e.g. signal size, signal stability, peak shape, oxide interferences, resolution, etc.) requires specialized expertise and is beyond the scope of this chapter.

Box 3.10: Internal Standard Addition for ICP-MS

At NIOZ, the internal standard contains a mixture of In and Lu owing to both their low (sub pmol/L) concentrations in natural seawater and their resin recoveries of >98% using Nobias chelating PA1 resin (Middag et al. 2015a). Internal standards are used to allow the pre-concentration factor of the SeaFAST method to be determined without the need to measure the weight of the sample before and after the extraction step. The pre-concentration factor is accounted for since the ratios between the internal standard and the trace metal of interest do not change after addition of the standard spike to the sample and remain the same in the sample and in the eluent when the recoveries are quantitative. Therefore, any change in the volume of the sample or the eluent (by, for example, evaporation) becomes irrelevant since the ratio of the element of interest to the internal standard will stay unchanged. During ICP-MS data analysis, the signal of the trace metals of interest is normalized to the In and Lu signal which accounts for the pre-concentration factor as well as any variations in sensitivity, i.e. drift.

At NIOZ, first an eluent standard addition calibration curve (standard additions to eluent acid) is measured to assess recovery, together with some elemental standards to calculate the polyatomic interference of MoO^+ on Cd, if Cd is an analyte of interest (see Biller and Bruland (2012) for more details), followed by the SeaFAST extracts. After every 12 SeaFAST extracts, a drift standard is measured followed by two separate sub-samples of the eluent acid (the acid taken from the reagent bottle, without going through the SeaFAST). The drift standard is a standard from the eluent standard addition calibration curve (~2/3 of the maximum concentration in this case) that is used to correct for any element specific drift that is different from the internal standard element (and thus cannot be accounted for by the internal standard). Such a drift occurs, for example, if the change in sensitivity during an analytical run for an analyte of interest is different from the change in sensitivity for the used internal standards (In and Lu).

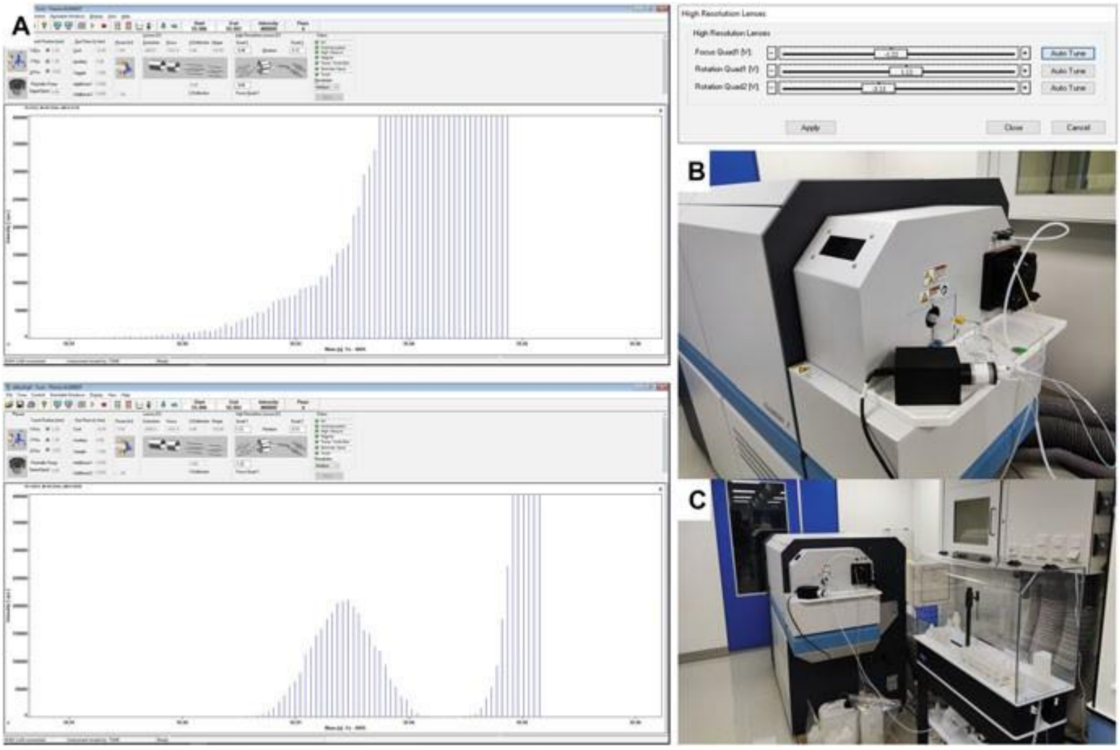

Fig. 3.18 Thermo Fisher Element HR-SF-ICP-MS set-up. Scans at the mass of ^{56}Fe in medium resolution on the NIOZ HR-SF-ICP-MS with (panel a, lower graph) and without (panel a, upper graph) tuning to resolve ^{56}Fe from ^{40}Ar^{16}O (a), Thermo Fisher Element HR-SF-ICP-MS (b) and MicroFAST sample introduction system (c) (Figure and photograph credit: Patrick Laan, NIOZ, Netherlands)

Changes in sensitivity are very common when running SeaFAST extracts, especially in long analytical runs, due to build-up of traces of salt still present in such samples on the ICP-MS cones. Element-dependent drift, however, is quite rare in our experience, but should be monitored and corrected for if needed. The two separate sub-samples of the elution acid are necessary to check (and correct for) any changes in metal background concentration originating from the sample introduction system or the ICP-MS itself during the analytical run. The first sample solely serves the purpose of reducing any carry-over to the second elution acid sample, which is actually used for the background correction of the seawater samples. This background correction, especially for very low open ocean seawater concentrations, can significantly influence the results, and thus care should be taken in applying the right background correction. Overall, measured values are always corrected for the background (metals in the elution acid and derived from the ICP-MS and sample introduction system) and subsequently normalized to the internal standard. Thereafter, a blank (metals added during the SeaFAST extraction and sample handling) correction is applied, and last, if needed, an element- dependent drift correction or interference correction (e.g. for Cd) is applied.

3.5.1.2 Flow Injection Analysis

Flow injection techniques were the standard for measuring trace metal concentrations such as Fe prior to the development of ICP-MS techniques (Sohrin and Bruland 2011) and remained popular into the GEOTRACES programme. At the present day, while matrix removal followed by ICP-MS analysis is now the most used method (that also has the added benefit of multiple element detection), there is one very significant drawback: it cannot be used at sea. Therefore, there is a valuable use for flow injection analysis techniques (FIA) on board ship (or when a more expensive ICP-MS is not available back on shore). FIA systems can be used for shipboard determinations of Al, Mn, Fe, Cu, Zn and Cd (e.g. Middag et al. 2015b; Rijkenberg et al. 2018; van Hulten et al. 2017). A FIA set-up only allows one trace metal to be measured at a time, but the system is quite compact, can easily be used at sea in a shipboard (mobile) clean laboratory or 'bubble' and is often automated with electronic valves and an autosampler that allows semi-autonomous operation of the system (Worsfold et al. 2013). Shipboard metal determination is a highly valuable technique used to guide at sea sampling efforts (e.g. if looking for high- concentration features such as hydrothermal plumes), to check for inadvertent contamination of bottle samplers, to measure short-lived species such as dissolved Fe^{2+} or to inform shipboard experiments such as Fe addition bioassays (e.g. Bowie et al. 2002; Cutter and Bruland 2012; de Baar et al. 1990). More recently, sequential injection analysis has been used for determination of individual marine trace metal concentrations (Grand et al. 2016), but this method that uses minimal reagents and is amenable to autonomous deployment is not yet mature and is still being developed. In a FIA system, the analyte of interest is separated from the interfering bulk seawater matrix by being pumped through a chelating resin column (see Sect. 3.5.1.1 for more details on chelating resins). After this separation and subsequent elution, a reaction between the analyte of interest and the reagent leads to the formation of a complex or a reaction that can be detected using a specific detector (Figs. 3.19 and 3.20), e.g. chemiluminescence can be detected using a photomultiplier or a formed coloured analyte-reagent complex can be detected using a spectrometer (Bowie et al. 2004). The loading time in a FIA system is standardized and automated, which should ensure that the same amount of sample is loaded onto the column every time. Reagents are continuously pumped through the FIA system using a peristaltic pump, where automated valves either pass buffered seawater sample (denoted load), deionized water (DI, often UHPW; denoted rinse) or elution acid (denoted elute) over the chelating resin column.

Here, we describe an example using a chemiluminescence reaction for the analysis of Fe concentrations in seawater (Figs. 3.19 and 3.20), using SPE. During the loading of the sample of interest, prevalent metals including Fe are retained by the resin column and thereby separated from the seawater matrix. The loading pH is crucial, since Fe binds at a much lower pH compared to Mn, allowing either metal to be measured by changing the loading pH (Klunder et al. 2011; Middag et al. 2011). Acidified samples are usually buffered in-line, and the loading pH can be changed by using a stronger or different buffer solution, where the resulting loading pH should be regularly checked. Samples for Fe should be acidified to a $pH < 2$ prior to analysis (Johnson et al. 2007) and left to equilibrate at least 12 hrs (Klunder et al. 2011) or heated to reduce this time (Lohan et al. 2006). Depending on the used method, a reducing (e.g. sodium sulfate) or oxidizing reagent (e.g. hydrogen peroxide) should be added to ensure Fe is present only as either Fe^{2+} or Fe^{3+} (Bowie et al. 2004; Johnson et al. 2007), or FIA can also be used to measure Fe^{2+} specifically if the redox speciation of Fe is the research aim (e.g. Bowie et al. 2002).

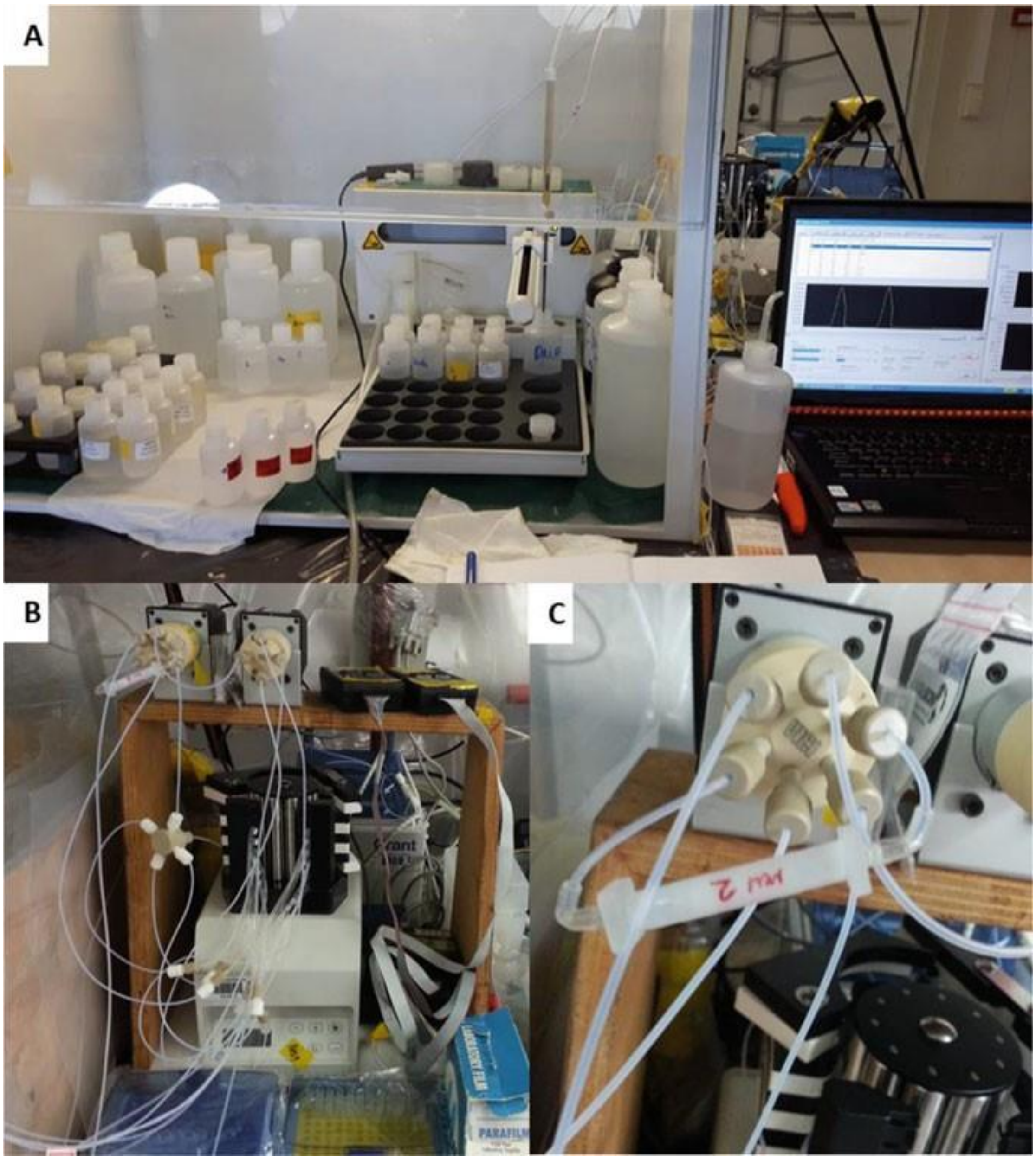

Fig. 3.19 Photo of a shipboard flow injection analysis (FIA) set-up including an autosampler, a computer and a water bath (a), a peristaltic pump and two automated valves (b) and a resin column on a valve (c) (photograph credits: Patrick Laan, NIOZ, Netherlands)

During the rinsing step, the seawater matrix is washed from the column and goes to waste (i.e. a dedicated waste line). At the same time, the reagent stream (in our example, luminol and peroxide as reactants and ammonium hydroxide to maintain the required reaction pH) flows continuously via the detector providing a baseline signal, i.e. the reaction between luminol and peroxide already produces chemiluminescence. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) is a compound that emits light upon oxidation with reactive oxygen species, a reaction catalysed by Fe (Borman et al. 2009). After loading and rinsing, the retained sample Fe is eluted from the column and is injected into the reagent stream that is kept at optimum temperature in a water bath, where the Fe catalyses the reaction between luminol and peroxide, resulting in increased chemiluminescence. The increase in chemiluminescence scales to the amount of Fe after pre-concentration. With this system, one first observes a dip in the baseline as first the UHPW in the column (result of the rinsing step) is injected, followed by a peak due to the catalysing effect of the Fe (Figs. 3.19 and 3.20).

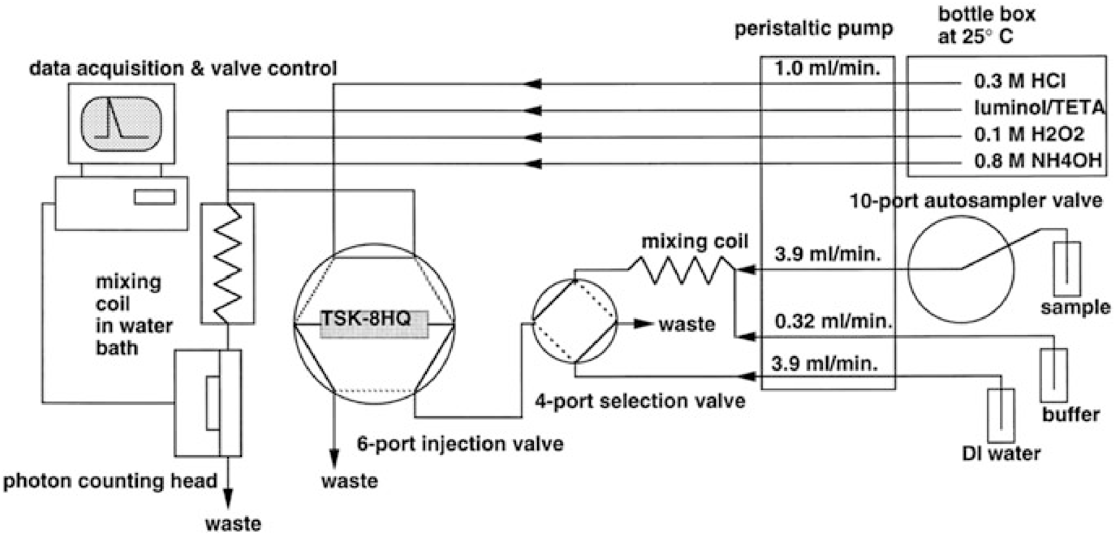

Fig. 3.20 Schematic overview of a flow injection system (FIA) for iron in seawater (de Jong et al. 1998)

Ideally, the peak is sharp and the signal returns back to baseline right away, but FIA systems are known to be ‘temperamental’. Thus, it can take significant tuning to get good peaks and sufficient sensitivity. Moreover, the chemiluminescence-based FIA method is prone to drift in sensitivity, for example, due to changes in temperature. In particular, a sample shows a higher signal at a higher temperature, and thus drift standards have to be run regularly to correct for temperature effects (Bowie et al. 2003; Bowie et al. 2004; Floor et al. 2015; Worsfold et al. 2019c). The calibration is done using standard additions, where care should be taken to measure samples in the calibrated and linear range of the chemiluminescence response (Borman et al. 2009). Moreover, despite the automated and standardized loading time, it has been shown that variations of up to 5% in the loaded mass of seawater samples occur. Combined with variation in sensitivity during a run and the uncertainty associated with the calibration (estimation of the sensitivity), this leads to an overall uncertainty in the order of 10–15% for the chemiluminescence-based FIA system for Fe (Floor et al. 2015). Thus, proper consideration should always be given to the analytical uncertainty associated with measurements of trace metals (see Sects. 3.5.1.4 and 3.6) to avoid overinterpretation of the data (Worsfold, et al. 2019).

3.5.1.3 In Situ Metal Analysis Systems

The sampling and analysis approaches detailed thus far largely deal with discrete samples that are representative for a given moment in time. However, to capture the spatial and temporal variabilities of trace metals at sub-nanomolar concentrations in dynamic (open) ocean settings, ideally in situ sensors or analysers that capture data continuously are implemented (Grand et al. 2019). Progress is being made in this area, and a combination of wet chemical analysers with electrochemical sensors may become available in the coming years or decades, but currently developed systems lack the accuracy and precision needed for oceanic trace metal concentrations and speciation. Readers interested in the state of the art and remaining challenges are referred to Grand et al. (2019) for an in-depth overview.

3.5.1.4 Data Quality Control for Trace Metal Concentration Measurements

Here, we briefly describe analytical considerations that are needed to produce consistent high-quality trace metal concentration data, including ‘blanks’, detection limits and uncertainty (accuracy and reproducibility) of analytical techniques. For a more in-depth discussion of good practices to obtain and report good quality data, the reader is referred to Worsfold et al. (2019) and Wurl (2009).

Obtaining low and consistent blanks, commonly referred to as ‘procedural blanks’, is key for both validating the method of choice and for ensuring contamination-free trace metal analysis during the implemented procedure (Wurl 2009). The blank is defined as the amount of analyte that is added (inadvertently) during the overall procedure, and differentiation can be made between various components of the procedure such as a sampling blank (contamination during taking of the sample), storage blank (contamination between sample collection and analysis) and/or analysis blank (contamination from the analytical procedure, including sample handling). The focus usually lies on the analysis blank associated with the use of reagents, i.e. systematic contamination, as this can be quantified. Nevertheless, levels of contamination during sampling and storage should also be assessed as detailed in the previous sections (Sects. 3.2, 3.3 and 3.4). The analytical limit of detection (LOD), which is a criterion for the performance of an analytical method and/or technique, indicates the lower limit at which a technique can differentiate between a signal due to background noise and a signal derived from the target analyte. Depending on the method, this can be closely related to the instrumental LOD (i.e. the limit at which the used instrument can differentiate between an analyte signal and background noise), but often the details of the analytical procedure are responsible for changes in the LOD (e.g. variation in extraction efficiency with SPE columns, or variations in pipetted sample volumes) and hence the analytical LOD should be determined throughout the whole procedure, not just from the instrumental LOD. The LOD is inherently linked to the blank value and the variations therein, and so the LOD is commonly defined as three times the standard deviation of the analytical blank (Wurl 2009). To be able to calculate a standard deviation and subsequently the LOD, the blank should be measured at least three times; however, a better approach is to measure the blank more often throughout an analytical run and also use the blank values from discrete runs over a longer time period to calculate the LOD in order to account for any intra- and inter-run variability. For trustworthy trace metal data, the metal concentration of interest of the target sample must be higher than the LOD (Wurl 2009). Common values for blanks and LODs of the six bio-essential metals from NIOZ for the SeaFAST technique can be found in Table 3.1 (Seyitmuhammedov 2021), showing a similar range as those generated by other approaches (e.g. Biller and Bruland 2012; Rapp et al. 2017).

Expressing uncertainty (error) on any dataset is vital. This uncertainty is typically expressed via accuracy (do we have the right number?) and precision (how reproducible is the number?). For seawater trace metal concentration data, accuracy of data can be compared in two ways: (1) measuring trace metal concentrations in a reference material (RM) and comparing these values to published or consensus values and/or (2) comparing measurements made by multiple laboratories in the same natural samples or on samples collected from the same location (see Sect. 3.6). Such activities are essential steps to confirm that the obtained results have acceptable accuracy relative to the variability being studied Worsfold et al. 2019; Wurl 2009). Certified reference materials (CRM) for ICP-MS can either be commercially available certified RMs and/or obtained from bodies such as the US National Institute for Standards and Technology (NIST). For good practice, RMs should have the same matrix as the sample of interest, their concentrations should be in the same range as those of the target samples, and obtained concentrations should agree with published concentrations (within the uncertainty range) of the RM (Worsfold et al. 2019; Wurl 2009). Because of the challenges of collecting and storing open-ocean seawater, there is no commercial CRM available for open ocean trace metal concentrations, and trace metal investigators thus often use in-house reference samples (e.g. a large volume homogenized seawater sample), or aliquots of reference seawater that GEOTRACES has made available upon request (e.g. Cutter et al. 2017; Johnson et al. 2007; see https://www.geotraces.org/standards-and-reference-materials/) for which the average accepted values are known as 'community consensus values'. Analysts will typically make (and report) regular measurements of these reference samples as RM alongside samples in order to assess the accuracy of the technique (see Sect. 3.5.1.1). When the procedure returns inaccurate reference sample values, action is needed by the investigator to identify and eliminate sources for the inconsistency of the data (Wurl 2009).

Evaluating the reproducibility (precision) of trace metal concentration data is also essential and should be done via replicate measurements of samples, blanks and RMs (Worsfold et al. 2019). Additionally, it is recommended to evaluate precision at concentrations appropriate to those of the samples being studied—for instance, the precision of low-concentration samples may be much poorer than high- concentration samples. Commonly, duplicate or triplicate measurements are used to assess precision. However, it should be noted that triplicate measurements of a single sample are not necessarily representative of the overall precision, if sampling also contributes to uncertainty.

	HR-SF-ICP-MS						
	DMn pmol L^{-1}	**DFe** nmol L^{-1}	**DCo** pmol L^{-1}	**DNi** nmol L^{-1}	**DCu** pmol L^{-1}	**DZn** nmol L^{-1}	**DCd** Pmol L^{-1}
Blank	2	0.03	2	0.03	7	0.03	3
SD	1	0.01	1	0.04	7	0.02	3
n	24	24	24	24	24	24	24
LOD	4	0.02	3	0.1	20	0.07	8
	DMn nmol/L	**DFe** nmol/L	**DCo** pmol/L	**DNi** nmol/L	**DCu** pmol/L	**DZn** nmol/L	**DCd** pmol/L
GSP 239	0.79	0.15	7	2.56	0.60	0.04	3
±SD	0.01	0.01	1	0.03	0.01	0.02	2
n	14	14	14	14	14	14	14
Consensus value	0.78 ± 0.04	0.16 ± 0.05	-	2.6 ± 0.1	0.57 ± 0.06	0.03 ± 0.06	2 ± 2

Table 3.1 Typical blank values, detection limits (LOD) and an example of a CCRS (Geotraces Surface Pacific; GSP, consensus values reported 2020; https:// www.geotraces.org/standards-and-reference-materials/) results for various dissolved trace metals (DFe, DCu, DZn, DMn, DNi, DCo and DCd) of using a SeaFAST pre-concentration step with subsequent HR-SF-ICP-MS analysis at NIOZ. The procedural

blank was UHPW acidified to the same pH as seawater samples. Please note that the LOD (3 x SD) may appear different from three times the reported SD due to rounding

For this it is best to collect replicate samples in successive sampling efforts at the same depth and location, which is done during some expeditions (e.g. sampling at the same depth twice on overlapping casts by the US GEOTRACES programme), but this is not currently routine practice for all sampling endeavours, usually due to the associated extra time and cost. For RMs and blanks, replicate measurements performed in a single day enable statements on the repeatability, while replicate measurements over longer time periods can be used to determine laboratory reproducibility, accounting for drifts in detector response or laboratory conditions, i.e. temperature (Worsfold et al. 2019). For blanks, the average values are commonly subtracted from the sample values obtained in the same run. In all cases, it is recommended that data is reported as means including standard deviations (Worsfold et al. 2019). Often samples are measured only once, as trace metal analysis is time-consuming and costly. As a compromise, the standard deviation (SD) for samples can be calculated as the square root of the sum of the internal and external SD. The internal SD is the SD of the ICP-MS measurement (instrumental precision), and the external SD is the SD of the specific analyte of an (in house) RM measured regularly throughout an analytical run (Gerringa et al. 2021a), where it is recommended to use RMs at different concentration levels as the precision is likely lower at lower concentrations. Good precision (% SD) for trace metal concentrations assessed by replicate analyses of RM or seawater by SeaFAST, ICP-MS, FIA and isotope dilution from isotopic techniques, from a range of laboratories, has been assessed as ±0–5%. Reported precision is often better for less contamination-prone metals such as Cd and also better at higher trace metal concentrations (e.g. Conway et al. 2013; Jensen et al. 2020; Jensen et al. 2019; Lagerström et al. 2013; Middag et al. 2015a; Minami et al. 2015; Rapp et al. 2017; Rijkenberg et al. 2014; Wuttig et al. 2019), but may range from ±2 to 20% for FIA depending on the metal (e.g. Resing et al. 2015; Sedwick et al. 2015; Wyatt et al. 2014).

However, while reporting the precision (% SD) of good quality data is key, it is perhaps more realistic to estimate and report the overall or so-called ‘combined’ uncertainty of the obtained data (Worsfold et al. 2019). Such combined uncertainty considers the contributions from all the uncertainty contributions during sample analysis, including analyte uncertainty, blank uncertainty, pipette uncertainty for all volumes pipetted (sample and reagents), slope uncertainty of the calibration line, uncertainty of metal concentrations in reagents and volume uncertainties (Worsfold et al. 2019). Such accounting for the combined uncertainty may lead to larger relative uncertainties obtained for both ICP-MS and FIA techniques (e.g. 10–30%; Clough et al. 2015; Rapp et al. 2017; with more uncertainty at the lowest concentrations), but can also be of similar size to internal precision for ICP-MS (see Sect. 3.5.2.3). Larger uncertainty from combined uncertainty highlights that reporting only instrumental precision on data can underestimate the overall uncertainty of the obtained data, depending on the technique. For more information on combined uncertainty including example applications, the reader is referred to Worsfold et al. (2019) and references therein. Perhaps the best way to assess data precision and accuracy is to compare data from the same samples or same location produced by different laboratory groups, analytical techniques and/or sampling systems. Indeed, within the GEOTRACES programme, besides analysis and reporting of RMs, there has been a strong emphasis on intercalibration using crossover stations (see Sect. 3.6) to assess the comparability of datasets obtained via different sampling and/or analytical techniques.

3.5.2 Trace Metal (Fe, Ni, Cu, Zn, Cd) Isotope Ratio Measurement Techniques

3.5.2.1 Background

With the onset of improved clean collection and handling techniques and the development of HR multi-collector ICP-MS (MC-ICP-MS) (e.g. Douthitt 2008) has come the ability to measure the stable isotopic ratios of transition metals even at the low concentrations of these metals found in seawater (pmol L^{-1}-nmol L^{-1}). The promise of such measurements is that isotopic ratios may offer more insight into marine trace metal cycling than concentrations alone. The first isotopic measurements for Cd, Cu and Zn in seawater were published in 2006 (Bermin et al. 2006; Lacan et al. 2006), followed by Fe in 2007 (De Jong et al. 2007). Despite these efforts, by the official beginning of the GEOTRACES field programme in 2010, data remained sparse, with only about 10–50 data points having been published for each element (Conway et al. 2021). Since 2010, however, stimulated largely by GEOTRACES, the field of transition metal isotopes in seawater has undergone a figurative explosion, adding Cr and Ni isotopes to the toolbox and with data coverage increasing dramatically (Conway et al. 2021). The other bio-essential trace metals discussed in this chapter, Co and Mn, are monoisotopic for stable isotopes, and thus ratios cannot be determined. The first ocean 'sections' of dissolved trace metal isotope ratios were published for the Atlantic in 2014 (e.g. Zn, Conway and John 2014a); Fe, Conway and John 2014b)), and now a number of other such sections are published and available from several groups in the GEOTRACES Intermediate Data Products (Schlitzer et al. 2018). Indeed, sufficient data now exists for each isotope system to provide comprehensive insights into the biogeochemical cycling of each trace metal. This insight includes tracing Fe and Zn sediment sources to the ocean (Homoky et al. 2016), balancing the oceanic Zn and Cu budgets (Little et al. 2014; Little et al. 2016), understanding redox cycling of Fe and Ni (Rolison et al. 2018; Vance et al. 2016) and showing how scavenging, biological uptake and water mass mixing influence Ni, Zn and Cd (e.g. Abouchami et al. 2011; Archer et al. 2020; John and Conway 2014; Ripperger et al. 2007).

Here, we briefly describe the chemical and analytical challenges for making measurements of transition metal isotope ratios in seawater and the methods developed by different groups to overcome these—including the typical methods used at the University of South Florida (USF) for measuring δ^{56}Fe, δ^{66}Zn and δ^{114}Cd in seawater. For further reading on the distributions of the trace metal isotope ratios throughout the ocean, as well as the current state of understanding of the processes which influence these distributions, we refer the reader to several recent synthesis review articles (e.g. Anderson 2020; Horner et al. 2021 and references therein).

3.5.2.2 Chemical Processing for Trace Metal Isotope Analysis

There are two critical challenges to overcome to measure trace metal isotope ratios in seawater, which are common to each of the five trace metals described here (Fe, Ni, Cu, Zn, Cd). First, akin to concentration measurements (see Sect. 3.5.1.1), the sea salt matrix means that samples cannot be measured directly by mass spectrometry. Instead, the matrix must be separated and the trace metal of interest pre-concentrated. Second, mass spectrometric techniques for measuring trace metal concentrations (see Sect. 3.5.1.1) rely on measuring the whole metal pool or the most abundant isotope of each metal. For isotopic ratio measurement, however, the minor abundance isotopes must also be measured. These minor isotopes are typically present at even lower concentrations (often >50x lower), compounding the problems of clean handling and sample volumes needed. For example, Fe has four stable isotopes with abundances of 6% (^{54}Fe), 92% (^{56}Fe), 2% (^{57}Fe) and 0.3% (^{58}Fe). Thus, in a typical deep ocean seawater sample of 0.5 nmol L^{-1} Fe, ^{54}Fe is only present at only 30 pmol L^{-1}, and just 1.5 pmol L^{-1} are ^{58}Fe. In order to accurately measure a 56/54 or 58/54 Fe ratio, Fe must therefore be pre-concentrated cleanly from much larger volumes of sample (e.g. 1–20 L; e.g. Conway et al. 2013; Lacan, et al. 2008) than those typically needed for concentration analysis (e.g. 30 mL; see Sect. 3.5.1.1). Such large volume requirements mean that it has been historically challenging to make clean measurements at high spatial resolution in the ocean.

Typically, chemical methods to prepare samples for isotope analysis involve two to three purification stages, with one stage removing the bulk of the sea salt matrix (see below) and later stages purifying the element of interest from any minor elements which are potential isobaric interferences, as well as removing any remaining salts (see below). A range of approaches is described here, but whichever method is used, the key requirements are for clean (ideally procedural blank concentrations of S1 ng) and quantitative recovery (ideally ~100%) of the trace metal of interest.

A further challenge with trace metal isotope analyses is that, as with early concentration methods, most early isotopic methods focused on a single metal isotope system of interest (e.g. just Cd; Lacan et al. 2006; Ripperger and Rehkämper 2007). For multiple trace metal isotope measurements, separate aliquots of seawater were needed, meaning even larger total volumes of clean seawater were required. More recently, however, methods have been developed that can process the same sample for multiple metals in seawater for later isotopic analysis (e.g. Fe, Zn and Cd by Conway et al. 2013; or Ni, Cu and Zn by Takano et al. 2017) (see Box 3.11). These multiple element approaches have played an important role in facilitating the rapid application of multiple isotopic systems as tracers of oceanic processes as part of GEOTRACES.

Sea Salt Matrix Removal Stage

The first step of any seawater processing method for isotope analysis is to pre-concentrate the metal of interest cleanly from 1–4+ L down to a small volume for analysis (<1 mL), while removing the seawater matrix (see Box 3.11; Fig. 3.21). Due to the large volumes (and thus the larger total amount of salts) needed for isotopic compared to concentration measurements, automated processing techniques such as the SeaFAST are not yet commercially available, although in development (e.g. Field et al. 2019). Instead, pre-concentration methods have focused on in-house methods developed by different laboratory groups. These methods include using chelating resins, organic solvent separation (e.g. Thompson et al., 2013; Ellwood et al., 2014) or co-precipitation with Mg or Al (e.g. Bermin et al. 2006; Cameron and Vance 2014; De Jong et al. 2007; Staubwasser et al. 2013; Xue et al. 2012) to cleanly separate the trace metal(s) of interest from seawater. For the former, studies have made use of Qiagen NTA, Nobias PA-1, Chelex-100 or Bio-Rad AG1-X8 ion-exchange resins to extract metals from seawater; resin beads are either added directly to seawater, shaken and filtered out, or seawater is pumped through resin- packed columns (e.g. Abouchami et al. 2011; Bermin et al. 2006; Conway et al. 2013; John and Adkins 2010; Lacan, et al. 2008; Ripperger and Rehkämper 2007; Vance et al. 2016; Xue et al. 2012). The transition metal cations (or metal bromide or chloride complexes if anion resin) ‘stick’ to the functional groups of the resin and are thus removed from the seawater leaving the salts behind. At the time of writing, most isotope groups have moved to make use of Nobias PA-1 techniques (e.g. Archer et al. 2020; Ellwood et al. 2020; Sieber et al. 2021; Yang et al. 2020), principally due to the low blank and the high extraction efficiency of this resin for multiple trace metals. However, other resins continue to be used for single elements (e.g. NTA for Cu by Baconnais et al. 2019; AG1-X8 for Cd by Xie et al. 2015).

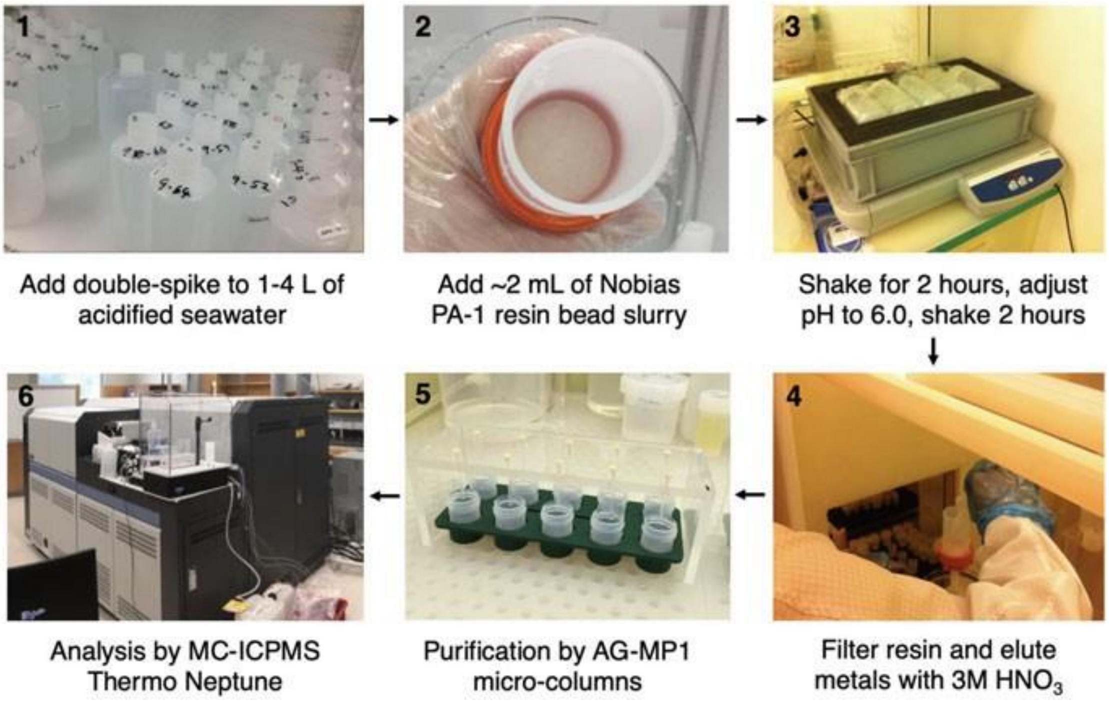

Fig. 3.21 Step-by-step scheme for multiple trace metal isotope ratio analysis (photograph credits: Tim Conway, University of Florida, USA)

Box 3.11: Chemical and Analytical Scheme for Multiple Trace Metal Isotope Ratio Analysis

The figure below shows a simplified schematic of the typical chemical method that is being used at ETH Zürich (Switzerland) and the University of South Florida (USA) to process seawater for Fe, Cd and Zn isotope ratios (follows Conway et al. 2013, and Sieber et al. 2021) (Fig. 3.21). Double spikes of ^{57}Fe-^{58}Fe, ^{64}Zn-^{67}Zn and ^{111}Cd-^{113}Cd are typically added prior to processing to allow for correction of both procedural and instrumental isotopic fractionation (see Box 3.15 for more explanation of double spikes).

Following extraction from a water sample, the trace metals can then be eluted from resins or redissolved from precipitates into a small volume of HNO_3 or HCl for further purification steps (e.g. on micro-columns, see below) and then analysis (see Sect. 3.5.2.3). For marine particles or sediments, following a suitable leaching or digestion process (see Sect. 3.4.3), samples can be redissolved for similar purification. However, care must be taken to establish that any leaching process does not fractionate isotope ratios (e.g. Revels et al. 2015).

Purification Stage

Although the first stage described above removes most of the salt from a seawater sample, the functional groups of ion-exchange resins often bind a range of elements, meaning that the resulting acidic solution contains not just the metal of interest, but often many other transition metals and traces of remnant salts such as Ca and Mg (e.g. for Nobias PA-1; Sohrin et al. 2008; see Box 3.12; Fig. 3.22). If a precipitation technique is used, the precipitate typically contains other elements as well. These other elements cause a range of problematic isobaric (e.g. ^{58}Ni on ^{58}Fe) and polyatomic (e.g. ^{40}Ca^{16}O on ^{56}Fe) interferences during mass spectrometric analysis, which adversely affect the accuracy of isotope ratios or the ionization of elements (see Sect. 3.5.1.1. for more background on interferences). As such, further purification of the sample is required prior to analysis of isotope ratios.

Box 3.12: Elution of Different Transition Metals from AGMP-1 Resin

The figure below shows an example of a second-stage AGMP-1 column purification scheme, showing the separate elution of Cu, Fe, Zn and Cd from major salts and interfering elements when acid strength and type through the resin are varied (Conway et al. 2013), with volume being cumulative throughout the column scheme (Fig. 3.22).

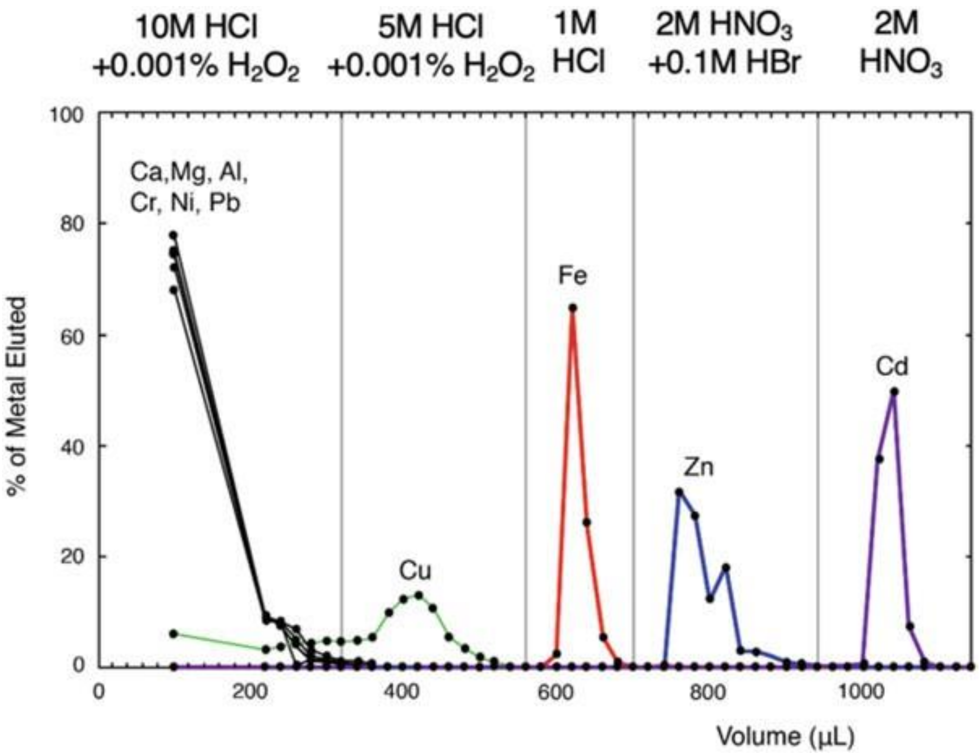

Fig. 3.22 Elution of different transition metals from an AGMP-1 resin (modified from Conway et al. 2013)

The goal of the second purification step is thus to achieve only the single metal of interest being dissolved in a small volume of weak acid (e.g. 0.5–1 mL of 0.2 M HNO_3), ready for isotope analysis. This purification is typically achieved by taking the sample through a single (or several) anion-exchange resin-packed columns, usually filled AGMP-1 or AG1-X8 anion-exchange resins (from Bio-Rad). AGMP-1 is perhaps the most widely used resin for this purpose (e.g. Archer and Vance 2004), although AG1-X8 has been widely used for Cd purification, usually followed by an additional Eichrom TruSpec column to further separate Cd from Sn and Mo (e.g. Abouchami et al. 2011; Ripperger and Rehkämper 2007; Xue et al. 2012). In each case, the functionality of these anion-exchange resins relies on metals having different distribution coefficients—that metals ‘stick’ variably to the resin—as the strength or type of acid is varied (e.g. Strelow 1980). Such AGMP-1 techniques were first applied for separation of Cu, Zn or Fe from rocks for isotope analysis (e.g. Beard and Johnson 1999; Maréchal et al. 1999). For seawater, where the transition metal concentrations are much lower, and thus blanks must be kept suitably low, studies have modified these techniques to work with smaller volumes of resin. Archer and Vance (2004) developed an AGMP-1 column separation scheme for Cu and Zn that lowered the blank contamination of the method by an order of magnitude (down to 0.5–1 ng) compared to earlier techniques. AGMP-1 micro-column techniques have since been developed to purify Fe or Cd and then Cu, Zn, Fe and Cd from seawater for isotope analysis (e.g. see Box 3.12; Conway et al. 2013; John and Adkins 2010; Lacan et al. 2006; Takano et al. 2013). These micro-columns have typical blanks of less than 0.2 ng per element (e.g. Conway et al. 2013).

When using AGMP-1 resin (see Box 3.12), the sample from the first purification stage is typically dried down (evaporated to dryness), so that it can be dissolved in a specific reagent (>5 M HCl) and then ‘loaded’ on the AGMP-1 resin column. Different metals are then ‘eluted’ from the resin column by changing the acid strength or acid type (see Box 3.12 for an example column elution scheme). Such procedures have been shown to successfully separate Cu, Fe, Zn and Cd from elements such as Ca, Mg, Ni, Cr, Sn, etc. (Conway et al. 2013). Nickel, however, elutes from the columns with elements such as Na, Al, Ca and Mg (see Box 3.12), and so further purification steps (e.g. Nobias PA-1 or multiple AGMP-1 columns) are typically needed to remove interfering elements before isotope analysis of Ni is possible (Archer et al. 2020; Wang et al. 2019; Yang et al. 2020).

3.5.2.3 Analytical Procedures for Trace Metal Isotope Analysis

Here, we provide a brief overview of the details of trace metal isotope analysis, with a focus on the bioactive metals measured in seawater, often using Fe as an example. For a fuller discussion on the detail of isotope systematics and details of isotope ratio analysis for the bioactive metals, we point the reader elsewhere (e.g. Dauphas et al. 2017; Johnson et al. 2020; Moynier et al. 2017; Rehkämper et al. 2012; Teng et al. 2017). The concepts reviewed in the following sections apply to all transition metals discussed here, although we note each element has its own quirks and interferences to account for during both chemistry and analysis.

Isotope Ratio Basics, Nomenclature and ‘Zero’ Isotope Standards

Following chemical purification, transition metal isotope ratios are measured by mass spectrometer. However, the natural range of mass-dependent variability for transition metal isotope ratios (e.g. ^{56}Fe/^{54}Fe), while measurable, is typically very small, usually at the 0.001 or permil (‰) level (e.g. Horner et al. 2021; Johnson and Beard 1999). Further, instrumental ‘mass’ bias, or the systematic loss of lighter ions during analysis, means that it is difficult to measure absolute isotope ratios at this level of precision. Thus, isotope ratios are typically measured relative to a measured standard RM, usually in ‘delta notation’ (see Box 3.13). For example, Fe isotope ratios are typically expressed as:

$$\delta^{56}Fe = \left[\frac{(^{56}\mathrm{Fe}/^{54}\mathrm{Fe})_{sample}}{(^{56}\mathrm{Fe}/^{54}\mathrm{Fe})_{IRMM-014}} - 1\right] \times 1000$$

In this case the ^{56}Fe/^{54}Fe of the sample is not expressed as an absolute isotope ratio, but rather relative to the ^{56}Fe/^{54}Fe of the international Fe isotope standard IRMM-014 (an isotope zero standard) (Taylor et al. 1993) that is measured by the same mass spectrometer. For each element, data is expressed in the form of a common isotope pair (or range of pairs) and typically relative to one international ‘zero’ standard (see Table 3.2). It is worth noting that some groups report in other notations (e.g. epsilon units; Table 3.2), which is calculated by replacing the x1000 in the delta equation with x10,000, meaning that, for example, $\varepsilon^{114/110}$Cd is equivalent to 10 x δ^{114}Cd.

Table 3.2 *Typical notation and isotope zero standards for trace metal isotope ratios in seawater. Where multiple notations exist, the common notation and isotope pairs (as included in the GEOTRACES Intermediate Data Product) are shown in bold. Ranges for deep seawater (expressed in the common notation) are from Horner et al. (2021); for isotope standards see Abouchami, et al. (2013); Dauphas et al. (2017).; Moynier et al. (2017); and Elliott and Steele (2017). A more detailed discussion of conversion between isotope ratios and notation for Cd can be found in Rehkämper et al. (2012).*

Element	**Notation**	**Isotope Pair(s)**	**Isotope Zero Standard**	**Range in deep seawater (‰)**
Fe	**δ^{56}Fe**, δ^{57}Fe	**^{56}Fe/^{54}Fe**, ^{57}Fe/^{54}Fe	IRMM-014	-2.4 to +1.5

Ni	δ^{60}Ni	**^{60}Ni/^{58}Ni**	NIST SRM 986	+1.2 to +1.5
Cu	δ^{65}Cu	**^{65}Cu/^{63}Cu**	NIST SRM 976	+0.6 to +0.8
Zn	δ^{66}Zn	**^{66}Zn/^{64}Zn**	JMC-Lyon	-0.2 to +0.6
Cd	**δ^{114}Cd**, δ^{112}Cd, $\varepsilon^{114/110}$Cd, $\varepsilon^{112/110}$Cd	**^{114}Cd/^{110}Cd**, ^{112}Cd/^{110}Cd	NIST SRM 3108	+0.2 to +0.4

For converting between isotope ratio pairs, typically a simple mass difference approach can be applied; for example, $\delta^{56/54}$Fe ¼ ~2/3 x $\delta^{57/54}$Fe, or $\delta^{114/110}$Cd ¼ ~ 2 x $\delta^{112/110}$Cd.

For some 'metallic' elements such as B or Li, there is large enough (~10‰) mass-dependent isotope variability in nature to allow isotope ratios to be measured by relatively imprecise instruments such as single-detector SF-ICP-MS instruments (e.g. precision of +0.5‰; Misra et al. 2014). However, for the transition metals, which display much lower levels of natural isotopic variability (e.g. 1–4‰; Horner et al. 2021), higher precision (<0.2‰) is needed. Such levels of precision require 'multi-collector' (MC) mass spectrometers, which operate by collecting a series of ions simultaneously into multiple 'faraday cup' ion-counting detectors, allowing isotope abundances to be measured simultaneously rather than sequentially, as is the case for a single collector SF-ICP-MS (e.g. Walder and Freedman 1992). MC instruments take two forms, classified depending on how the sample is introduced and ionized, either ICP- (inductively coupled plasma) or TI- (thermal ionization) MS. MC-ICP-MS instruments introduce a sample via an ionized plasma (as described in Sect. 3.5.1.1), while for MC-TI-MS a sample is loaded on a metal 'filament' which is then heated and the sample vaporized/ionized (see Johnson and Beard 1999; Schmitt et al. 2009). Of the two, the most widely applied technique in seawater literature for trace metal isotope ratios is MC-ICP-MS, making use of one of three instruments: the Thermo Scientific Neptune (9–10 laboratory groups; Fe, Ni, Cu, Zn and Cd), Nu Instruments Plasma I or II (6 groups; mostly Cd) or the GV Instruments

Box 3.13: Transition Metal Isotope Standards

The choice of isotope standard reference material (RM) and 'zero' standards are established by consensus between laboratory groups and can take the form of in-house shared materials (e.g. JMC-Lyon; Moynier et al. 2017) or commercially available standard RM (e.g. NIST-3108 Cd; Abouchami et al. 2013), with the isotope ratio difference between different isotope standards established by consensus (e.g. Abouchami et al. 2013; Archer et al., 2017). For in-house shared materials, investigators typically acquire an aliquot via personal communication with the original laboratory. For commercial RM, which are often designed as concentration standards, it is important to acquire the correct batch. In both cases, the limited availability of such RM often means that isotope zero standards can become depleted and must be replaced by others. This depletion has already occurred with JMC-Lyon for Zn, with AA-ETH being suggested as a replacement solution (Archer et al., 2017). In this case, it is encouraged to measure ratios relative to AA-ETH but report ratios relative to JMC-Lyon using the multi-laboratory established offset of 0.28‰ between the standards so that new data can most easily be compared with literature data (Archer et al., 2017).

IsoProbe (1 group; Zn). One group utilizes TIMS (Thermo Fisher Triton instrument) to measure Cd isotope ratios (e.g. Abouchami et al. 2011). It is worth noting that for Cd, which is the only isotope system to be measured in aliquots of the same deep seawater sample by TI-MS and both Nu and Thermo MC-ICP-MS, excellent agreement has been shown, meaning that data from all three methods are inter-comparable (e.g. Boyle et al. 2012). For a more detailed discussion of the comparison of MC-ICP-MS and TI-MS for Cd isotope analysis, see Rehkämper et al. (2012).

MC-ICP-MS Analytical Techniques and Mass Bias Correction Techniques

For simplicity, here we focus on the measurement of trace metal isotope ratios by MC-ICP-MS, as used by most trace metal isotope groups, and specifically give a brief overview of the systematics of the Neptune Plus as used at the University of South Florida (for a general introduction of the mechanism of sample introduction by ICP-MS, see Sect. 3.5.1.1). The concepts discussed here for Neptune MC-ICP- MS analysis are generally applicable to the Nu Plasma instruments, and mass bias correction techniques are typically similar for MC-ICP-MS and TI-MS. We do not provide detailed discussion of TIMS, but instead point the reader to Schmitt et al. (2009), Abouchami et al. (2011) and Rehkämper et al. (2012) for further reading.

For seawater samples, where metal concentrations are low (especially surface waters), the main concern for isotope analysis is to maximise signal size in order to reduce uncertainty on isotope ratios (e.g. John and Adkins 2010). Compounding this issue for some elements (e.g. Fe), which have isobaric interferences that are large and similar in mass ($^{56}Fe^{+}$ and $^{40}Ar^{16}O^{+}$), ‘high’-resolution (HR) mode must be used on the Neptune MC-ICP-MS (Weyer and Schwieters 2003). While HR mode allows successful measurement of the Fe isotope peaks separate from their Argide interferences, it also comes at a cost – approximately six to seven times reduction in signal size compared to low resolution (LR; Weyer and Schwieters 2003).

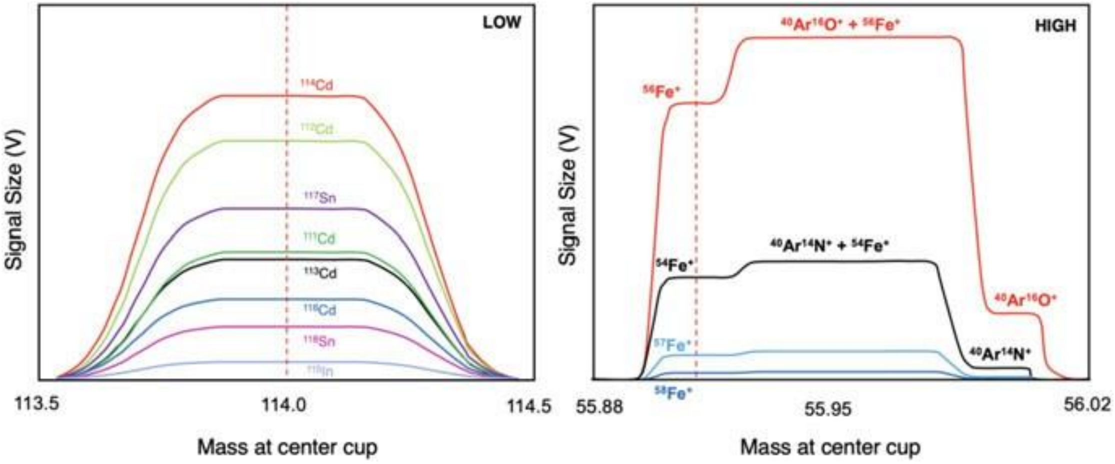

Fig. 3.23 Peak alignment for measurement of trace metal isotope ratios on the Thermo Neptune MC-ICP-MS in low-resolution mode (left) and high-resolution mode (right) (Cartoon credit: Tim Conway, University of Florida, USA). Dashed vertical lines indicate measurement mass on each peak

As such, every attempt is made to boost signal size, which can be done in two ways, either by dissolving the sample in the smallest volume of acid possible (often<0.5 mL) or by optimising the MC-ICP-MS introduction system. For the latter, there are two aspects which are helpful: (i) choosing the optimal interface cone combination and (ii) choosing the right introduction system. Neptune MC-ICP-MS methods often boost signal size (i.e. V per ng mL^{-1}) by making use of the high- sensitivity combination of Jet sampler and X-skimmer interface cones (e.g. Conway et al. 2013). Most MC-ICP-MS methods also use a desolvation system, such as the commercially available Cetac Aridus I/II, ESI Apex Q or ESI Apex Omega (e.g. Bermin et al. 2006; Conway et al. 2013; Sieber et al. 2021), as a suitable introduction system. Desolvation systems use a series of heated and cooled spray chambers, together with added Ar or N_2 gases (or both), to boost the signal size of the element of interest while minimising oxide interferences. It should be noted, however, that specific care must be taken when customising the introduction system set-up, since both desolvators and high-sensitivity cones can induce larger interference formation (e.g. CdH on Cd or ZnH on Zn; Archer et al., 2017; Sieber et al., 2019) or mass bias effects (Archer and Vance 2004; Bermin et al. 2006), compared to regular H-cones or a spray chamber. For example, larger mass bias effects attributed to the Aridus desolvator have led to δ^{65}Cu being more commonly measured using a spray chamber and H cones (e.g. Bermin et al. 2006; Takano et al. 2013; Little et al. 2014).

Once the introduction system is set up and the plasma lit, the instrument must be tuned for optimal performance, including signal size, signal stability and peak shape, by tuning the gas flows, torch position and lenses. Peak shape is tuned to generate wide, flat-topped peaks in LR and sharply resolved peaks in HR (see Box 3.14, Fig. 3.23). This tuning is akin to what is needed for concentration measurements (Sect. 3.5.1.1). In addition, the Faraday detectors or 'cups' must also be positioned to detect the isotopes of interest, with cup positions collectively termed a 'cup configuration'. Lastly, a specific measurement mass on the peaks must be chosen (see Box 3.14). Similar to the metal concentration measurements, this set-up requires extensive training of the analysts.

Once peaks are aligned and measurement position has been chosen, each cup reports the raw voltage of a single isotope during analysis. The raw voltages must then be corrected for both instrumental background and isobaric interferences before isotope ratios are calculated simply as the ratio of corrected voltages. Instrumental background on the Neptune MC-ICP-MS is typically corrected for in two ways: first, the gain and baseline function are used to correct instrumental noise on the detectors (prior to an analytical session), and second the instrumental background or 'blank' is corrected by subtracting the voltage from each detector in a solution of the acid used for sample dissolution, prior to sample measurement (or sometimes the average of two blank analyses before and after sample). A careful sequence of rinsing between samples is also necessary to ensure that the introduction system returns to background values. As with concentration measurements by ICP-MS (see Sect. 3.5.1.1), isobaric interferences are corrected for mathematically.

Background and isobaric interference corrected isotope ratios must then be further corrected for the inaccuracy caused by instrumental mass bias, which is the effect of preferentially 'losing' lighter isotopes during ICP-MS analysis leading to ratios biased towards heavy isotopes (see Johnson et al. 2020 for a recent discussion). Although the causes of mass bias are not completely understood, instrumental mass bias is systematic and can be corrected for using one of two empirically derived mass bias equations (e.g. Rehkämperab and Halliday 1998). The size of instrumental mass bias in MC-ICP-MS is also typically specific to each instrument and introduction system and predictably increases with mass difference between isotope ratios, but decreases with atomic mass (Johnson et al. 2020). For measuring transition metal isotope ratios via MC-ICP-MS or

TIMS, instrumental mass bias is typically corrected in one of three ways, either by sample-standard bracketing, by doping with a second element, or by ‘double-spiking’ (see Bermin et al. 2006 for extended discussion of the choice for Cu and Zn in seawater). In the first case, an isotope standard reference solution is analysed immediately before and after each sample in an analytical session, and the sample isotope ratio is expressed relative to these two ‘bracketing’ standards (e.g. for Fe; John and Adkins 2010). Sample-standard bracketing is used routinely for elements where a double spike is not possible (e.g. Cu), but may struggle to account for very rapid changes in mass bias from sample to sample. While care is taken to matrix-match samples to standards for MC-ICP-MS, any change in the sample matrix (e.g. presence of organics) may also induce mass bias effects which are not accounted for in the standards. Doping samples with a second element to correct for instrumental mass bias has also been used for transition metal isotope analysis (e.g. Zn for Cu; Maréchal et al. 1999), often combined with sample-standard bracketing for seawater samples (e.g. Takano et al. 2013; Yang et al. 2020).

Box 3.14: Peak Alignment for Measurement of Trace Metal Isotope Ratios by MC-ICP-MS

The following text gives a brief peak alignment example for isotope ratio measurements on the Thermo Neptune MC-ICP-MS (Fig. 3.23). For the low-resolution mode (e.g. for Zn and Cd), the cups (denoted by different colours in Fig. 3.23) are aligned so that the broad flat-topped peaks line up and the isotope voltages are simultaneously measured at the centre of each peak (red dashed line; left panel; Fig. 3.23). Interference peaks (in this case Sn and In on Cd) are also measured to facilitate correction of isobaric interferences. For the high-resolution mode (e.g. Fe), the cups are aligned so that the sharp, flat-topped compound peaks are lined up along the left side of the peak (right panel; Fig. 3.23). A measurement mass on the left hand ‘shoulder ‘of the peak, which is free from polyatomic interferences, is chosen (red dashed line; Fig. 3.23). Note the much smaller mass range in high- resolution mode in the figure below. For more details on high-resolution measurements, see Weyer and Schwieters (2003).

The double spike technique (Dodson 1963; Russell et al. 1978) involves the addition of an unnatural spike of two isotopes of the element being measured (e.g. ^{57}Fe and ^{58}Fe; Johnson and Beard 1999; Lacan, et al. 2008) to the natural sample, prior to chemical processing and analysis. Any fractionation of isotope ratios in the natural sample during processing or analysis will similarly affect the spike isotopes, and so mass bias can be corrected for mathematically using a series of equations in a three-dimensional data reduction scheme (see Siebert et al. 2001 for more details). Addition of a double spike also allows for the calculation of precise concentration data by isotope dilution (see Box 3.15), provided that the spike amount and the sample weight are known. A double spike technique requires three isotope ratios (e.g. 56/54, 57/54 and 58/54 for Fe; Dodson 1963), and so while it can be used for Ni, Fe, Zn and Cd, it is not suitable for Cu that only has two naturally occurring isotopes. Use of a double spike is advantageous because it accounts for matrix effects or rapid mass bias changes that can cause problems for sample- standard bracketing, as well as accounts for any fractionation of isotope ratios during chemical processing. Use of a double spike is not without challenges, however, as the spike must be made (by purchasing high-purity single spikes as metal ingots or compounds, dissolving and mixing them) and calibrated (see Box 3.15). If a multi-element technique is being used, multiple double spikes must be 'cleaned' to prevent contamination from the spike solution biasing other metal isotope ratios (e.g. Sun et al. 2021). Typically, such cleaning methods will involve scaled-up versions of the metal purification approach used for seawater samples, but may require adjustments (e.g. Sieber et al., 2019).

Different laboratory groups use slightly different approaches for analytical protocols, but a typical analytical session at USF would begin by analysing a series of mixtures of the 'natural' zero standard and the calibrated double spike at a range of concentrations and ratios to check that the same delta value (ideally 0 ± 0.1‰) is obtained for the zero standard across the range of intended sample compositions. For example, for Fe, these solutions would be made from IRMM-014 and the double spike and typically take the form of 5:1, 2:1, 1:1, 1:2 and 1:5 (natural/spike ratio) and 5:10, 10:20, 25:50, 50:100 and 100:200 (natural ng/g: spike ng/g) concentrations. After this, groups of 5–6 seawater samples are analysed, with an IRMM-014:double spike mixture measured before and after each group. Sample δ^{56}Fe values are then calculated using the double spike iterative technique (Siebert et al. 2001) and subsequently expressed relative to the average (or 'zero') of the δ^{56}Fe of the two mixtures.

Box 3.15: Double Spike Calibration

Once the double spike is purified, the three isotope ratios required for double spike calculations must be established in both the natural zero standard and the spike. The isotope ratios of the spike and natural metal must then be entered into the double spike calculation scheme, which can then be used to correct for instrumental mass bias in samples. Establishment or 'calibration' of the isotope ratios in the spike and natural metal (usually in the zero standard) is not trivial, however. The calibration process typically requires measurement of both natural and spike solutions and then mathematical optimisation of the spike composition by ensuring a set of natural-double spike mixture solutions generate a delta of 0‰ in the double spike calculation scheme. The spike calibration process can either be carried out once, after preparation of the spike, or more frequently if required. For use in concentration measurements in samples via isotope dilution, the concentration of the spike must also be first established by inverse isotope dilution with a concentration RM.

Uncertainty (Precision and Accuracy) on Trace Metal Isotope Ratios Uncertainty on isotope measurement by MC-ICP-MS is typically expressed as a combination of 'accuracy' and 'reproducibility' (precision). Accuracy is influenced by mass bias and interference corrections, with insufficiently corrected isobaric or polyatomic interferences leading to significant inaccuracy (e.g. several ‰) on isotope ratios.

Precision can be expressed as the internal statistics of a single MC- ICP-MS measurement, where uncertainty arises principally from a combination of detector noise, ion counting statistics and plasma flicker within the MC-ICP-MS (e.g. John and Adkins 2010), or external precision that also includes any effects during sampling, chemical extraction and interference correction, as well as day-to- day run variability. A large and/or variable procedural blank to signal ratio can drive inaccuracy and reduce precision. Different groups express accuracy and precision on isotope ratios using different approaches, which are summarised here. In all cases, however, it is crucial to robustly establish both types of uncertainty in order to assess data quality, before any data variability can be interpreted.

Accuracy of δ^{56}Fe, δ^{60}Ni, δ^{63}Cu, δ^{66}Zn and δ^{114}Cd in seawater can be assessed in several ways: (1) measurement of the isotope ratio of a standard reference material (e.g. Abouchami et al. 2013); (2) 'doping' a standard reference material into low-metal seawater and then comparing obtained values to literature values (e.g. Bermin et al. 2006; Conway et al. 2013); or (3) comparing isotope measurements obtained by different groups in reference seawater (e.g. SAFe) or at GEOTRACES crossover stations (e.g. Boyle et al. 2012; Conway et al., 2016). The challenges of collection of sufficient volume for multiple laboratories have restricted intercomparison, but efforts have taken place in the Southern Ocean, North and South Atlantic and Southwest Pacific (Boyle et al. 2012; Xue, et al. 2013; Xie, et al. 2019; Ellwood et al. 2020), and multiple groups have now published Cd and Zn isotope ratios for the SAFe standards (e.g. Cd is summarised in Sieber, et al. 2019). These exercises generally show good agreement between groups, but highlight a need for more intercomparison, especially for low-concentration surface samples for elements like Cd (Janssen et al. 2019).

To establish reproducibility on isotope measurements (also referred to as analytical precision), there are several approaches which can be taken. The simplest is to look at the internal statistics of a single ICP-MS analysis (the 2x standard error; 2SE), which depends largely on the concentration for sample limited analyses—for example, for a set of North Atlantic seawater samples, 2SE internal error varied from ~0.01‰ for samples with high concentrations of Fe (>1 nM in 1 L) to 0.1‰ for low-concentration surface samples (~0.1 nM in 1 L; Conway et al. 2013). However, this estimation of precision does not consider within run or run-to-run uncertainty, or uncertainties associated with sample processing. A fuller assessment of precision (external precision) is to calculate the 2x standard deviation (2SD) of the isotope ratio of a sample by measuring multiple aliquots of samples that have been through complete chemical processing and ideally measured over multiple analytical sessions (e.g. Bermin et al. 2006; Xue et al. 2012; Ellwood et al. 2020). For Fe, external precision can be on the order of 0.04 to 0.07‰ (Conway et al., 2016 Ellwood et al. 2020). For seawater, however, this approach is usually prohibited by the volume requirements of large numbers of replicate samples (ideally 30+ samples). Instead, assessment of external precision on isotope ratios for seawater samples commonly uses the 2SD of the isotope ratio of replicate measurements of an isotope standard RM over multiple analytical periods. Such external precision is typically greater than internal precision and on the order of 0.03–0.09‰ depending on element and laboratory. For example, while the internal 2SE of a δ^{56}Fe measurement at USF by Neptune MC-ICP-MS can be as low as 0.01 or 0.02‰, the 2SD of measurement of the NIST 3126 Fe reference material is

0.04‰, equivalent to the 0.04‰ established from replicate analyses of seawater at USF (Sieber et al. 2021). As such, such estimates of external precision should typically be quoted as the best estimate of uncertainty on a seawater measurement, unless the internal error is larger (and then the internal error should be used; e.g. Sieber et al. 2021).

3.5.3 Trace Metal Speciation Measurement Techniques

Biological effects and the geochemical behaviour (solubility, reactivity, residence times) of trace metals in the ocean are highly dependent on the physical and chemical speciation of the metal of interest. Thus, measurements of dissolved metal concentrations alone may not yield sufficient information for understanding the fate of trace metals in the ocean (Gledhill and Buck 2012; Tessier and Turner 1995; Vraspir and Butler 2009). For marine chemists, the redox speciation and organic complexation (including the free ion concentration) of bioactive metals in the dissolved fraction are of particular interest to better understand the toxicity, bioavailability and geochemical behaviour of trace metals in natural waters (Achterberg et al. 2018; Hirose 2006). Chemical speciation refers to the specific chemical form of an element, while speciation analysis is an analytical process for identifying and/or measuring the quantities of a chemical species of interest. Though redox speciation plays an important role in the cycling and fate of dissolved marine trace metals, redox speciation is beyond the scope of this chapter, but some redox species can be quantified in seawater samples (e.g. Boye et al. 2006; Bruland et al. 2014; Gledhill and van den Berg 1995; Han and Pan 2021; Oldham et al. 2021; Pađan et al. 2019; Sander et al. 2009; Stumm and Morgan 1996). Here, we focus solely on the chemical speciation, i.e. complexation, including free metal ion concentration (M^+, with M being the metal of interest) and the organic complexation of trace metals (ML, with L being the organic ligand) in the marine environment (see Fig. 3.1). Inorganic metal complexation (MY, with Y referring to, e.g. OH^-, $CO_2{}^{-3}$, Cl^-, etc., Fig. 3.1) also exists but is not discussed in detail here.

The speciation of many dissolved metals, namely, Fe but also Cu, Co, Mn, Zn and Ni, in marine systems is controlled by complexation processes with organic binding ligands, consisting of organic low molecular weight compounds up to large macromolecules (Donat et al. 1994; Ellwood and Van den Berg 2000; Gledhill and Buck 2012; Saito and Moffett 2001; Vraspir and Butler 2009; Whitby et al. 2018). The organic ligands can form stable complexes with various metals, keeping them in solution – thus both possibly inhibiting their biological uptake and/or reducing their adsorption to particles and thereby removal from the water column (Gledhill and Buck 2012; Vraspir and Butler 2009). For Fe, however, ligands can be a blessing, facilitating biological Fe uptake in Fe-limited environments such as the HNLC regions (Hassler et al. 2011b). Examples of organic metal-binding ligands are humic substances derived from terrestrial humus, as well as thiols, siderophores and exo-polymeric substances (e.g. Hassler et al. 2011a; b; Hirose 2006; Velasquez et al. 2016; Velasquez et al. 2011; Vraspir and Butler 2009). Most organic ligands are, however, yet to be identified and their contributions quantified. Similarly, the processes, sources, dynamics and driving factors of organic metal-binding ligand complexation in the ocean are still poorly understood and are thus an area of ongoing research (e.g. Buck et al. 2016; Campos and van den Berg 1994; Hartland et al. 2019; Hirose 2006; Kleint et al. 2016; Laglera et al. 2011; Laglera et al. 2020; Sander et al. 2007; Whitby et al. 2020; Whitby et al. 2018). The quantitative characterization of metal complexation with organic ligands is currently largely carried out via the estimation of ambient organic ligand concentrations ([L]) and conditional stability constants ($\log K^{\mathrm{cond}}_{\mathrm{ML}}$, with M being the metal of interest) of the metal-ligand complex via electrochemical techniques, i.e. voltammetry (Henze 1990; Hirose 2006; Vraspir and Butler 2009). Conditional stability constants reflect the strength of a complex between a metal ion and an organic ligand at a specific temperature, pressure, pH, and ionic strength. These conditional stability constants are complex specific and are thus an important parameter to indirectly identify the character of organic ligands and their ecological roles in the marine environment (Hirose 2006; Vraspir and Butler 2009) (see Box 16 for more information on the characterization of ligands). For instance, $\log K^{\mathrm{cond}}_{\mathrm{ML}}$ are commonly divided into various classes; in the case of Cu-binding ligands two ligand classes are usually distinguished, i.e. L_1 referring to strong ligands ($\log K_1{}^{\mathrm{cond}}_{\mathrm{CuL,\ Cu^{2+}}}$ >12 and up to 16) and L_2 referring to weak ligands ($\log K_2{}^{\mathrm{cond}}_{\mathrm{CuL,\ Cu^{2+}}}$ <12) (Croot et al., 2000; Vraspir and Butler, 2009, Bundy et al., 2013; Muller and Batchelli, 2013,Whitby et al., 2018). However, ligand classes with considerably lower $\log K_2{}^{\mathrm{cond}}_{\mathrm{CuL,\ Cu^{2+}}}$ have been quantified in some studies of coastal and estuarine settings (e.g. Heller and Croot 2015; Sander, et al. 2015). Information about the nature of naturally observed ligand classes can be obtained by comparison to measured $\log K^{\mathrm{cond}}_{\mathrm{ML}}$ of known organic molecules studied in the laboratory. Such a comparative approach can give a preliminary indication of the prevalence of different marine organic ligands, for example actively and/or passively produced by phytoplankton and/or originating from humic substances.

Box 3.16: Molecular Characterization of Metal-Binding Organic Ligands

Molecular characterization and quantification of naturally occurring marine organic ligands in the ocean, while largely an emerging discipline, can be done via excitation-emission matrix (EEM) fluorescence combined with parallel factor analysis (PARAFAC) (Yamashita et al. 2011), nuclear magnetic resonance (NMR) (Hertkorn et al. 2006; Rehman et al. 2017), high-performance liquid chromatography mass spectrometry (HPLC-MS) (Boiteau et al. 2016; Mawji et al. 2008; Velasquez et al. 2016) and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) (Pohlabeln and Dittmar 2015). Studies making use of such techniques have provided new insights, for example, into the distribution and form of certain organic binding molecules for Fe, Ni and Cu in the Pacific Ocean (e.g. Boiteau and Repeta 2015; Boiteau et al. 2016; Bundy et al., 2018; Boiteau et al., 2019), or to investigate siderophores associated with nitrogen fixers (Gledhill et al., 2019). However, most of the methods for characterising and quantifying marine organic- binding compounds are still in development or refinement or limited to a small number of laboratories and yet to be widely applied. As such, we do not provide a detailed discussion here but point readers to the references above

The following sections will focus on the description of the quantitative and semi-qualitative voltammetric techniques which are widely used for trace metal speciation analysis in the scientific community.

3.5.3.1 Voltammetric Techniques

Voltammetry can be used for measuring total dissolved concentrations of various trace metals, but the efficiency of quantifying metal concentrations using voltammetric methods lags behind ICP-MS techniques and is thus no longer extensively used. However, voltammetric methods are nowadays still used to quantify the speciation of various metals in marine waters both onboard ship and in the laboratory (Campos and van den Berg 1994; Croot et al. 1999; Gerringa et al. 2015; Gledhill and van den Berg 1995; Plavšić et al. 2009; Rue and Bruland 1995; Ružić 1982; Saito and Moffett 2001; Van den Berg 1982; Van Den Berg 1986). While other methods for metal speciation determination in aquatic environments such as ion-selective electrodes (ISE), diffusive gradients in thin films (DGT; see Sect. 3.3.1.2) and ion-exchange techniques (IET) exist (Florence, 1986; Gerringa et al. 1991; Achterberg and Braungardt, 1999), voltammetry is the most used method for metal speciation analysis due to its selectivity, its suitability for ultra-low-concentration levels, particularly in saline waters (detection limit of 10^{-9} to 10^{-12} mol L^{-1}) (Campos and van den Berg 1994; Croot et al. 1999; Gledhill and van den Berg 1995; Rue and Bruland 1995; Ružić 1982; Van den Berg 1982), and the fact that this method needs no sample treatment such as salt removal. Voltammetric methods also have the advantage that they permit the discrimination between the free ionic form of a metal and a metal ion complexed with an organic ligand, including calculation of $[\mathrm{L}]$ and $\log K_{\mathrm{ML}}^{\mathrm{cond}}$ of the prevalent metal ligand complexes (Gerringa et al. 2014;Hudson et al. 2003; Omanović et al. 2010; Omanović et al. 2015; Pižeta et al. 2015; Rue and Bruland 1995; Ružić 1982). These parameters can then be used to evaluate the reactivity (e.g. bioavailability, toxicity, geochemical behaviour) of a trace metal of interest and thus lead to interpretations of its fate in the water column.

The free and labile metal ion is usually defined as the ionic form of a metal, e.g. Cu^{2+} for Cu and Fe^{3+} and Fe^{2+} for Fe, but can also include all forms of the metal which are reactive or labile and can be detected by a particular analytical method (Henze 1990). Thus, in practice, these forms often also include metals complexed by inorganic ligands (Bruland et al. 2014). For voltammetry, the reactive species of a metal is specifically the fraction that is readily reactive at a working electrode (Henze 1990) usually a mercury-based electrode. The mercury (Hg) electrode was developed and defined by Jaroslav Heyrovsky in the early 1920s (Zuman 2001) and further refined by Matson et al. (1965). However, due to Hg being a chemical of major health concern, Hg electrodes are currently being progressively replaced with other materials such as carbon or noble metals (gold, silver, platinum) which are often modified with films, nanostructured materials or reagents, to improve the selectivity and/or sensitivity of the analysis (Borrill et al. 2019; Worsfold et al. 2019c). Voltammetry is a method based on current(redox)-potential-response measurements in a three-electrode system—the working electrode, the reference electrode and the counter electrode, which is also known as the auxiliary electrode (Han and Pan 2021; Henze 1990). The registered analytical signal is the change in the current-potential behaviour during a redox reaction of a metal of interest at the stationary working electrode, which itself is immersed into an electro-active solution, e.g. a saltwater sample (Henze 1990). The reference electrode is used as a reference for measuring and controlling the potential of the working electrode (Henze 1990). The counter electrode serves as a source or sink of electrons to balance the current observed at the working electrode (Henze 1990).

Fig. 3.24 Photograph of an automated voltammetry set-up (left) and a three-electrode voltammetric cell (right) (photograph credits: Dario Omanović, Rudjer Boskovic Institute, Croatia)

The electron transfer between redox species at the working electrode and the counter electrode generates a current which provides (1) quantitative information on the analyte of interest, that is, the signal is proportional to the metal concentration, and (2) qualitative information of the analyte since the potential where the signal is detected is analyte specific (Achterberg et al. 2018; Borrill et al. 2019; Han and Pan 2021; Henze 1990). The latter allows voltammetry to be used for mono-elemental or multi-elemental determination. Many laboratories use a three-electrode configuration that is composed of a hanging mercury drop electrode (HMDE) as the working electrode, an Ag|AgCl|3 M KCl reference electrode and a glassy carbon or platinum counter electrode. In general, the basic instrumentation for voltammetric measurements consists of a potentiostat (device that controls the potential between the electrodes while measuring the resulting current flow, i.e. the signal); a three- electrode cell, as described above; and a computer for automated measurements and data acquisition (Fig. 3.24) (e.g. Achterberg et al. 2018; Borrill et al. 2019; Han and Pan 2021; Henze 1990). The obtained signal is used to estimate [L] and $\log K_{ML}^{cond}$, which together with the metal concentration can be used to calculate the free metal ion concentration of the analyte of interest.

Stripping voltammetry (SV) is a subdivision of voltammetry and constitutes the most widely used voltammetric technique in electroanalytical chemistry since it is the most sensitive electrochemical technique currently available (Achterberg et al. 2018; Borrill et al. 2019; Han and Pan 2021; Henze 1990). The high sensitivity and selectivity of stripping voltammetry are the result of the separation of the analytical technique into two steps, that is, a pre-concentration step and a so-called stripping step. The pre-concentration step consists of an electrochemical deposition of a metal species onto or into the working electrode at a constant potential, necessary to isolate the metal of interest from the matrix. This first step can involve either an anodic or a cathodic potential. This step is followed by the second ‘stripping’ step during which the analyte of interest is ‘stripped’ back into the solution. The resulting current is proportional to the analyte concentration in the sample (Achterberg et al. 2018; Borrill et al. 2019; Han and Pan 2021; Henze 1990). Depending on the reduction or oxidation of analytes during the potential sweep, SV can be classed as either anodic SV (ASV; oxidation with reductive pre-concentration step) or cathodic SV (*CSV*; reduction with oxidative pre-concentration step) (see Box 3.17). Currently, competitive ligand exchange-adsorptive cathodic striping voltammetry (CLE-AdCSV) is the most widely used voltammetric method for metal speciation analysis, especially in seawater matrices (Han and Pan 2021). The principle of this method is the addition of a well-characterized artificial ligand (AL), establishing a competitive equilibrium between the AL and the natural ligands (L) in a seawater sample (Achterberg et al. 2018; Borrill et al. 2019; Han and Pan 2021; Henze 1990; Rue and Bruland 1995).

The AL forms an electrochemically active complex with the metal of interest, and after titration with increasing concentrations of M, the natural [L] and $\log K_{\mathrm{ML}}^{\mathrm{cond}}$ can be determined. The main advantage of the technique is its greater sensitivity compared to conventional ASV and CSV and that the $[\mathrm{M}^{+}]$ can be easily determined based on the M-AL concentration (Borrill et al. 2019; Han and Pan 2021; Henze 1990; Ružić 1982; Van den Berg 1982). Various synthetic ligands are available. For Fe, salicylaldoxime (SA; Abualhaija and van den Berg 2014; Campos and van den Berg 1994; Rue and Bruland 1995), 1-nitroso-2-naphthol (NN; Gledhill and van den Berg 1994), thiazolylazo-p-cresol (TAC; Croot and Johansson 2000) and dihydroxynaphthalene (DHN; Laglera et al. 2011; van den Berg 2006) are commonly used ligands (Han and Pan 2021). Depending on the ligand groups of interest, with respect to their $\log K^{\mathrm{cond}}$, the analyst can choose the appropriate detection window (D), with D being defined as the product of the concentration of AL and the conditional stability constant of M-AL (e.g. Apte et al. 1988; Laglera and Filella 2015).

Box 3.17: Metal Determination: ASV versus CSV

Both CSV and ASV are similar but differ in the nature and direction of the pre-concentration and stripping steps, as well as in the metals that can be determined (Han and Pan 2021; Worsfold et al. 2019c). ASV is employed to detect metal species that can be reduced, accumulated on and then reoxidized from the surface of the working electrode under appropriate potentials (Han and Pan 2021). Metals that can be determined by ASV are Pb, Cd and Zn in natural waters. ASV-labile metal fractions include free metal ions, inorganically bound metals and weakly organically bound metals (Achterberg et al. 2018; Borrill et al. 2019; Han and Pan 2021; Henze 1990; Van Den Berg 1986), and thus the labile fraction obtained by ASV generally corresponds well with the concentration of bioavailable metals (Han and Pan 2021). CSV is widely used for the determination and speciation analysis of more than 30 elements that cannot be reduced on electrode surface and determined by ASV, such as Fe, Co and Ni (Han and Pan 2021).

The following section describes in brief the analytical aspects and procedures of CLE-AdCSV used by various marine laboratories for the analysis of Cu and Fe speciation. For a more detailed description of the analytical steps and procedures, the reader is referred to available literature belonging to the specific application including Rue and Bruland

(1995) and Campos and van den Berg (1994).

3.5.3.2 Voltammetric Analysis of Metal Complexation by Ligand Titration Using CLE-AdCSV

Currently, the quantification of metal speciation ([L] and $\log K_{ML}^{cond}$) in discrete samples is widely undertaken using a titration approach with an artificial ligand (AL), i.e. CLE-AdCSV. The CLE-AdCSV method can be implemented for fresh or thawed unacidified seawater samples. Briefly, a seawater sample is divided into typically 10–15 sub-samples, which should be pipetted into trace metal clean and previously conditioned voltammetric cups. Preconditioning of voltammetric cups with a matrix matched seawater sample, including metal additions and buffer, is necessary to avoid adsorption of the metal of interest on the cup wall prior to the measurements. First, the samples are generally buffered to a method specific pH using a particular buffer. For Fe and Cu, the pH has to be buffered to ambient seawater pH (~8.04) using, e.g. a borate buffer or a HEPES buffer ((4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; a zwitterionic sulfonic acid buffering agent), to ensure efficient electrodeposition at the Hg drop (Borrill et al. 2019). Lately, however, some methods have been introduced that quantify metal speciation at natural pH without the need for buffer addition (Sanvito and Monticelli 2021). This step is followed by the addition of the AL of choice. Note that the added AL must be in great excess (factor 1000 approximately) of the metal concentration of interest to obtain reliable results. Typically, metal concentrations are quantified in sub-samples using ICP-MS prior to the voltametric measurements. Before the AL addition, increasing amounts of the metal of interest are added to the vials. After the addition of AL, buffer and metal, an equilibration period (often overnight owing to slow kinetics) is applied during which the naturally present ligands compete (i.e. equilibration) with the synthetic ligand for the metal of interest in a controlled manner. Once equilibrated, the metal speciation in the samples can be determined via CSV (see Box 3.18 and 3.19).

Box 3.18: Forward and Reverse Titration

It should be noted that the metal titration approach described here, also termed forward titration, is suitable only for conditions where the natural organic ligand concentrations exceed the dissolved metal concentrations in the sample, which is the case for most oceanic conditions (Achterberg et al. 2018). However, in estuarine, coastal and hydrothermal waters that receive significant metal inputs, metal concentrations can exceed the natural ligand concentrations and then a reverse titration rather than a forward titration has to be applied, by increasing the concentration of the added ligand rather than the metal concentration (Hawkes et al. 2013).

Prior to analysis, the sample may be de-aerated with an inert gas (N_2 or argon) for at least 2 min to remove reactive dissolved oxygen from solution in order to reduce interferences of the reactive oxygen in the voltammogram and to improve the sensitivity of the voltammetric determination (e.g. Campos and van den Berg 1994; Henze 1990; Ružić 1982; Van den Berg 1982). No purge (e.g. Buck et al. 2007) or compressed air is also used by some laboratories for some CLE-AdCSV methods including for Fe, e.g. with SA (Abualhaija and van den Berg 2014) or DHN (Sanvito and Monticelli 2021). No purge or compressed air is used in order to equilibrate the solution with the above standing atmospheric pressure and/or avoid carbon dioxide removal and thus changes in sample pH (Sanvito and Monticelli 2021). A deposition potential is then applied to the working electrode to cause the analyte of interest to be adsorbed on the Hg drop. The solution is commonly stirred during deposition to maximize electrode-analyte contact and to decrease the diffusion layer of the Hg drop. The selection of the deposition potential depends upon the analyte of interest and the redox process (reduction or oxidation). Deposition times can be increased to acquire more sensitivity (distinctive peaks) or decreased to avoid electrode saturation issues of high metal samples. Subsequently, the sample is scanned in a certain potential range (cathodic or anodic) to strip the analyte of interest from the electrode while measuring the resulting current. For quality assurance, each measurement should be repeated at least two times, each with a freshly generated Hg drop. The pH of each sample should be monitored in sample aliquots (the pH electrode must not be immersed into the sample as it may introduce contamination), preferably before the titration, to ensure reliable results—the efficiency of the electrodeposition at the Hg drop is pH dependent. Once the titrations are completed, data transformation allows the calculation of [L] and $\log K^{cond}$, in addition to the free aqueous metal concentration (Omanović et al. 2015; Pižeta et al. 2015; Sander et al. 2011; Wells et al. 2013). Data transformation can be facilitated using software packages such as proMCC developed by Omanović et al. (2015) or the R-based software package developed by Gerringa et al. (2014). Both system operation and data fitting require extensive training of the analyst.

Box 3.19: Limitations of Voltammetric Methods

While voltammetry remains an indispensable tool for evaluating metal speciation, this method suffers from several limitations that can lead to significant misrepresentation of metal complexation parameters in seawater samples. Some of the major issues and uncertainties of the electrochemical method are listed below.

1. The voltammetric technique works under the assumption that natural organic ligands exist in well-defined classes, which can be operationally distinguished and measured independently from each other (Town and Filella 2000). However, in complex and heterogeneous natural saline waters, it is much more likely that a ‘ligand soup’ exists without specific partitioning into different ligand classes (Town and Filella 2000).
2. It is largely assumed that metal-ligand complexation processes occupy a 1: 1 metal-ligand stoichiometry (Omanović et al. 2010), even though ligands with multiple binding sites could be expected and might be more realistic (Tipping, 1998).
3. Organic compounds, sulphides in hydrothermal samples and multiple prevalent metals may shift or distort the stripping peaks for the analyte of interest. These problems can often be minimized by adjusting the deposition time, changing the deposition potential or adding a pre-treatment step. For instance, sulphide-rich hydrothermal samples can undergo a pretreatment to remove acid volatile sulphides (AVS) from the sample solutions, which improves the electrochemical measurement of the analyte of interest

(Sander et al. 2007; Kleint et al. 2016; Cotte et al., 2018).

3.5.3.3 Data Quality Control for Trace Metal Speciation Measurements At present, no reference material exists for the *speciation* of trace metals. Thus, method validation is currently limited to measuring the repeatability and reproducibility of the procedure, i.e. by measuring some samples in duplicate. In addition, it is recommended that the accuracy of the voltammetric method be evaluated by measuring [L] of UV-oxidized seawater samples spiked with a known amount of artificial ligand or humic acid using the procedure described above (e.g. Gerringa et al. 2021a, b; Whitby et al. 2020, 2018).

3.6 Considerations of Data Quality, Inter-Comparability and Accessibility

Data produced by the marine trace metal community is critical to improve our current understanding of the biogeochemistry of the ocean and its variability, to force models of ocean biogeochemistry and thus enhance predictions for future ocean changes and to help design effective data-driven management and mitigation strategies (Worsfold et al. 2019). Further, biogeochemical data, including time series data, are becoming more and more critical in informing climate modelers and climate-relevant decisions of governments and the civic society, and thus, there is an urgent need for good practice and good quality data using the FAIR principles (i.e. data has to be findable, accessible, interoperable and reusable) (Tanhua et al. 2019; Worsfold et al. 2019). Now, national funding agencies and journals often require that data is publicly deposited in online repositories, with attached metadata and data quality assessments. Data quality assurance (accuracy, reproducibility; see Sects. 3.5.1.4 and 3.5.2.3) is particularly important for trace metal research as many commonly used methods and analytical techniques operate at or close to their limit of detection, especially in open ocean waters (Worsfold et al. 2019). Since many techniques are designed or customised by individual laboratories, metadata (information on, e.g. used methods and procedures and availability of ancillary data) is also essential.

In order to achieve the best possible trace metal data, GEOTRACES recommends intercalibration exercises including designated ‘crossover’ stations between expeditions, the analysis of RM alongside samples and the practice of reporting overall uncertainty as well as the internal instrumental precision (Cutter 2013; Worsfold et al. 2019). Crossover stations are when multiple different expeditions occupy the same geographic location and sample the water column, albeit at different times. Such crossovers are essential for combining multiple expedition datasets into usable global GEOTRACES products. Crossover stations allow intercomparison of the fidelity of sampling systems and/or the analytical techniques used to measure metals of interest by different laboratories/groups on different expeditions (e.g. Middag et al. 2015a, b). The fidelity of analytical techniques can also be assessed by different laboratories on aliquots of the same sample using different techniques (e.g. Middag et al. 2015a, b; Jensen et al. 2020). Such intercomparison exercises, especially when using different sampling systems, test not only inter-laboratory precision but also any uncertainty resulting from sample collection and handling. For example, Jensen et al. (2019) compared Zn from full water column profiles in the Arctic Ocean analysed by three different techniques by three laboratories (two SeaFAST ICP-MS and one FIA) and found a RSD of 6% for the average of the three methods, similar to the stated internal precision of those individual techniques. Similarly, Middag et al. (2015a, b) found that for the full water column of the North Atlantic, two laboratories using ICP-MS largely statistically agreed within stated analytical precision, except for the surface samples which was attributed to temporal variation between station occupations. Strong agreement has also been found between laboratories in trace metal isotope intercomparison exercises (e.g. Boyle et al. 2012; Conway et al. 2013), but the field is less mature than that of trace metal concentrations due to the smaller number of laboratories that routinely make these measurements, and the degree of agreement still depends on the element being studied. Voltammetric techniques are also seldom intercalibrated owing to the time-consuming analysis technique and the small number of laboratories routinely measuring metal speciation. However, published intercomparison studies showed that consensus values could be largely obtained between different laboratories and using different voltametric methods, but that the number of identified ligand classes present and the groups of ligands resolved in a target sample need more attention in future work (e.g. Bruland et al. 2000; Buck et al. 2016, 2012; Pižeta et al. 2015).

Going forward, continuing to include information on laboratory precision and accuracy, combined with metadata and intercomparison between different laboratories and sampling systems, will enhance the ability of the trace metal community to maintain consistent data quality and to compare datasets obtained by different investigators using different instruments, analytical protocols and/or techniques (Worsfold et al. 2019). In fact, GEOTRACES requires evidence of data quality and intercomparison to be assessed by a committee before data can be included in a publicly available GEOTRACES data product, to ensure synthesized global datasets (Schlitzer et al. 2018). Such close attention to quality control, combined with free public accessibility, means that GEOTRACES datasets can serve as a reliable baseline to assess future change. GEOTRACES data and derived insights will likely be an invaluable tool to develop diagnostic and predictive models on the biogeochemical cycles that ultimately drive life on our planet that is increasingly perturbed by anthropogenic influences.

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References

Abadie C et al (2017) Iron isotopes reveal distinct dissolved iron sources and pathways in the intermediate versus deep Southern Ocean. Proc Natl Acad Sci 114(5):858–863

Abouchami W et al (2011) Modulation of the Southern Ocean cadmium isotope signature by ocean circulation and primary productivity. Earth Planet Sci Lett 305(1–2):83–91

Abouchami W et al (2013) A common reference material for cadmium isotope studies–NIST SRM 3108. Geostand Geoanal Res 37(1):5–17

Abualhaija MM, van den Berg CMG (2014) Chemical speciation of iron in seawater using catalytic cathodic stripping voltammetry with ligand competition against salicylaldoxime. Mar Chem 164:60–74

Achterberg EP, Braungardt C (1999) Stripping voltammetry for the determination of trace metal speciation and in-situ measurements of trace metal distributions in marine waters. Anal Chim Acta 400(1–3):381–397

Achterberg EP et al (2013) Natural iron fertilization by the Eyjafjallajökull volcanic eruption. Geophys Res Lett 40(5):921–926

Achterberg EP, Gledhill M, Zhu K (2018) Voltammetry—cathodic stripping. Elsevier

Adams WJ, Chapman PM (2007) Assessing the hazard of metals and inorganic metal substances in aquatic and terrestrial systems. CRC Press

Allan IJ et al (2008) Chemcatcher® and DGT passive sampling devices for regulatory monitoring of trace metals in surface water. J Environ Monit 10(7):821–829

Anderson RF (2020) GEOTRACES: accelerating research on the marine biogeochemical cycles of trace elements and their isotopes. Annu Rev Mar Sci 12(1):49–85

Anderson RF et al (2014) GEOTRACES: changing the way we explore ocean chemistry. Oceanography 27(1):50–61

Aparicio-González A, Duarte CM, Tovar-Sánchez A (2012) Trace metals in deep ocean waters: A review. J Mar Syst 100:26–33

Apte SC, Gardner MJ, Ravenscroft JE (1988) An evaluation of voltammetric titration procedures for the determination of trace metal complexation in natural waters by use of computers simulation. Anal Chim Acta 212:1–21

Apte SC, Batley G, and Maher WA (2002) Monitoring of trace metals and metalloids in natural waters. Environmental Monitoring Handbook

Archer C, Vance D (2004) Mass discrimination correction in multiple-collector plasma source mass spectrometry: an example using Cu and Zn isotopes. J Anal At Spectrom 19(5):656–665

Archer C, Andersen MB, Cloquet C, Conway TM, Dong S, Ellwood M et al (2017) Intercalibration of a proposed new primary reference standard AA-ETH Zn for zinc isotopic analysis. J Anal At Spectrom 32(2):415–419

Archer C et al (2020) The oceanic biogeochemistry of nickel and its isotopes: new data from the South Atlantic and the Southern Ocean biogeochemical divide. Earth Planet Sci Lett 535: 116118

Arrigo KR (2005) Marine microorganisms and global nutrient cycles. Nature 437(7057):349–355 Asadi S et al (2019) Aerosol emission and superemission during human speech increase with voice loudness. Sci Rep 9(1):1–10

Baconnais I et al (2019) Determination of the copper isotope composition of seawater revisited: a case study from the Mediterranean Sea. Chem Geol 511:465–480

Beard BL, Johnson CM (1999) High precision iron isotope measurements of terrestrial and lunar materials. Geochim Cosmochim Acta 63(11–12):1653–1660

Bell J, Betts J, Boyle E (2002) MITESS: a moored in situ trace element serial sampler for deep-sea moorings. Deep-Sea Res I Oceanogr Res Pap 49(11):2103–2118

Berger CJM et al (2008) Application of a chemical leach technique for estimating labile particulate aluminum, iron, and manganese in the Columbia River plume and coastal waters off Oregon and Washington. J Geophys Res Oceans 113:C2

Bergquist BA, Wu J, Boyle EA (2007) Variability in oceanic dissolved iron is dominated by the colloidal fraction. Geochim Cosmochim Acta 71(12):2960–2974

Bermin J et al (2006) The determination of the isotopic composition of Cu and Zn in seawater. Chem Geol 226(3–4):280–297

Biller DV, Bruland KW (2012) Analysis of Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb in seawater using the Nobias-chelate PA1 resin and magnetic sector inductively coupled plasma mass spectrometry (ICP-MS). Mar

Chem 130–131:12–20

Bishop JKB, et al. (1985) A multiple-unit large-volume in situ filtration system for sampling oceanic particulate matter in mesoscale environments

Bishop JKB, Lam PJ, Wood TJ (2012) Getting good particles: accurate sampling of particles by large volume in-situ filtration. Limnol Oceanogr Methods 10(9):681–710

Boiteau RM, Repeta DJ (2015) An extended siderophore suite from Synechococcus sp. PCC 7002 revealed by LC-ICPMS-ESIMS. Metallomics 7(5):877–884

Boiteau RM et al (2016) Structural characterization of natural nickel and copper binding ligands along the US GEOTRACES Eastern Pacific Zonal Transect. Front Mar Sci 3:243

Boiteau RM, Till CP, Coale TH, Fitzsimmons JN, Bruland KW, Repeta DJ (2019) Patterns of iron and siderophore distributions across the California current system. Limnol Oceanogr 64(1):376– 389

Borman CJ et al (2009) The use of flow-injection analysis with chemiluminescence detection of aqueous ferrous iron in waters containing high concentrations of organic compounds. Sensors 9(6):4390–4406

Borrill AJ, Reily NE, Macpherson JV (2019) Addressing the practicalities of anodic stripping voltammetry for heavy metal detection: a tutorial review. Analyst 144(23):6834–6849

Bowie AR, Lohan MC (2009) Determination of iron in seawater. In: Practical guidelines for the analysis of seawater. CRC Press, pp 247–270

Bowie AR et al (2002) Real-time monitoring of picomolar concentrations of iron(II) in marine waters using automated flow injection-chemiluminescence instrumentation. Environ Sci Technol 36(21):4600–4607

Bowie AR et al (2003) Shipboard analytical intercomparison of dissolved iron in surface waters along a north–south transect of the Atlantic Ocean. Mar Chem 84(1–2):19–34

Bowie AR, Sedwick PN, Worsfold PJ (2004) Analytical intercomparison between flow injection-chemiluminescence and flow injection-spectrophotometry for the determination of picomolar concentrations of iron in seawater. Limnol Oceanogr Methods 2(2):42–54

Boyd PW et al (2017) Biotic and abiotic retention, recycling and remineralization of metals in the ocean. Nat Geosci 10(3):167–173

Boye M et al (2006) The chemical speciation of iron in the north-East Atlantic Ocean. Deep-Sea Res I Oceanogr Res Pap 53(4):667–683

Boyle EA, Sclater F, Edmond JM (1976) On the marine geochemistry of cadmium. Nature 263(5572):42–44

Boyle EA et al (2012) GEOTRACES IC1 (BATS) contamination-prone trace element isotopes Cd, Fe, Pb, Zn, Cu, and Mo intercalibration. Limnol Oceanogr Methods 10(9):653–665

Brewer PG, Spencer DW, Robertson DE (1972) Trace element profiles from the GEOSECS-II test station in the Sargasso Sea. Earth Planet Sci Lett 16(1):111–116

Brewer PG (1975) Minor elements in sea water. In: Riley JP, Skirrow G (eds) Chemical oceanography. Academic Press, Waltham, pp 415–496

Bruland KW (1983) CHAPTER 45—trace elements in sea-water. In: Riley JP, Chester R (eds) Chemical oceanography. Academic Press, pp 157–220

Bruland KW, Franks RP (1983) Trace metals in seawater. Chem Oceanogr 8:157–220 Bruland KW, Lohan MC (2003) Controls of trace metals in seawater. Treatise Geochem 6:625

Bruland KW, Knauer GA, Martin JH (1978) Zinc in north-East Pacific water. Nature 271(5647): 741–743

Bruland KW et al (1979) Sampling and analytical methods for the determination of copper, cadmium, zinc, and nickel at the nanogram per liter level in sea water. Anal Chim Acta 105: 233–245

Bruland KW, Donat JR, Hutchins DA (1991) Interactive influences of bioactive trace metals on biological production in oceanic waters. Limnol Oceanogr 36(8):1555–1577

Bruland KW et al (2000) Intercomparison of voltammetric techniques to determine the chemical speciation of dissolved copper in a coastal seawater sample. Anal Chim Acta 405(1–2):99–113

Bruland KW et al (2003) Treatise on geochemistry. Oceans Mar Geochem 6:23–48

Bruland KW, Middag R, Lohan MC (2014) 8.2—controls of trace metals in seawater A2—Holland, Heinrich D. In: Turekian KK (ed) Treatise on geochemistry, 2nd edn. Elsevier, Oxford, pp 19–51

Buck KN et al (2007) Dissolved iron speciation in two distinct river plumes and an estuary: implications for riverine iron supply. Limnol Oceanogr 52(2):843–855

Buck KN et al (2012) The organic complexation of iron and copper: an intercomparison of competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV) techniques. Limnol Oceanogr Methods 10(7):496–515

Buck KN, Gerringa LJA, Rijkenberg MJA (2016) An intercomparison of dissolved iron speciation at the

Bermuda Atlantic Time-series Study (BATS) site: results from GEOTRACES crossover station a. Front Mar Sci 3:262

Buma AGJ et al (1991) Metal enrichment experiments in the Weddell-scotia seas: effects of iron and manganese on various plankton communities. Limnol Oceanogr 36(8):1865–1878

Bundy RM, Barbeau KA, Buck KN (2013) Sources of strong copper-binding ligands in Antarctic Peninsula surface waters. Deep-Sea Res II Top Stud Oceanogr 90:134–146

Bundy, R.M., Boiteau, R.M., McLean, C., Turk-Kubo, K.A., McIlvin, M.R., Saito, M.A., Van Mooy, B.A. and Repeta, D.J., 2018. Distinct siderophores contribute to iron cycling in the mesopelagic at station ALOHA. Frontiers in Marine Science, 5, p.61.

Cameron V, Vance D (2014) Heavy nickel isotope compositions in rivers and the oceans. Geochim Cosmochim Acta 128:195–211

Campos AM, van den Berg CMG (1994) Determination of copper complexation in sea water by cathodic stripping voltammetry and ligand competition with salicylaldoxime. Anal Chim Acta 284(3):481–496

Capodaglio G, Barbante C, Cescon P (2001) Trace metals in Antarctic Sea water. In: Environmental contamination in Antarctica. Elsevier, pp 107–154

Caroli S, Cescon P, and Walton DWH (2001) Trace metals in Antarctic sea water. Environmental Contamination in Antarctica: A Challenge to Analytical Chemistry:107

Chester R (1990) Trace elements in the oceans. In: Marine geochemistry. Springer, pp 346–421

Cleanroom-Technology (2011) Datex study into human particle shedding. GIT Verlag

Clough R et al (2015) Uncertainty contributions to the measurement of dissolved co, Fe, pb and V in seawater using flow injection with solid phase preconcentration and detection by collision/ reaction cell—quadrupole ICP–MS. Talanta 133:162–169

Conway TM, John SG (2014a) The biogeochemical cycling of zinc and zinc isotopes in the North Atlantic Ocean. Glob Biogeochem Cycles 28(10):1111–1128

Conway TM, John SG (2014b) Quantification of dissolved iron sources to the North Atlantic Ocean. Nature 511(7508):212–215

Conway TM et al (2013) A new method for precise determination of iron, zinc and cadmium stable isotope ratios in seawater by double-spike mass spectrometry. Anal Chim Acta 793:44–52 Conway TM et al (2021) A decade of progress in understanding cycles of trace elements and their isotopes in the oceans. Chem Geol:120381

Conway TM, John SG, Lacan F (2016) Intercomparison of dissolved iron isotope profiles from reoccupation of three GEOTRACES stations in the Atlantic Ocean. Mar Chem 183:50–61

Cotte L, Omanović D, Waeles M, Laës A, Cathalot C, Sarradin PM, Riso RD (2018) On the nature of dissolved copper ligands in the early buoyant plume of hydrothermal vents. Environ Chem 15 (2):58–73

Croot PL, Johansson M (2000) Determination of iron speciation by cathodic stripping voltammetry in seawater using the competing ligand 2-(2-Thiazolylazo)-p-cresol (TAC). Electroanalysis: an international journal devoted to fundamental and practical aspects of. Electroanalysis 12(8): 565–576

Croot PL, Moffett JW, Luther III GW (1999) Polarographic determination of half-wave potentials for copper-organic complexes in seawater. Mar Chem 67(3–4):219–232

Cullen JT, Sherrell RM (1999) Techniques for determination of trace metals in small samples of size-fractionated particulate matter: phytoplankton metals off Central California. Mar Chem 67(3):233–247

Cunliffe M, Wurl O (2014) Guide to best practices to study the ocean's surface. Plymouth, UK, Marine Biological Association of the United Kingdom for SCOR, 118 pp. (Occasional Publications of the Marine Biological Association of the United Kingdom). https://doi.org/10. 25607/OBP-1512

Cutter GA (2013) Intercalibration in chemical oceanography—getting the right number. Limnol Oceanogr Methods 11(7):418–424

Cutter GA, Bruland KW (2012) Rapid and noncontaminating sampling system for trace elements in global ocean surveys. Limnol Oceanogr Methods 10:425–436

Cutter GA, et al. (2017) Sampling and sample-handling protocols for GEOTRACES cruises, Version 3

Dauphas N, John SG, and Rouxel O (2017) 11 iron isotope systematics. Non-Traditional Stable Isotopes:415–510

Davison W, Zhang H (1994) In situ speciation measurements of trace components in natural waters using thin-film gels. Nature 367(6463):546–548

de Baar HJW et al (1990) On iron limitation of the Southern Ocean: experimental observations in the Weddell and Scotia Seas. Mar Ecol Prog Ser:105–122 de Baar HJW (1994) von Liebig's law of the minimum and plankton ecology (1899–1991). Prog Oceanogr 33(4):347–386. https://doi.org/10.1016/0079-6611(94)90022-1

De Baar HJW et al (2008) Titan: a new facility for ultraclean sampling of trace elements and isotopes in the deep oceans in the international Geotraces program. Mar Chem 111(1–2):4–21

de Baar HJW, van Heuven SMAC, Middag R (2018) Ocean biochemical cycling and trace elements. In: White WM (ed) Encyclopedia of geochemistry: a comprehensive reference source on the chemistry of the earth. Springer International Publishing, Cham, pp 1–21

de Jong JTM et al (1998) Dissolved iron at subnanomolar levels in the Southern Ocean as determined by ship-board analysis. Anal Chim Acta 377(2–3):113–124

De Jong J et al (2007) Precise measurement of Fe isotopes in marine samples by multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS). Anal Chim Acta 589(1): 105–119

Dodson MH (1963) A theoretical study of the use of internal standards for precise isotopic analysis by the surface ionization technique: part I-general first-order algebraic solutions. J Sci Instrum 40(6):289

Donat JR, Lao KA, Bruland KW (1994) Speciation of dissolved copper and nickel in South San Francisco Bay: a multi-method approach. Anal Chim Acta 284(3):547–571

Douthitt CB (2008) The evolution and applications of multicollector ICPMS (MC-ICPMS). Anal Bioanal Chem 390(2):437–440

Ellwood MJ, Nodder SD, King AL, Hutchins DA, Wilhelm SW, Boyd PW (2014) Pelagic iron cycling during the subtropical spring bloom, east of New Zealand. Mar Chem 160:18–33

Ebling AM, Landing WM (2015) Sampling and analysis of the sea surface microlayer for dissolved and particulate trace elements. Mar Chem 177:134–142

Elliott T, Steele RCJ (2017) The isotope geochemistry of Ni. Rev Mineral Geochem 82(1):511–542

Ellwood MJ, Van den Berg CMG (2000) Zinc speciation in the northeastern Atlantic Ocean. Mar Chem 68(4):295–306

Ellwood MJ et al (2018) Insights into the biogeochemical cycling of iron, nitrate, and phosphate across a 5,300 km South Pacific zonal section (153 E–150 W). Glob Biogeochem Cycles 32(2): 187–207

Ellwood MJ et al (2020) Distinct iron cycling in a Southern Ocean eddy. Nat Commun 11(1):1–8 EPA, US (1996) Sampling ambient water for trace metals at EPA water quality criteria levels. Office of Water Engineering and Analysis Division Washington, EPA Method 1669

EPA (1997) Determination of trace elements in water by preconcentration and inductively coupled plasma-mass spectrometry. U.S. Environmental Protection Agency Office of Water Office of Science and Technology Engineering and Analysis Division (4303)

Falkner KK, Edmond JM (1990) Gold in seawater. Earth Planet Sci Lett 98(2):208–221

Farley KJ, Morel FM (1986) Role of coagulation in the kinetics of sedimentation. Environ Sci Technol 20(2):187–195

Field MP, Conway TM, Summers BA, Saetveit N, and Sakowski JC (2019) Automated processing of seawater samples for iron isotope ratio determination. Analytical Poster Note, Goldschmidt 2019, Elemental Scientific, Omaha, Nebraska, USA

Fitzsimmons JN, Boyle EA (2012) An intercalibration between the GEOTRACES GO-FLO and the MITESS/vanes sampling systems for dissolved iron concentration analyses (and a closer look at adsorption effects). Limnol Oceanogr Methods 10(6):437–450

Fitzsimmons JN, Boyle EA (2014a) Assessment and comparison of Anopore and cross flow filtration methods for the determination of dissolved iron size fractionation into soluble and colloidal phases in seawater. Limnol Oceanogr Methods 12(4):246–263

Fitzsimmons JN, Boyle EA (2014b) Both soluble and colloidal iron phases control dissolved iron variability in the tropical North Atlantic Ocean. Geochim Cosmochim Acta 125:539–550

Fitzsimmons JN et al (2015a) The composition of dissolved iron in the dusty surface ocean: an exploration using size-fractionated iron-binding ligands. Mar Chem 173:125–135

Fitzsimmons JN et al (2015b) Partitioning of dissolved iron and iron isotopes into soluble and colloidal phases along the GA03 GEOTRACES North Atlantic Transect. Deep-Sea Res II Top Stud Oceanogr 116:130–151

Floor GH et al (2015) Combined uncertainty estimation for the determination of the dissolved iron amount content in seawater using flow injection with chemiluminescence detection. Limnol Oceanogr Methods 13(12):673–686

Florence TM (1986) Electrochemical approaches to trace element speciation in waters. A review. Analyst 111(5):489–505

Garbe-Schönberg (2006) KIPS -a new multiport valve-based all-Teflon fluid sampling system for ROVs. Geology

Gasparon M (1998) Trace metals in water samples: minimising contamination during sampling and storage. Environ Geol 36(3):207–214

GEOTRACES-Group (2006) GEOTRACES Science Plan. Scientific Committee on Oceanic Research, Baltimore, Maryland

Gerringa LJA, Van der Meer J, Cauwet G (1991) Complexation of copper and nickel in the dissolved phase of marine sediment slurries. Mar Chem 36(1–4):51–70

Gerringa LJA et al (2014) A critical look at the calculation of the binding characteristics and concentration of iron complexing ligands in seawater with suggested improvements. Environ Chem 11(2):114–136

Gerringa LJA et al (2015) Organic complexation of iron in the West Atlantic Ocean. Mar Chem 177:434–446 Gerringa LJA et al (2020) Dissolved trace metals in the Ross Sea. Front Mar Sci 7:874

Gerringa LJA, Rijkenberg MJA, Slagter HA, Laan P, Paffrath R, Bauch D et al (2021a) Dissolved Cd, Co, Cu, Fe, Mn, Ni, and Zn in the Arctic Ocean. J Geophys Res Oceans 126(9): e2021JC017323

Gerringa LJA et al (2021b) Comparing CLE-AdCSV applications using SA and TAC to determine the Fe binding characteristics of model ligands in seawater. Biogeosci Discuss:1–40

Gillain G, et al. (1982) Sampling techniques and analytical methods. Distribution, Transport and Fate of Heavy Metals in the Belgian Coastal Marine Environment, Actions de Recherche Concertées-Geconcerteerde Onderzoeksacties, Oceanologie, final report 2:13–39

Gledhill M, Buck KN (2012) The organic complexation of iron in the marine environment: a review. Front Microbiol 3:69

Gledhill M, van den Berg CMG (1994) Determination of complexation of iron (III) with natural organic complexing ligands in seawater using cathodic stripping voltammetry. Mar Chem 47(1): 41–54

Gledhill M, van den Berg CMG (1995) Measurement of the redox speciation of iron in seawater by catalytic cathodic stripping voltammetry. Mar Chem 50(1–4):51–61

Gledhill M, Basu S, Shaked Y (2019) Metallophores associated with Trichodesmium erythraeum colonies from the Gulf of Aqaba. Metallomics 11(9):1547–1557

Goldberg, M.M. 1996 Trace metal clean room. National Environmental Research Laboratory, United States Environmental Protection Agency EPA/600/R-96/018

Goldhaber SB (2003) Trace element risk assessment: essentiality vs. toxicity. Regul Toxicol Pharmacol 38(2):232–242

Grand MM et al (2016) Determination of trace zinc in seawater by coupling solid phase extraction and fluorescence detection in the lab-on-valve format. Anal Chim Acta 923:45–54

Grand MM et al (2019) Developing autonomous observing systems for micronutrient trace metals. Front Mar Sci 6:35

Hales B, Takahashi T (2002) The pumping SeaSoar: a high-resolution seawater sampling platform. J Atmos Ocean Technol 19(7):1096–1104

Han H, Pan D (2021) Voltammetric methods for speciation analysis of trace metals in natural waters. Trends Environ Anal Chem:e00119

Hartland A et al (2019) Aqueous copper bioavailability linked to shipwreck-contaminated reef sediments. Sci Rep 9(1):1–13

Hassler CS et al (2011a) Exopolysaccharides produced by bacteria isolated from the pelagic Southern Ocean—role in Fe binding, chemical reactivity, and bioavailability. Mar Chem 123(1–4):88–98

Hassler CS et al (2011b) Saccharides enhance iron bioavailability to Southern Ocean phytoplankton. Proc Natl Acad Sci 108(3):1076–1081

Hawkes JA et al (2013) Characterisation of iron binding ligands in seawater by reverse titration. Anal Chim Acta 766:53–60

Heller MI, Croot PL (2015) Copper speciation and distribution in the Atlantic sector of the Southern Ocean. Mar Chem 173:253–268

Henderson GM, Achterberg EP, Bopp L (2018) Changing trace element cycles in the 21st Century Ocean. Elements 14(6):409–413

Henze G (1990) Application of polarographic and voltammetric techniques in environmental analysis. In: Metal speciation in the environment. Springer, pp 391–408

Hertkorn N et al (2006) Characterization of a major refractory component of marine dissolved organic matter. Geochim Cosmochim Acta 70(12):2990–3010

Hirose K (2006) Chemical speciation of trace metals in seawater: a review. Anal Sci 22(8): 1055–1063

Homoky WB et al (2016) Quantifying trace element and isotope fluxes at the ocean–sediment boundary: a review. Philos Trans R Soc A Math Phys Eng Sci 374(2081):20160246

Homoky WB et al (2021) Iron colloids dominate sedimentary supply to the ocean interior. Proc Natl Acad Sci 118(13):e2016078118

Honeyman BD, Santschi PH (1989) A Brownian-pumping model for oceanic trace metal scavenging: evidence from Th isotopes. J Mar Res 47(4):951–992

Horner TJ, Little SH, Conway TM, Farmer JR, Hertzberg JE, Janssen DJ, Lough AJM, McKay JL, Tessin A, Galer SJG, Jaccard SL (2021) Bioactive trace metals and their isotopes as paleoproductivity proxies: an assessment using GEOTRACES-era data. Glob Biogeochem Cycles 35(11):e2020GB006814

Hudson RJM, Rue EL, Bruland KW (2003) Modeling complexometric titrations of natural water samples. Environ Sci Technol 37(8):1553–1562

Hunter P (2008) A toxic brew we cannot live without: micronutrients give insights into the interplay between geochemistry and evolutionary biology. EMBO Rep 9(1):15–18

Hutchins DA, Boyd PW (2016) Marine phytoplankton and the changing ocean iron cycle. Nat Clim Change 6(12):1072–1079

Jackson SL et al (2018) Determination of Mn, Fe, Ni, Cu, Zn, Cd and Pb in seawater using offline extraction and triple quadrupole ICP-MS/MS. J Anal At Spectrom 33(2):304–313

Janssen DJ et al (2019) Particulate cadmium stable isotopes in the subarctic Northeast Pacific reveal dynamic Cd cycling and a new isotopically light Cd sink. Earth Planet Sci Lett 515:67–78

Jensen LT et al (2019) Biogeochemical cycling of dissolved zinc in the Western Arctic (Arctic GEOTRACES GN01). Glob Biogeochem Cycles 33(3):343–369

Jensen LT et al (2020) Assessment of the stability, sorption, and exchangeability of marine dissolved and colloidal metals. Mar Chem 220:103754

John SG, Adkins JF (2010) Analysis of dissolved iron isotopes in seawater. Mar Chem 119(1–4): 65–76

John SG, Conway TM (2014) A role for scavenging in the marine biogeochemical cycling of zinc and zinc isotopes. Earth Planet Sci Lett 394:159–167

Johnson CM, Beard BL (1999) Correction of instrumentally produced mass fractionation during isotopic analysis of Fe by thermal ionization mass spectrometry. Int J Mass Spectrom 193(1): 87–99

Johnson KS et al (2007) Developing standards for dissolved iron in seawater. EOS Trans Am Geophys Union 88(11):131–132

Johnson C, Beard B, Weyer S (2020) Iron geochemistry: an isotopic perspective. Springer

Karl DM, Lukas R (1996) The Hawaii Ocean time-series (HOT) program: background, rationale and field implementation. Deep-Sea Res II Top Stud Oceanogr 43(2–3):129–156

Kleint C et al (2016) Voltammetric investigation of hydrothermal iron speciation. Front Mar Sci 3: 75

Klunder MB et al (2011) Dissolved iron in the Southern Ocean (Atlantic sector). Deep-Sea Res Part Ii-Topical Studies in Oceanography 58(25–26):2678–2694

Klunder MB et al (2012) Dissolved iron in the Arctic Ocean: important role of hydrothermal sources, shelf input and scavenging removal. J Geophys Res Oceans 117:C4

Knutsson J (2013) Passive sampling for monitoring of inorganic pollutants in water. Chalmers University of Technology

Koprivnjak JF et al (2009) Chemical and spectroscopic characterization of marine dissolved organic matter isolated using coupled reverse osmosis–electrodialysis. Geochim Cosmochim Acta 73(14):4215–4231

Lacan F et al (2006) Cadmium isotopic composition in the ocean. Geochim Cosmochim Acta 70(20):5104–5118

Lacan F, Radic A, Jeandel C, Poitrasson F, Sarthou G, Pradoux C, Freydier R (2008) Measurement of the isotopic composition of dissolved iron in the open ocean. Geophys Res Lett 35(24)

Lagerström ME et al (2013) Automated on-line flow-injection ICP-MS determination of trace metals (Mn, Fe, Co, Ni, Cu and Zn) in open ocean seawater: application to the GEOTRACES program. Mar Chem 155:71–80

Laglera LM, Filella M (2015) The relevance of ligand exchange kinetics in the measurement of iron speciation by CLE–AdCSV in seawater. Mar Chem 173:100–113

Laglera LM, Battaglia G, van den Berg CMG (2011) Effect of humic substances on the iron speciation in natural waters by CLE/CSV. Mar Chem 127(1–4, 134):–143

Laglera LM et al (2020) Iron organic speciation during the LOHAFEX experiment: iron ligands release under biomass control by copepod grazing. J Mar Syst 207:103151

Lam PJ, Ohnemus DC, Auro ME (2015) Size-fractionated major particle composition and concentrations from the US GEOTRACES North Atlantic zonal transect. Deep-Sea Res II Top Stud Oceanogr 116:303–320

Landing WM, Bruland KW (1981) The vertical distribution of iron in the Northeast Pacific. EOS: Transactions of the American Geophysical Union 62:906

Lane TW, Morel FMM (2000) A biological function for cadmium in marine diatoms. Proc Natl Acad Sci 97(9):4627–4631

Lane TW et al (2005) A cadmium enzyme from a marine diatom. Nature 435(7038):42–42

Lee J-M et al (2011a) Analysis of trace metals (Cu, Cd, Pb, and Fe) in seawater using single batch nitrilotriacetate resin extraction and isotope dilution inductively coupled plasma mass spectrometry. Anal Chim Acta 686(1–2):93–101

Lee W-J, et al. (2011b) Spherical PF resin beads prepared from phenol-liquefied Bambusa dolichoclada with suspension polymerization

Little SH et al (2014) The oceanic mass balance of copper and zinc isotopes, investigated by analysis of their inputs, and outputs to ferromanganese oxide sediments. Geochim Cosmochim Acta 125:673–693

Little SH et al (2016) Key role of continental margin sediments in the oceanic mass balance of Zn and Zn isotopes. Geology 44(3):207–210

Lohan MC, Aguilar-Islas AM, Bruland KW (2006) Direct determination of iron in acidified (pH 1.7) seawater samples by flow injection analysis with catalytic spectrophotometric detection: application and intercomparison. Limnol Oceanogr Methods 4(6):164–171

Maréchal CN, Télouk P, Albarède F (1999) Precise analysis of copper and zinc isotopic compositions by plasma-source mass spectrometry. Chem Geol 156(1–4):251–273

Martin JH, Gordon RM (1988) Northeast Pacific iron distributions in relation to phytoplankton productivity. Deep Sea Res Part A Oceanographic Research Papers 35(2):177–196

Martin JH, Bruland KW, and Broenkow WW (1976) Cadmium transport in the California current [Water pollutants]

Martin JH, Gordon RM, Fitzwater SE (1990) Iron in Antarctic waters. Nature 345(6271):156–158 Martin JH et al (1994) Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature 371(6493):123–129

Matamoros V (2012) 1.13 – Equipment for water sampling including sensors. In: Pawliszyn J (ed) Comprehensive sampling and sample preparation. Academic Press, Oxford, pp 247–263

Matson WR, Roe DK, Carritt DE (1965) Composite graphite-mercury electrode for anodic stripping voltammetry. Anal Chem 37(12):1594–1595

Mawji E et al (2008) Collision-induced dissociation of three groups of hydroxamate siderophores: ferrioxamines, ferrichromes and coprogens/fusigens. Rapid Commun Mass Spectrom 22(14): 2195–2202

Mawji E, Schlitzer R, Dodas EM, Abadie C, Abouchami W, Anderson RF, Baars O, Bakker K, Baskaran M, Bates NR, Bluhm K (2015) The GEOTRACES intermediate data product 2014. Mar Chem 177:1–8

McDonnell AMP et al (2015) The oceanographic toolbox for the collection of sinking and suspended marine particles. Prog Oceanogr 133:17–31

Measures CI et al (2008) A commercially available rosette system for trace metal—clean sampling. Limnol Oceanogr Methods 6(9):384–394

Michaels AF, Knap AH (1996) Overview of the US JGOFS Bermuda Atlantic time-series study and the Hydrostation S program. Deep-Sea Res II Top Stud Oceanogr 43(2–3):157–198

Middag R et al (2011) Dissolved manganese in the Atlantic sector of the Southern Ocean. Deep Sea Res Part Ii-Topical Studies in Oceanography 58(25–26):2661–2677

Middag R et al (2015a) Intercomparison of dissolved trace elements at the Bermuda Atlantic time series station. Mar Chem 177(Part 3):476–489

Middag R et al (2015b) Dissolved aluminium in the ocean conveyor of the West Atlantic Ocean: effects of the biological cycle, scavenging, sediment resuspension and hydrography. Mar Chem 177(Part 1):69–86

Middag R et al (2020) The distribution of nickel in the West-Atlantic Ocean, its relationship with phosphate and a comparison to cadmium and zinc. Front Mar Sci 7:105

Mills MM et al (2004) Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. Nature 429(6989):292–294

Minami T et al (2015) An off-line automated preconcentration system with ethylenediaminetriacetate chelating resin for the determination of trace metals in seawater by high-resolution inductively coupled plasma mass spectrometry. Anal Chim Acta 854:183–190 Misra S et al (2014) Determination of δ11B by HR-ICP-MS from mass limited samples: application to natural carbonates and water samples. Geochim Cosmochim Acta 140:531–552

Moffett JW, German CR (2020) Distribution of iron in the Western Indian Ocean and the eastern tropical south pacific: an inter-basin comparison. Chem Geol 532:119334

Moigne L, Frédéric AC et al (2014) Sequestration efficiency in the iron-limited North Atlantic: implications for iron supply mode to fertilized blooms. Geophys Res Lett 41(13):4619–4627

Moore CM et al (2006) Iron limits primary productivity during spring bloom development in the Central North Atlantic. Glob Chang Biol 12(4):626–634

Moore CM et al (2009) Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability. Nat Geosci 2(12):867–871

Moore CM et al (2013) Processes and patterns of oceanic nutrient limitation. Nat Geosci 6(9): 701–710

Morel FMM, Milligan AJ, Saito MA (2014) 8.5—marine bioinorganic chemistry: the role of trace metals in the oceanic cycles of major nutrients. In: Holland HD, Turekian KK (eds) Treatise on geochemistry, 2nd edn. Elsevier, Oxford, pp 123–150

Morrison, Archie TIII, John D Billings, and Kenneth W Doherty (2000) The McLane WTS-LV: a large volume, high accuracy, oceanographic sampling pump. OCEANS 2000 MTS/IEEE Conference and Exhibition. Conference Proceedings (Cat. No. 00CH37158), 2000. Vol. 2, pp. 847–852. IEEE

Moynier F et al (2017) The isotope geochemistry of zinc and copper. Rev Mineral Geochem 82(1): 543–600

Muller FL, Batchelli S (2013) Copper binding by terrestrial versus marine organic ligands in the coastal plume of River Thurso, North Scotland. Estuar Coast Shelf Sci 133:137–146

Mueller AV, et al. (2018) A novel high-resolution trace metal clean sampler for stationary, profiling, and mobile deployment. 2018 OCEANS-MTS/IEEE Kobe Techno-Oceans (OTO), 2018, pp. 1–8. IEEE

Murray JW et al (1992) EqPac: a process study in the central equatorial Pacific. Oceanography 5(3): 134–142 Nehme C (2020) HVAC DESIGN FOR CLEAN ROOMS: Charles Nehme

Nielsdóttir MC et al (2009) Iron limitation of the postbloom phytoplankton communities in the Iceland Basin. Glob Biogeochem Cycles 23:3

Nishioka J, et al. (2020) Iron supply from the marginal seas and its influence on biological production in the North Pacific Ocean. Ocean Sciences Meeting 2020, 2020. AGU

Noble AE et al (2020) A review of marine water sampling methods for trace metals. Environ Forensic 21(3–4):267–290

Obata H et al (2017) Dissolved iron and zinc in Sagami Bay and the Izu-Ogasawara Trench. J Oceanogr 73(3):333–344

Ohnemus DC et al (2014) Laboratory intercomparison of marine particulate digestions including piranha: a novel chemical method for dissolution of polyethersulfone filters. Limnol Oceanogr Methods 12(8):530–547

Okamura K et al (2013) Development of a 128-channel multi-water-sampling system for underwater platforms and its application to chemical and biological monitoring. Methods Oceanogr 8: 75–90

Oldham VE et al (2021) Inhibited manganese oxide formation hinders cobalt scavenging in the Ross Sea. Glob Biogeochem Cycles 35(5):e2020GB006706

Olesik JW (2014) 15.17—inductively coupled plasma mass spectrometers. In: Holland HD, Turekian KK (eds) Treatise on geochemistry (second edition). Elsevier, Oxford, pp 309–336

Omanović D et al (2010) Significance of data treatment and experimental setup on the determination of copper complexing parameters by anodic stripping voltammetry. Anal Chim Acta 664(2):136–143

Omanović D, Garnier C, Pižeta I (2015) ProMCC: an all-in-one tool for trace metal complexation studies. Mar Chem 173:25–39

Pađan J et al (2019) Improved voltammetric methodology for chromium redox speciation in estuarine waters. Anal Chim Acta 1089:40–47

Pađan J et al (2020) Organic copper speciation by anodic stripping voltammetry in estuarine waters with high dissolved organic matter. Front Chem:8

Patterson CC (1965) Contaminated and natural lead environments of man. Arch Environ Health 11(3):344–360

Patterson C, Settle D, Glover B (1976) Analysis of lead in polluted coastal seawater. Mar Chem 4(4):305–319 Pilson MEQ (2012) An introduction to the chemistry of the sea. Cambridge University Press

Pižeta I et al (2015) Interpretation of complexometric titration data: an intercomparison of methods for estimating models of trace metal complexation by natural organic ligands. Mar Chem 173:3– 24

Planquette H, Sherrell RM (2012) Sampling for particulate trace element determination using water sampling bottles: methodology and comparison to in situ pumps. Limnol Oceanogr Methods 10(5):367–388

Plavšić M et al (2009) Determination of the copper complexing ligands in the Krka river estuary. Fresenius Environ Bull 18(3):327–334

Pohlabeln AM, Dittmar T (2015) Novel insights into the molecular structure of non-volatile marine dissolved organic sulfur. Mar Chem 168:86–94

Price NM, Morel FMM (1990) Cadmium and cobalt substitution for zinc in a marine diatom. Nature 344(6267):658–660

Protti P (2001) Introduction to modern voltammetric and polarographic analisys techniques. AMEL srl:p10

Rapp I et al (2017) Automated preconcentration of Fe, Zn, Cu, Ni, Cd, Pb, Co, and Mn in seawater with analysis using high-resolution sector field inductively-coupled plasma mass spectrometry. Anal Chim Acta 976:1–13

Rauschenberg S, Twining BS (2015) Evaluation of approaches to estimate biogenic particulate trace metals in the ocean. Mar Chem 171:67–77

Rehkämper M et al (2012) Natural and anthropogenic Cd isotope variations. In: Handbook of environmental isotope geochemistry. Springer, pp 125–154

Rehkämperab M, Halliday AN (1998) Accuracy and long-term reproducibility of lead isotopic measurements by multiple-collector inductively coupled plasma mass spectrometry using an external method for correction of mass discrimination. Int J Mass Spectrom 181(1–3):123–133 Rehman ZU et al (2017) Advanced characterization of dissolved organic matter released by bloom-forming marine algae. Desalin Water Treat 69:1–11

Resing JA et al (2015) Basin-scale transport of hydrothermal dissolved metals across the South Pacific Ocean. Nature 523(7559):200–203

Revels BN et al (2015) Fractionation of iron isotopes during leaching of natural particles by acidic and circumneutral leaches and development of an optimal leach for marine particulate iron isotopes. Geochim Cosmochim Acta 166:92–104

Richter R (2003) Clean chemistry: techniques for the modern laboratory. Milestone Press Shelton, CT

Rijkenberg MJA et al (2014) The distribution of dissolved iron in the West Atlantic Ocean. PLoS One 9(6):e101323

Rijkenberg MJA et al (2015) "PRISTINE", a new high volume sampler for ultraclean sampling of trace metals and isotopes. Mar Chem 177(Part 3):501–509

Rijkenberg MJA et al (2018) Dissolved Fe in the deep and upper Arctic Ocean with a focus on Fe limitation in the Nansen Basin. Front Mar Sci 5:88

Riley GA, Van Hemert D, Wanngersky PJ (1965) Organic aggregates in surface and DEEP waters of the Sargasso Sea 1. Limnol Oceanogr 10(3):354–363

Riley JS et al (2012) The relative contribution of fast and slow sinking particles to ocean carbon export. Glob Biogeochem Cycles 26:1

Ripperger S, Rehkämper M (2007) Precise determination of cadmium isotope fractionation in seawater by double spike MC-ICPMS. Geochim Cosmochim Acta 71(3):631–642

Ripperger S et al (2007) Cadmium isotope fractionation in seawater—a signature of biological activity. Earth Planet Sci Lett 261(3–4):670–684

Rolison JM et al (2018) Iron isotope fractionation during pyrite formation in a sulfidic Precambrian Ocean analogue. Earth Planet Sci Lett 488:1–13

Rue EL, Bruland KW (1995) Complexation of iron (III) by natural organic ligands in the central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. Mar Chem 50(1–4):117–138

Russell WA, Papanastassiou DA, Tombrello TA (1978) Ca isotope fractionation on the earth and other solar system materials. Geochim Cosmochim Acta 42(8):1075–1090

Ružić I (1982) Theoretical aspects of the direct titration of natural waters and its information yield for trace metal speciation. Anal Chim Acta 140(1):99–113

Ryan-Keogh TJ et al (2013) Spatial and temporal development of phytoplankton iron stress in relation to bloom dynamics in the high-latitude North Atlantic Ocean. Limnol Oceanogr 58(2): 533–545

Saito MA, Moffett JW (2001) Complexation of cobalt by natural organic ligands in the Sargasso Sea as determined by a new high-sensitivity electrochemical cobalt speciation method suitable for open ocean work. Mar Chem 75(1–2):49–68

Saito MA, Goepfert TJ, Ritt JT (2008) Some thoughts on the concept of colimitation: three definitions and the importance of bioavailability. Limnol Oceanogr 53(1):276–290

Saito MA et al (2013) Slow-spreading submarine ridges in the South Atlantic as a significant oceanic iron source. Nat Geosci 6(9):775–779

Sander S et al (2005) Effect of UVB irradiation on Cu^{2+}–binding organic ligands and Cu2+ speciation in alpine lake waters of New Zealand. Environ Chem 2(1):56–62

Sander SG et al (2007) Organic complexation of copper in deep-sea hydrothermal vent systems. Environ Chem 4(2):81–89

Sander SG, Hunter K, and Frew R (2009) 14-sampling and measurements of trace metals in seawater. Practical Guidelines for the Analysis of Seawater 305

Sander SG et al (2011) Numerical approach to speciation and estimation of parameters used in modeling trace metal bioavailability. Environ Sci Technol 45(15):6388–6395

Sander SG, Buck KN, Wells M (2015) The effect of natural organic ligands on trace metal speciation in San Francisco Bay: implications for water quality criteria. Mar Chem 173:269–281

Sanderson MP et al (1995) Primary productivity and trace-metal contamination measurements from a clean rosette system versus ultra-clean go-Flo bottles. Deep-Sea Res II Top Stud Oceanogr 42(2–3):431–440

Santschi PH (2018) Marine colloids, agents of the self-cleansing capacity of aquatic systems: historical perspective and new discoveries. Mar Chem 207:124–135

Sanvito F, Monticelli D (2021) Exploring bufferless iron speciation in seawater by competitive ligand equilibration-cathodic stripping voltammetry: does pH control really matter? Talanta 229:122300

Schintu M, Marrucci A, Marras B (2014) Passive sampling technologies for the monitoring of organic and inorganic contaminants in seawater. In: Current environmental issues and challenges. Springer, pp 217–237

Schlitzer R, Anderson RF, Dodas EM, Lohan M, Geibert W, Tagliabue A, Bowie A, Jeandel C, Maldonado MT, Landing WM, Cockwell D (2018) The GEOTRACES intermediate data product 2017. Chem Geol 493:210–223

Schmitt A-D, Galer SJG, Abouchami W (2009) High-precision cadmium stable isotope measurements by double spike thermal ionisation mass spectrometry. J Anal At Spectrom 24(8):1079–1088

Sclater FR, Boyle E, Edmond JM (1976) On the marine geochemistry of nickel. Earth Planet Sci Lett 31(1):119–128

Sedwick PN et al (2015) A zonal picture of the water column distribution of dissolved iron (II) during the US GEOTRACES North Atlantic transect cruise (GEOTRACES GA03). Deep-Sea Res II Top Stud Oceanogr 116:166–175

Seewald JS et al (2002) A new gas-tight isobaric sampler for hydrothermal fluids. Deep-Sea Res I Oceanogr Res Pap 49(1):189–196

Seyitmuhammedov K (2021) Biogeochemical cycling of trace metals in shelf regions: the importance of cross-shelf exchange. University of Otago

Shah SB (2021) Heavy metals in scleractinian corals. Springer Nature

Sherrell RM, Boyle EA (1992) The trace metal composition of suspended particles in the oceanic water column near Bermuda. Earth Planet Sci Lett 111(1):155–174

Sieber M, Conway TM, de Souza GF, Hassler CS, Ellwood MJ, Vance D (2019) High-resolution Cd isotope systematics in multiple zones of the Southern Ocean from the Antarctic Circumnavigation Expedition. Earth Planet Sci Lett 527:115799

Sieber M et al (2021) Isotopic fingerprinting of biogeochemical processes and iron sources in the iron-limited surface Southern Ocean. Earth Planet Sci Lett 567:116967

Siebert C, Nägler TF, Kramers JD (2001) Determination of molybdenum isotope fractionation by double-spike multicollector inductively coupled plasma mass spectrometry. Geochem Geophys Geosyst 2:7

Sohrin Y, Bruland KW (2011) Global status of trace elements in the ocean. TrAC Trends Anal Chem 30(8):1291–1307

Sohrin Y et al (2008) Multielemental determination of GEOTRACES key trace metals in seawater by ICPMS after preconcentration using an ethylenediaminetriacetic acid chelating resin. Anal Chem 80(16):6267–

6273

Sonstadt E (1872) On the presence of gold in sea water. Chem News 26(671):159–161 Staubwasser M et al (2013) Isotope fractionation between dissolved and suspended particulate Fe in the oxic and anoxic water column of the Baltic Sea. Biogeosciences 10(1):233–245

Strady E et al (2008) PUMP–CTD-system for trace metal sampling with a high vertical resolution. A test in the Gotland Basin. Baltic Sea Chemosphere 70(7):1309–1319

Strelow FEW (1980) Improved separation of iron from copper and other elements by anionexchange chromatography on a 4% cross-linked resin with high concentrations of hydrochloric acid. Talanta 27(9):727–732

Stumm W, Morgan JJ (1996) Aquatic chemistry: chemical equilibria and rates in natural waters, paperback. Wiley, New York

Sun M, Archer C, and Vance D (2021) New methods for the chemical isolation and stable isotope measurement of multiple transition metals, with application to the earth sciences. Geostandards and Geoanalytical Research

Sunda W (2012) Feedback interactions between trace metal nutrients and phytoplankton in the ocean. Front Microbiol 3:204

Tagliabue A et al (2016) How well do global ocean biogeochemistry models simulate dissolved iron distributions? Glob Biogeochem Cycles 30(2):149–174

Tagliabue A et al (2017) The integral role of iron in ocean biogeochemistry. Nature 543(7643): 51–59

Takano S et al (2013) Determination of isotopic composition of dissolved copper in seawater by multi-collector inductively coupled plasma mass spectrometry after pre-concentration using an ethylenediaminetriacetic acid chelating resin. Anal Chim Acta 784:33–41

Takano S et al (2017) A simple and rapid method for isotopic analysis of nickel, copper, and zinc in seawater using chelating extraction and anion exchange. Anal Chim Acta 967:1–11

Tanhua T et al (2019) Ocean FAIR data services. Front Mar Sci 6:440

Taylor PDP et al (1993) Stable isotope analysis of iron by gas-phase electron impact mass spectrometry. Anal Chem 65(21):3166–3167

Teng F-Z, Dauphas N, Watkins JM (2017) Non-traditional stable isotopes: retrospective and prospective. Rev Mineral Geochem 82(1):1–26

Tessier A, Turner DR (1995) Metal speciation and bioavailability in aquatic systems. Wiley, Chichester

Tessier A, Campbell PGC, Bisson MJAC (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51(7):844–851

Thompson CM, Ellwood MJ, Wille M (2013) A solvent extraction technique for the isotopic measurement of dissolved copper in seawater. Anal Chim Acta 775:106–113

Tipping E (1998) Humic ion-binding model VI: an improved description of the interactions of protons and metal ions with humic substances. Aquat Geochem 4(1):3–47

Tonnard M et al (2020) Dissolved iron in the North Atlantic Ocean and Labrador Sea along the GEOVIDE section (GEOTRACES section GA01). Biogeosciences 17(4):917–943

Tovar-Sánchez A (2012) 1.17 – Sampling approaches for trace element determination in seawater. In: Pawliszyn J (ed) Comprehensive sampling and sample preparation. Academic Press, pp 317– 334, ISBN 9780123813749. https://doi.org/10.1016/B978-0-12-381373-2.00017-X

Tovar-Sánchez A et al (2014) Spatial gradients in trace metal concentrations in the surface microlayer of the Mediterranean Sea. Front Mar Sci 1:79

Tovar-Sánchez A et al (2020) Characterizing the surface microlayer in the Mediterranean Sea: trace metal concentrations and microbial plankton abundance. Biogeosciences 17(8):2349–2364

Town RM, Filella M (2000) A comprehensive systematic compilation of complexation parameters reported for trace metals in natural waters. Aquat Sci 62(3):252–295

Trull TW, et al. (2010) The Australian integrated marine observing system Southern Ocean time series facility. OCEANS'10 IEEE SYDNEY, 2010, pp. 1–7. IEEE

Turk J (2001) Field guide for surface water sample and data collection. USDA Forest Service, Washington (DC), p 67

Twining BS, Baines SB (2013) The trace metal composition of marine phytoplankton. Annu Rev Mar Sci 5(1):191–215

Twining BS et al (2015a) Metal contents of phytoplankton and labile particulate material in the North Atlantic Ocean. Prog Oceanogr 137:261–283

Twining BS et al (2015b) Comparison of particulate trace element concentrations in the North Atlantic Ocean as determined with discrete bottle sampling and in situ pumping. Deep-Sea Res II Top Stud Oceanogr 116:273–282

US-Geological-Survey 2006 Chapter A4. Collection of water samples. Report, 09-A4

Ussher SJ et al (2010) Distribution of size fractionated dissolved iron in the Canary Basin. Mar Environ Res 70(1):46–55

Van den Berg CMG (1982) Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO2 I. Theory Mar Chem 11(4):307–322

Van Den Berg CM (1986) Determination of copper, cadmium and lead in seawater by cathodic stripping voltammetry of complexes with 8-hydroxyquinoline. J Electroanal Chem Interfacial Electrochem 215(1–2):111–121

van der Merwe P et al (2019) The autonomous clean environmental (ACE) sampler: a trace-metal clean seawater sampler suitable for open-ocean time-series applications. Limnol Oceanogr Methods 17(9):490–504

Van Dorn WG (1956) Large-volume water samplers. EOS Trans Am Geophys Union 37(6): 682–684

van den Berg CM (2006) Chemical speciation of iron in seawater by cathodic stripping voltammetry with dihydroxynaphthalene. Anal Chem 78(1):156–163

van Hulten M et al (2017) Manganese in the West Atlantic Ocean in the context of the first global ocean circulation model of manganese. Biogeosciences 14(5):1123–1152

Vance D et al (2016) The oceanic budgets of nickel and zinc isotopes: the importance of sulfidic environments as illustrated by the Black Sea. Philos Trans R Soc A Math Phys Eng Sci 374(2081):20150294

Vance D et al (2017) Silicon and zinc biogeochemical cycles coupled through the Southern Ocean. Nat Geosci 10(3):202–206

Velasquez I et al (2011) Detection of hydroxamate siderophores in coastal and sub-Antarctic waters off the South Eastern Coast of New Zealand. Mar Chem 126(1–4):97–107

Velasquez IB et al (2016) Ferrioxamine siderophores detected amongst iron binding ligands produced during the remineralization of marine particles. Front Mar Sci 3:172

Vink S et al (2000) Automated high resolution determination of the trace elements iron and aluminium in the surface ocean using a towed fish coupled to flow injection analysis. DeepSea Res I Oceanogr Res Pap 47(6):1141–1156

Von Damm KL et al (1985) Chemistry of submarine hydrothermal solutions at 21° N, East Pacific rise. Geochim Cosmochim Acta 49(11):2197–2220

Vraspir JM, Butler A (2009) Chemistry of marine ligands and siderophores. Annu Rev Mar Sci 1:43

Waeber T, Marie-Louise SS, Slaveykova V (2012) Trace metal behavior in surface waters: emphasis on dynamic speciation, sorption processes and bioavailability. Arch Sci 65:119–142 Walder AJ, Freedman PA (1992) Communication. Isotopic ratio measurement using a double focusing magnetic sector mass analyser with an inductively coupled plasma as an ion source. J Anal At Spectrom 7(3):571–575

Wang R-M et al (2019) Zinc and nickel isotopes in seawater from the Indian sector of the Southern Ocean: the impact of natural iron fertilization versus Southern Ocean hydrography and biogeochemistry. Chem Geol 511:452–464

Weber T et al (2018) Biological uptake and reversible scavenging of zinc in the global ocean. Science 361(6397):72–76

Wells ML, Goldberg ED (1992) Marine submicron particles. Mar Chem 40(1):5–18

Wells M, Buck KN, Sander SG (2013) New approach to analysis of voltammetric ligand titration data improves understanding of metal speciation in natural waters. Limnol Oceanogr Methods 11(9):450–465

Weyer S, Schwieters JB (2003) High precision Fe isotope measurements with high mass resolution MC-ICPMS. Int J Mass Spectrom 226(3):355–368

Whitby H et al (2018) Copper-binding ligands in the NE Pacific. Mar Chem 204:36–48

Whitby H et al (2020) A call for refining the role of humic-like substances in the oceanic iron cycle. Sci Rep 10(1):1–12

Wilde, Franceska D, and Dean B Radtke (1998) Handbooks for Water-resources Investigations: National field manual for the collection of water-quality data. Field measurements: US Department of the Interior, US Geological Survey

Worsfold PJ et al (2013) Flow injection analysis as a tool for enhancing oceanographic nutrient measurements—a review. Anal Chim Acta 803:15–40

Worsfold PJ et al (2014) Determination of dissolved iron in seawater: a historical review. Mar Chem 166:25–35

Worsfold PJ et al (2019) Estimating uncertainties in oceanographic trace element measurements. Front Mar Sci 5:515

Worsfold, Paul, et al. 2019c Encyclopedia of analytical science: Elsevier

Wu JF (2007) Determination of picomolar iron in seawater by double Mg(OH)(2) precipitation isotope dilution high-resolution ICPMS. Mar Chem 103(3–4):370–381

Wurl O (2009) Sampling and sample treatments. In: Practical guidelines for the analysis of seawater. CRC Press, pp 13–44

Wuttig K et al (2019) Critical evaluation of a seaFAST system for the analysis of trace metals in marine samples. Talanta 197:653–668

Wyatt NJ et al (2014) Biogeochemical cycling of dissolved zinc along the GEOTRACES South Atlantic transect GA10 at 40 S. Glob Biogeochem Cycles 28(1):44–56

Xie RC et al (2015) The cadmium–phosphate relationship in the western South Atlantic—the importance of mode and intermediate waters on the global systematics. Mar Chem 177:110–123 Xue Z et al (2012) A new methodology for precise cadmium isotope analyses of seawater. Anal Bioanal Chem 402(2):883–893

Xue Z, Rehkämper M, Horner TJ, Abouchami W, Middag R, van de Flierd T, de Baar HJ (2013) Cadmium isotope variations in the Southern Ocean. Earth Planet Sci Lett 382:161–172

Xie RC, Rehkämper M, Grasse P, van de Flierdt T, Frank M, Xue Z (2019) Isotopic evidence for complex biogeochemical cycling of Cd in the eastern tropical South Pacific. Earth Planet Sci Lett 512:134–146

Yamamoto A et al (2019) Glacial CO2 decrease and deep-water deoxygenation by iron fertilization from glaciogenic dust. Clim Past 15(3):981–996

Yamashita Y et al (2011) Assessing the spatial and temporal variability of dissolved organic matter in Liverpool Bay using excitation–emission matrix fluorescence and parallel factor analysis. Ocean Dyn 61(5):569–579

Yang S-C et al (2020) A new purification method for Ni and Cu stable isotopes in seawater provides evidence for widespread Ni isotope fractionation by phytoplankton in the North Pacific. Chem Geol 547:119662

Zhang H, Davison W (1995) Performance characteristics of diffusion gradients in thin films for the in situ measurement of trace metals in aqueous solution. Anal Chem 67(19):3391–3400

Zuman P (2001) Electrolysis with a dropping mercury electrode: J. Heyrovsky's contribution to electrochemistry. Crit Rev Anal Chem 31(4):281–289