

学 位 論 文

Determination of subnanomolar zinc and its speciation in seawater with improved clean sampling and analytical method

(クリーンな採水と分析法を用いた海水中の
極微量亜鉛の濃度と存在状態に関する研究)

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Abstract

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Introduction

Zn is an essential micronutrient for the bacteria and phytoplankton in the ocean since it plays an important role in numerous enzyme systems involved in various metabolic processes (Vallee and Auld 1990). In the ocean, vertical profiles of dissolved Zn are nutrient-like and are strongly correlated with those of Si (Bruland 1980). In most surface waters, natural organic ligands strongly bind Zn and dominate the speciation of the total Zn pool. Organic complexation reduces the fraction of free metal ion (Zn^{2+}) to a level as low as 1 pM (Bruland et al., 1991; Ellwood and Van den Berg, 2000). Culture experiments have shown that a Zn^{2+} concentration of less than 1 pM limits the growth of some phytoplankton species (Brand et al., 1983; Sunda and Huntsman, 1995; 1992). Therefore, the study of Zn speciation is important for understanding biogeochemical cycling of Zn in the ocean. In the open oceans, around 95% of total dissolved Zn present in the form of organic complexes dominates the dissolved speciation of Zn in seawater shallower than 200 m (Bruland, 1989; Donat and Bruland, 1990; Ellwood and Van den Berg, 2000; Jakuba et al., 2012). Nevertheless, biogeochemical cycles of Zn have not been fully revealed yet in the ocean since determination of Zn in seawater is very difficult because of contamination problems (Fitzwater et al., 1982). Cathodic stripping voltammetry (CSV) is sufficiently sensitive to determine low concentrations of trace metals in seawater such as Zn. Important advantages of voltammetry are that it can be used to determine the chemical speciation as well as the concentration of dissolved species. In this study, I have established a new reliable determination method and clean sampling technique for subnanomolar level of Zn in seawater. Using these methods, vertical distributions of Zn and its speciation were investigated.

Materials and procedures

1) Sample collection and storage

For comparing the performance of trace metal clean sampling methods, seawater samples were collected using three different sampling methods in which acid-cleaned Teflon-coated X-type Niskin bottles were (1) fixed on a conductivity-temperature-depth carousel multi-sampling system (CTD-CMS, SBE-911plus and SBE-32 water sampler, Sea Bird Electronics, Inc.), with the Zn sacrificial anode being replaced

with Al to avoid the possibility of Zn contamination from the frame; (2) attached bottle by bottle to a Kevlar wire (6 mm diameter, Hikari-kogyo); and (3) attached bottle by bottle to a Ti wire equipped in R/V Hakuho-maru. Seawater samples were filtered using acid-cleaned 0.2 µm pore-size Acropak filter cartridge (PALL Co.). Samples for total dissolved Zn concentration were then stored after being acidified to achieve a pH < 1.8 by using ultra pure HCl (Tamapure AA-100). Samples for Zn speciation analysis were also filtered and frozen immediately onboard the ship.

2) Determination of total dissolved Zn and its speciation in seawater

On the land-based laboratory, total dissolved Zn concentration was determined by using cathodic stripping voltammetry (CSV) after UV-digestion in Teflon beaker. Zn speciation was determined by titration using competitive ligand equilibrium / adsorptive cathodic stripping voltammetry (Donat and Bruland, 1990; Van den Berg, 1985). This involves the establishment of a competitive equilibrium between zinc-complexing ligands naturally present in the sample and a competing organic ligand, ammonium 1-pyrrolidinedithiocarbamate (APDC), added to the sample. Briefly, the complexation of zinc in seawater by a natural ligand (L) can be defined as

$$K'_{ZnL,Zn^{2+}} = \frac{[ZnL]}{[Zn^{2+}][L']} \quad (1)$$

where $K'_{ZnL,Zn^{2+}}$ is the conditional stability constant of the zinc complex in seawater. $[L']$ is the concentration of L not complexed by zinc, $[ZnL]$ is the concentration of zinc complexed with the ligand L, and $[Zn^{2+}]$ is the free zinc ion concentration. The total ligand concentration (C_L) is defined as

$$C_L = [ZnL] + [L'] \quad (2)$$

Substitution for $[L']$ in Eq. (1) using Eq. (2) and rearranging gives

$$\frac{[Zn^{2+}]}{[ZnL]} = \frac{[Zn^{2+}]}{C_L} + \frac{1}{(K'_{ZnL,Zn^{2+}} \times C_L)} \quad (3)$$

When values of $[Zn^{2+}]/[ZnL]$ are plotted against corresponding values of $[Zn^{2+}]$ a linear relationship is usually obtained with a slope equal to $1/C_L$ and with the intercept yielding $1/(K'_{ZnL,Zn^{2+}} \times C_L)$ (Fig. 1).

3) Study areas

To confirm and establish the clean sampling and analytical techniques for determining trace levels of Zn, seawater samples were collected in the subtropical North and South Pacific (December 2011 to January 2012) and subarctic North Pacific (August to October 2012) during the Hakuho-maru KH-11-10 and KH-12-

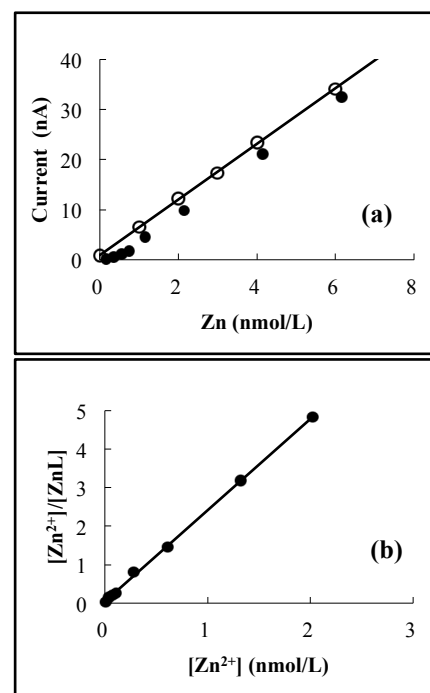


Fig. 1. Example of Zn titration data. (a) The response of Zn peak current with increasing Zn additions. The line and blank circles indicate the response of UV-irradiated sample. (b) Linear relationship obtained by transforming the titration data, which a ligand concentration of 0.4 nmol/L and $\log K'_{ZnL,Zn^{2+}}$ of 10.8 was calculated.

4 R/V cruises, respectively. Seawater samples for investigating total dissolved Zn and its speciation in the western North Pacific and its marginal seas, the Sea of Okhotsk and the Sea of Japan (East Sea) were collected during the R/V Hakuho-maru research cruise KH-10-2 (in June and July 2010, at stations CR-27, CR-30 and CR-47). Andaman Sea and the Bay of Bengal were also determined. Seawater samples for Andaman Sea and Bay of Bengal (July to August 2013) were collected during another R/V Hakuho-maru KH-13-4 cruise.

Result and Discussion

1) Procedural blank value, detection limit, and reference seawater for Zn analysis

To obtain a procedural blank for Zn analysis, surface seawater was passed through a chelating resin column (NOBIAS CHELATE-PA1, Hitachi High-Tec). By measuring the purified seawater repeatedly, the procedural blank value was calculated as 75 ± 3 pmol/L ($n = 7$). This obtained blank value was used for calculating the total dissolved Zn concentrations, which was subtracted from the measured values. Detection limit (calculated as three times the standard deviation of measurements of blank values for purified seawater) was 28 pmol/L. To confirm the precise Zn determination, reference seawater samples were determined. The results are in good agreement with consensus values. It indicates that precise seawater determination for Zn using CSV is accomplished.

2) Comparing three sampling methods

Three different seawater sampling methods, Teflon-coated X-type Niskin samplers were 1) fixed on CTD-CMS, 2) attached to Kevlar wire and 3) attached to titanium wire were compared. Because Zn is used as sacrificial anode in the research vessel, especially in main propellers of the Hakuho-Maru, Zn contamination was observed during Kevlar wire hydrocasts that were performed from the stern of the vessel. By minimizing the influence from the propellers, almost the same Zn concentrations within analytical errors among those three different sampling methods have obtained (Fig. 2).

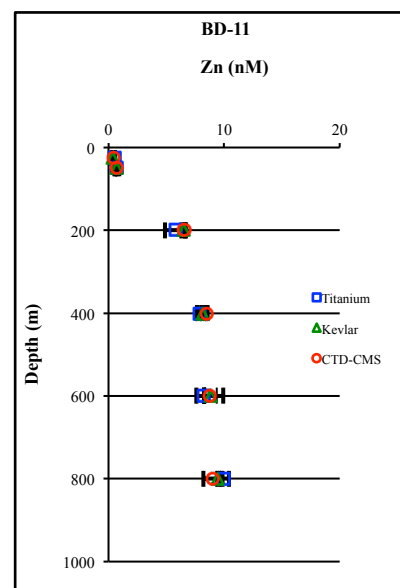


Fig. 2. Comparison of dissolved Zn concentrations in seawater collected with three different sampling methods, Niskin-X samplers with titanium wire hydrocasts, Kevlar wire hydrocasts and CTD-CMS

3) Distribution and speciation of dissolved zinc in the western North Pacific and its adjacent seas

Vertical distributions of Zn show nutrient-type vertical profiles, with a gradual increase in concentration from the surface to deep water. The dissolved Zn profiles are similar to those of Si in this study, which showed the same trends in the previous studies in the North Pacific Ocean (Bruland et al. 1978; Donat and Bruland 1990). In high latitude areas such as the subarctic North Pacific, the Sea of Okhotsk and the Sea of Japan, Zn complexing ligands in most samples are already saturated because of the presence of high concentrations of total dissolved Zn. Relatively high total ligand concentrations have been obtained in

the surface waters of the Sea of Okhotsk (2.6 nM) and the Sea of Japan (1.3 nM) compared with those in the open ocean (subtropical and subarctic North Pacific, 0.2–1.2 nM). In the western North Pacific, positive relationships were obtained between total ligands and chlorophyll *a* concentrations (Fig. 3), suggesting that Zn complexing ligands in the surface water might be derived from bacteria and phytoplankton in the western North Pacific. However, the relationships were completely different in the Sea of Okhotsk and the Sea of Japan (Fig. 3), implying that Zn complexed ligands in those marginal seas are derived from different sources.

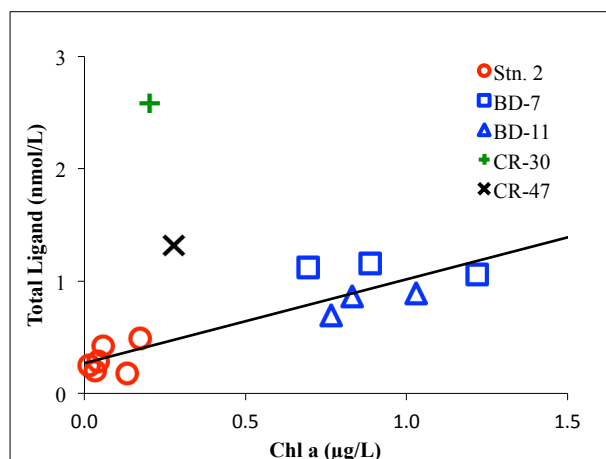


Fig. 3. Comparison of Chl *a* and total ligand concentrations in the western North Pacific (Stn. 2, BD-7 and BD-11), Sea of Okhotsk (CR-30) and Sea of Japan (CR-47). Black line indicates the relationship between Chl *a* and total ligand concentrations in the western North Pacific ($R^2 = 0.814$).

4) Distributions of total dissolved Zn and its speciation of the northeastern Indian Ocean and Andaman Sea

In the Andaman Sea, results show that there are high influence of fluvial discharge from Irrawaddy and Salween rivers. In the northern Andaman Sea, where high Chl *a* content has obtained, total dissolved Zn were relatively lower than those of southern Andaman Sea, may indicate removal of total dissolved Zn in the northern Andaman Sea by biological uptake. Vertical distributions of total dissolved Zn in the northeastern Indian Ocean (NR-1) and Andaman Sea (MY-1 and MY-3) show almost identical concentrations from surface to 1000 m depth. Below 1000 m depth, total dissolved Zn was diverged, indicates the deep water in the Andaman Sea appears to be rapidly replaced by the incoming waters from the northeastern Indian Ocean across the sills and then homogenized by vertical mixing. In the northernmost station of the Andaman Sea (MY-6), total ligand concentrations are correlated with Chl *a* contents (Fig. 4), which supports that phytoplankton and bacteria-excreted organic substances were dominant source for Zn complexing ligands. Other stations in the Andaman Sea, however, poor correlation have obtained (Fig. 4), might suggest that different sources of Zn complexing ligands were dominant at those stations.

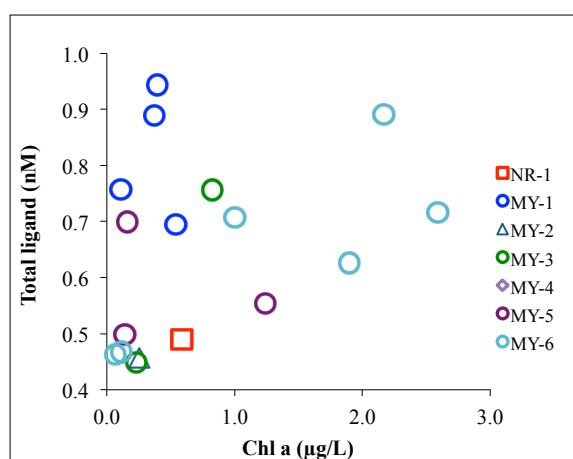


Fig. 4. Comparison of Chl *a* and total ligand concentrations in the northeastern Indian Ocean (NR-1) and Andaman Sea (MY-1 to MY-6).

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Chapter 1.

Introduction

1-1 Trace metals in seawater

Marine phytoplankton produces organic compounds with fixation of carbon by processing of photosynthesis, which is known as primary production (Lindeman, 1942). For this process, phytoplankton requires availability of light, appropriate temperature, and nutrients such as nitrogen, phosphorus, and silicon (for diatoms). These conditions are integral for the growth of phytoplankton in seawater. However, high nutrient low chlorophyll (HNLC) areas such as the Antarctic Ocean, Gulf of Alaska, and Equatorial Pacific area showed very low production rates even though there were still remained sufficient nutrients. This phenomenon has been unconfidently attributed to low temperature, insufficient of light, or grazing by zooplankton, but not clearly understood.

Recently, deficiency of essential metals, presenting at trace concentrations ($< 0.1 \mu\text{mol/L}$) in seawater, has been discussed. The metals, which are related to biological activities, often act as cofactors or part of cofactors in enzyme and as structural elements in proteins. Although the metals such as Mn, Fe, Co, Ni, Cu, Zn and Cd are enriched in rocks and soil, they are kept in very low concentrations in seawater because of their limited solubilities and effective removal from the water column, particularly in estuaries. As a result, their concentrations fall precipitously

within short distance of the coastline. Long-range atmospheric transport through aeolian dust is an important source for open oceans.

Most essential trace metals are depleted at the surface as a result of uptake by phytoplankton (Rue and Bruland, 1995). Determination of such extraordinarily low concentrations of trace metals in surface seawater is very difficult due to contamination problems. Because of the low concentrations, seawater samples are easily contaminated from reagents, analytical instruments, and also surrounding environment (Fitzwater et al., 1982). Accompanying with the developments of clean sampling methods, determination methods of some trace metals in seawater have been established. Therefore, reliable data of trace elements have been obtained, and it has been clarified that many essential trace metals shows nutrient-type vertical profiles whose concentrations are extremely low at surface water and gradually increasing with depth (Martin and Fitzwater, 1988). Determination of some trace metals in seawater, however, is still a big challenge. Also, biogeochemical cycles of such trace metals have not been fully revealed yet in the oceans.

1-2 Chemical behaviors of Zn in seawater

Zinc is known to exist as Zn^{2+} ion, inorganic complex and organic complex in seawater. Vertical distributions of Zn show the nutrient-type profile, which shows a gradual increase in concentration from the surface to deep water (Bruland et al., 1979). It suggests that Zn is involved in the biogeochemical cycle of biological uptake in the surface water and regeneration in deep waters.

Because of the low concentration of Zn in the surface water, Zn is speculated to be a biolimiting nutrient to phytoplankton in the open ocean (Anderson et al., 1978).

Based on the relationship between zinc concentrations in surface waters and their geographic distributions in the surface ocean, it was suggested that Zn availability is an important selective force acting on phytoplankton and ultimately affecting the structure of phytoplankton communities (Brand et al., 1983). In the ocean, vertical profiles of dissolved Zn are nutrient-like and are strongly correlated with those of Si (Bruland, 1980). However, previous study suggests that zinc is not involved in silicon uptake or in silicon transporter proteins (Thamatrakoln and Hildebrand, 2008). Ellwood and Hunter (2000) also showed that biogenic opal has low Zn content suggesting that zinc is not directly involved with silicon uptake and the amount of Zn incorporated into opal represents only 1–3% of the total amount of Zn taken up by diatoms. Low Zn concentrations could limit CO₂ uptake and ultimately the growth rate in some cells via the absence of the enzyme carbonic anhydrase (CA) (Morel et al., 1994). Low rates of phosphate uptake from dissolved organic phosphorus in oligotrophic waters has been linked to the limitation of available Zn in seawater via its central role in the enzyme alkaline phosphatase (Shaked et al., 2006). At high concentrations, Zn could be toxic to phytoplankton and bacteria (Chen et al., 2008; Sunda and Huntsman, 1998; 1996), but Zn concentrations in surface seawater could be biolimiting for phytoplankton growth (Brand et al., 1983; Sunda and Huntsman, 2000; 1998), although this Zn biolimiting has not yet been observed in field studies (Coale et al., 2003; Crawford et al., 2003; Jakuba et al., 2012; Lohan et al., 2005).

Total dissolved Zn concentrations in the surface waters have been reported to be very low in the eastern North Pacific (Bruland et al., 1978; Lohan et al., 2002). In most surface waters, natural organic ligands strongly bind Zn and dominate the speciation of the total Zn pool. Organic complexation reduces the fraction of free metal ion (Zn²⁺) to a level as low as 1 pM (Bruland et al., 1991; Ellwood and Van den

Berg, 2000). Culture experiments have shown that a Zn^{2+} concentration of less than 1 pmol/L limits the growth of some phytoplankton species (Brand et al., 1983; Sunda and Huntsman, 1995; 1992). Therefore, the study of Zn speciation is important for understanding biogeochemical cycling of Zn in the ocean. In the open oceans, studies of zinc complexation in surface water of the North Pacific and North Atlantic reveal that greater than 95% of zinc is complexed to organic ligands (Bruland, 1989; Donat and Bruland, 1990; Ellwood and Van den Berg, 2000; Jakuba et al., 2012), while Zn predominated as inorganic Zn in both surface and deep water of the Southern Ocean (Baars and Croot, 2011). In the marginal seas, although there are few studies, relatively high total ligand concentrations and relatively low conditional stability constants ($K'_{\text{ZnL,Zn}^{2+}}$) reveal in the surface waters of the Black Sea (Muller et al., 2001) and Bering Sea (Jakuba et al., 2012) compared with those in the open ocean. The main source of Zn complexing ligands could be humic substances (Campbell et al., 2002), phytoplankton and bacteria-excreted organic substances (Bruland, 1989) and pore waters from estuarine marine sediments (Skrabal et al., 2006). However, the identity and sources of these organic ligands for Zn are currently unknown.

1-3 Objective and originality of this thesis

The major focus of this study was to establish the clean technique not only for seawater sampling methods, but also a precise determination method of picomolar level of Zn in seawater without any contamination using cathodic stripping voltammetry (CSV). I aimed to confirm and be certified the precise Zn determination by comparing with consensus values of reference seawater samples, which was the first attempt for CSV method.

Using this improved analytical method, total dissolved Zn concentrations and chemical speciation of Zn in the western North Pacific and its adjacent seas have been determined, which were the first obtained data of whole vertical profiles for total dissolved Zn and its speciation at western North Pacific and its adjacent seas. Moreover, I determined total dissolved Zn and its speciation in the northeastern Indian Ocean and Andaman Sea, which were also the first time to be reported. Using these data, the geochemical processes of Zn in the ocean were investigated.

Chapter 2.

Methods

2-1 Clean materials and purification of reagents

For the determination of dissolved trace metals in the seawater, clean analytical procedures must be established. Determination of trace-level Zn is involved in complicated contamination problems. Zn is ubiquitous in the laboratory and is typically used as sacrificial anodes on equipments deployed in seawater, which causes serious contamination during the analytical and sampling processes of trace level Zn in seawater (Fitzwater et al., 1982). It is necessary to establish the procedures not only for cleaning of experimental instruments, but also for Zn measurements.

In this study, almost all the instruments were made of plastics such as Teflon, polyethylene and polycarbonate. Polyethylene gloves were also used during handling samples and reagents. All the procedures of this study on land were performed in a class-1000 clean room (Room # 460c) of the Atmosphere and Ocean Research Institute (Kashiwa campus).

2-1-1 Reagents for cleaning procedure

Deionized water (MQW), purified with a Milli-Q Gradient-A10 (Millipore) system, was used to clean experimental materials and prepare all solutions.

Alkaline surfactant (Extran MA01, Merck), diluted by MQW to 5%, was used to remove organic contaminants.

Neutral surfactant (Extran MA02, Merck), diluted by MQW to 5%, was used to remove organic contaminants.

Three types of HCl, for Analysis of Poisonous Metals (36 %, Wako Pure Chemical Industries), JIS special grade (36 %, Wako Pure Chemical Industries), and Tamapure AA-100 (20 %, Tama Chemicals), were used properly for various purposes as mentioned below.

HNO₃ (Tamapure AA-100, 68%, Tama Chemicals) was used to clean the chelating resin column (NOBIAS CHELATE-PA1, Hitachi High-Tec).

Acetone (JIS special grade, Wako Pure Chemical Industries) was also used to clean the chelating resin column.

Mixed acid was made by mixing of HNO₃ (61%, for analysis of poisonous metals, Wako Pure Chemical Industries), H₂SO₄ (97%, for analysis of poisonous metals, Wako Pure Chemical Industries) and HClO₄ (60%, for analysis of poisonous metals, Wako Pure Chemical Industries) as 1:1:1 volume ratios. The mixed acid was used to clean Teflon materials.

2-1-2 Cleaning apparatus

Low-density polyethylene (LDPE) bottles (Nalgene) were used for seawater sample preservation. For the cleaning, bottles were soaked in the alkaline surfactant overnight, and rinsed 7 times by MQW. After rinsing, LDPE bottles were soaked in 3 mol/L HCl (JIS special grade, Wako Pure Chemical Industries) overnight, and rinsed 5 times with MQW. Furthermore, bottles were filled with 0.5 mol/L HCl, heated

using microwave (500 kW) for 15 minutes, stood until their temperature were lowered to the room temperature, and rinsed 5 times with MQW. Finally, bottles were filled with MQW, and heated for 20 minutes. After their temperature was decreased to the same as room temperature, bottles were rinsed 5 times with MQW (Fig. 2-1).

Teflon materials: Teflon cells which were used at cathodic stripping voltammetry and Teflon beakers which were used during UV irradiation, were soaked in 5% alkaline surfactant overnight, and rinsed 7 times with MQW. After rinsing, cells and beakers were soaked in 3 mol/L HCl (JIS special grade, Wako Pure Chemical Industries) overnight, and rinsed 5 times with MQW. After that, Teflon cells and beakers were heated at 150 °C in mixed acid for 3~4 hours, then rinsed 5 times with MQW after the temperature was decreased to the same as room temperature. And, these were heated at 80 °C in 6 mol/L HCl (for analysis of poisonous metals, Wako Pure Chemical Industries) for 2~3 hours, then rinsed 5 times with MQW after the temperature was decreased to the same as room temperature. Finally, these were heated at 80 °C in MQW for 2~3 hours, then rinsed 5 times with MQW. Heating of cells and beakers in mixed acid was conducted only at their first use. From the second use, the cleaning procedure was conducted only for 6 mol/L HCl and MQW heating steps (Fig. 2-1).

Polycarbonate materials were soaked in 5% neutral surfactant for overnight, and washed 7 times with MQW. Then, those filled with 0.1 mol/L HCl (Tamapure AA-100, Tama Chemicals) were heated at 60°C for 2 hours. After temperature was decreased to the same as room temperature, those were washed 5 times with MQW. Finally, bottles were filled with MQW and heated at 60°C for 2 hours. After temperature was decreased to the same as room temperature, those were washed 5 times with MQW.

Quartz caps, used for covering the Teflon beaker during the UV irradiation, were soaked in 5% neutral surfactant overnight, and rinsed 7 times with MQW. After rinsing, bottles were soaked in 3 mol/L HCl (JIS special grade, Wako Pure Chemical Industries) overnight, and rinsed 5 times with MQW. After that, quartz caps were heated at 150 °C in mixed acid for 3~4 hours, then rinsed 5 times with MQW after the temperature was decreased to the same as room temperature. And, those were heated at 80 °C in 6 mol/L HCl (for analysis of poisonous metals, Wako Pure Chemical Industries) for 2~3 hours, then rinsed 5 times with MQW after the temperature was decreased to the same as room temperature. Finally, these were heated at 80 °C in MQW for 2~3 hours, then rinsed 5 times with MQW. Heating of quartz caps in mixed acid was conducted only at their first use. From the second use, the cleaning procedure was conducted only for 6 mol/L HCl and MQW heating steps.

For a chelating resin column (NOBIAS CHELATE-PA1, Hitachi High-Tec), which was used for removal of Zn from seawater, 5 mL of acetone (JIS special grade, Wako Pure Chemical Industries) was passed through the column. Then, 10 mL of 3 mol/L HNO₃ (Tamapure AA-100, Tama Chemicals) and MQW were passed through the column sequentially. 3 mol/L HNO₃ and MQW steps were repeated more than 3 times.

Acropak filter cartridge (PALL Co.) for filtration of seawater samples were filled with hot 0.1 mol/L HCl (Tamapure AA-100, Tama Chemicals) overnight. Then, 0.1 mol/L HCl was drained from the filter, and rinsed 5 times with MQW. After rinsing, filters were filled with hot MQW overnight. Then, MQW was drained from the filter, and rinsed 5 times with MQW.

2-2 Analytical method

2-2-1 Instrumentation

For determining trace levels of Zn (especially in surface waters), different analytical methods have been introduced (Table 2-1). Inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS) are multielement techniques that are routinely used to determine Zn concentrations. ICP-MS is a high-speed method that can yield precise data by using only a small volume of the sample. Sohrin et al. (2008) obtained excellent results by ICP-MS after column preconcentration using chelating resin (NOBIAS CHELATE-PA1, Hitachi High-Tec) to determine Zn concentration in seawater. This method was successfully applied to determine the concentrations of not only Zn, but also other trace metals in the seawater reference materials, including the surface open-ocean seawater, obtained during the SAFe program (Johnson et al., 2007).

Although ICP-MS makes it possible to measure the concentrations of many elements precisely, it is not applicable for onboard determination. For obtaining real-time data on the ship, other methods (e.g., voltammetry, flow injection analysis: FIA, and sequential injection analysis: SIA) must be applied. These methods allow for rapid measurement during a research cruise, and this may help avoid sample contamination during sampling or storage on board the ship. FIA combines the techniques of column preconcentration and detection by fluorescence (FL) of Zn complex with the ligand *p*-tosyl-8-aminoquinoline. Gosnell et al. (2012) reported a large-scale total dissolved Zn distribution in the southern Indian Ocean by FIA-FL with a low detection limit (0.06 nmol/L, 3 SD). The sequential injection analysis

laboratory-on-valve technique (SIA-LOV) was recently developed by Grand et al. (2011), but it is currently not sensitive enough for use in the open ocean.

Cathodic stripping voltammetry (CSV), which is the method used in this study, enables extremely sensitive and rapid analysis (Fig. 2-2a). One of the advantages of this technique is that enables direct determination not only of the total Zn concentration but also of Zn speciation in seawater. CSV allows direct measurements of labile Zn (Zn') (Donat and Bruland, 1990; Ellwood and Van den Berg, 2000; Van den Berg, 1985). Further, CSV could be useful for measuring the total dissolved Zn after the breakdown of interfering organic substances and metal complexing organic ligands by UV-irradiation (Lohan et al., 2002).

The technique of CSV is based on the measurement of the reduction current from Zn^{2+} to Zn^0 . CSV usually incorporates 3 steps, (1) deposition, (2) equilibrating, and (3) stripping. At the deposition step, Zn^{2+} and ammonium 1-pyrrolidinedithiocarbamate (APDC) chelator are complexed (Zn-PDC) while the stirrer is rotating (2000 rpm), and Zn-PDC complexes are absorbed onto the surface of the hanging mercury drop electrode (HMDE). The potential is held at a positive potential in this step. Appropriate deposition potential and period can be adjusted for the analyte. After the deposition step, stirring is stopped, and the electrode is kept at the lower potential. The purpose of this third step is to allow the deposited material to distribute more evenly in the mercury. The last step is stripping (reduction) of the analyte. The potential of the electrode was scanned to negative direction. The reduction current peak of Zn^{2+} in the adsorbed Zn-PDC complexes has been found at around -1.1 V. The concentrations of Zn in seawater were calibrated with a standard addition method (Lohan et al., 2002). In this study, 757 VA Computrace (Metrohm) was used as the voltammetric system (Fig. 2-2b). The reference electrode was

Ag/saturated AgCl, 3 mol/L KCl. The counter electrode was made of glassy carbon, and the working electrode was a hanging mercury drop electrode (HMDE). (Fig. 2-2a).

To decompose organic compound interfering with the Zn measurement, an UV irradiation system was developed (Lohan et al., 2002). The system was composed of a high pressure UV lamp (UM-453B-A, USHIO), and a stainless steel housing (Fig. 2-3). Teflon beakers filled with seawater samples were placed under the UV lamp. The beaker was covered with a quartz cap to prevent evaporation of seawater and contamination from the air.

2-2-2 Reagents

10 mmol/L APDC solution was made by adding 164 mg of APDC (Fluka) and 160 μ L of 20% NH_3 (Tamapure AA-100, Tama Chemicals) into 100 mL of MQW. APDC solution was stored into LDPE bottles (Nalgene) and kept in a refrigerator. The solution was remade to fresh APDC solution every 1-2 weeks.

Piperazine-1,4-bis (2-ethanesulfonic acid) (PIPES, Dojin) buffer solution for total Zn analysis was purified to remove impurities of Zn. 25g of PIPES, 150 mL of heated MQW, and 14 mL of 20% NH_3 (TamapureAA-100, Tama Chemicals) were added into a acid cleaned Teflon beaker and stirred. After PIPES was fully dissolved, 100 mL of 20% HCl (TamapureAA-100, Tama Chemicals) was added to the solution to produce precipitation of PIPES. The precipitate of PIPES in Teflon beaker was placed in a refrigerator overnight, then, filtered, washed with MQW, and dehydrated in a desiccator, which was placed on the clean room (class-1000). 0.5 mol/L of purified PIPES was made with 100 mL MQW and 20 % NH_3 (Tamapure AA-100,

Tama Chemicals). The PIPES buffer solution was adjusted to a pH value of around 7 by using UV irradiated seawater. The PIPES buffer solution was stored in a cleaned Teflon bottle.

1 mol/L Borate buffer solution for Zn speciation analysis was purified by passing through a chelating resin column (NOBIAS CHELATE-PA1, Hitachi High-Tec) to remove impurities of Zn. Zn-removed borate buffer was UV-digested under a UV lamp over 2 hours. The borate buffer solution was adjusted to a pH value of 8.2. The borate buffer solution was stored in a clean Teflon bottle.

Zn standard solution was made by diluting 1000 mg/L Zn standard solution (Wako Pure Chemical Industries) with MQW. Using 20% HCl (Tmapure AA-100, Tama Chemicals), Zn standard was adjusted to pH 2.

Mercury (JIS special grade, Wako Pure Chemical Industries) was used for a working electrode. 3 mol/L KCl (Metrohm) was used as internal solution for the reference electrode.

2-2-3 Total dissolved Zn analysis

A seawater sample was added into a Teflon beaker and UV irradiated for 40 minutes to destroy organic matters. After the UV irradiation, 10 mL of seawater sample was added into the Teflon cell with 40 μ L of 0.5 mol/L PIPES buffer solution, 100 μ L of 10 mmol/L APDC. Ammonia solution was also added to adjust the pH around 7. In the Teflon cell, then, sample was deaerated by nitrogen (over 99.9995%) to remove dissolved oxygen in the sample. The reduction current peak of Zn^{2+} in the adsorbed Zn-PDC complexes occurs around -1.1 V. The concentrations of Zn in seawater were calibrated by a standard addition method (Lohan et al., 2002). Fig. 2-4

shows typical voltammograms of Zn measurements with a standard addition method. During the measurement of trace Zn by CSV, a higher peak was obtained at the first measurement. But, the peak height was decreased slightly and became stable after the second measurement for the same seawater sample. This phenomenon might be caused by the adsorption equilibration between dissolved Zn in the solution and adsorbed Zn on the Teflon cell surface. To measure a precise value of peak height at the same condition, repeated measurements were carried out couple of times without adding Zn standard solution until the peak became stable.

2-2-4 Zn speciation analysis

Zn speciation was determined by titration using competitive ligand equilibrium / adsorptive cathodic stripping voltammetry (CLE-ACSV) (Donat and Bruland, 1990; Van den Berg, 1985), which uses a competitive equilibrium between zinc-complexing ligands naturally present in the sample and a competing ligand (APDC). A titration curve is produced by adding increasing concentrations of Zn. Once the natural ligands are saturated with Zn, the reduction peak current will be increased proportionally to the added Zn concentration (Fig. 2-5). The total dissolved Zn concentration (C_{Zn}) of a sample can be defined as

$$C_{Zn} = [Zn'] + [ZnL] + [ZnPDC] \quad (1)$$

where $[Zn']$ is the concentration of inorganic Zn, $[ZnPDC]$ is the concentration of Zn complexed with APDC, and $[ZnL]$ is the concentration of Zn complexed by natural ligands. Using a simple one-ligand model, the complexation of Zn in seawater by natural ligands can be defined as

$$K'_{ZnL,Zn^{2+}} = \frac{[ZnL]}{[Zn^{2+}][L']} \quad (2)$$

where $K'_{\text{ZnL,Zn}^{2+}}$ is the conditional stability constant of the Zn complex with respect to Zn^{2+} in seawater and $[\text{L}']$ is the concentration of ligand.

The total ligand concentration (C_L) of a sample can be defined as

$$C_L = [\text{ZnL}] + [\text{L}'] \quad (3)$$

Substitution for $[\text{L}']$ in Eq. (2) using Eq. (3) and rearranging gives the Van den Berg/Ružić linearization (Ružić, 1982; Van den Berg, 1982). The equation for the resulting line is

$$\frac{[\text{Zn}^{2+}]}{[\text{ZnL}]} = \frac{[\text{Zn}^{2+}]}{C_L} + \frac{1}{(K'_{\text{ZnL,Zn}^{2+}} \times C_L)} \quad (4)$$

When values of $[\text{Zn}^{2+}]/[\text{ZnL}]$ are plotted against corresponding values of $[\text{Zn}^{2+}]$, a linear relationship is obtained with a slope equal to $1/C_L$ and with the intercept yielding $1/(K'_{\text{ZnL,Zn}^{2+}} \times C_L)$.

The observed reduction peak current (i_p) is related to the concentration of Zn^{2+} by the equation:

$$[\text{Zn}^{2+}] = \frac{i_p}{S \times \alpha'} \quad (5)$$

where S is the sensitivity, which is calibrated by standard additions to UV-irradiated seawater (UVSW), and α' is the overall side reaction coefficient for Zn

$$\alpha' = \alpha_{\text{Zn}} + \alpha_{\text{ZnPDC}} \quad (6)$$

and where α_{Zn} is the inorganic side reaction coefficient for Zn. A value of 2.2 (Donat and Bruland, 1990; Jakuba et al., 2012; 2008; Turner et al., 1981) was used in this study. α_{ZnPDC} , the side reaction coefficient for Zn with PDC, is fixed by the concentration of PDC added to the sample. α_{ZnPDC} can be calculated as

$$\alpha_{\text{ZnPDC}} = K'_{\text{ZnPDC}}[\text{APDC}'] \quad (7)$$

where K'_{ZnPDC} is a conditional stability constant, and $[\text{APDC}']$ is the concentration of APDC not complexed by Zn^{2+} . This $[\text{APDC}']$ is much greater than that of Zn, so the

total APDC concentration ($[APDC']_T$) can be selected for calculations. A K'_{ZnPDC} value of $10^{4.4}$ was used for seawaters at pH 8.2 with borate buffer (Ellwood and Van den Berg, 2000). $[ZnL]$ can be calculated as

$$[ZnL] = C_{Zn} - \left(\frac{i_p}{S}\right) \quad (8)$$

where (i_p/S) is equal to labile Zn concentration ($= [Zn'] + [ZnPDC]$). Combining Eqs. (5) and (8), $[Zn^{2+}]/[ZnL]$ can be calculated as

$$\frac{[Zn^{2+}]}{[ZnL]} = \frac{i_p}{\alpha' \times (S \times C_{Zn}) - i_p} \quad (9)$$

Finally, once C_L and $K'_{ZnL,Zn^{2+}}$ have been determined, the concentration of Zn^{2+} can be calculated by the following quadratic equation (Ellwood and Van den Berg, 2000).

$$[Zn^{2+}]^2 \cdot \alpha_{Zn} \cdot K'_{ZnL,Zn^{2+}} + [Zn^{2+}] (K'_{ZnL,Zn^{2+}} \cdot C_L - K'_{ZnL,Zn^{2+}} \cdot C_{Zn} + \alpha_{Zn}) - C_{Zn} = 0 \quad (10)$$

The voltammetric system used was the same as that for total dissolved Zn determination. Acid cleaned Teflon vials were used for Zn titration. To minimize the effects of adsorption onto the vial walls, Teflon vials were rinsed twice with 10 mL of sample. After rinsing, 10 mL of seawater sample and 4 mmol/L borate buffer were added to the Teflon vial. Additions of borate buffer gave a pH of 8.2. Each vial was then spiked with Zn concentrations (range: 0.2–6.0 nmol/L) and allowed to equilibrate. After 2 h, 25 μ mol/L APDC was added to each vial. The APDC was allowed to equilibrate for 12 h (Ellwood and Van den Berg, 2000; Lohan et al., 2005). At this APDC concentration, the detection window of the method is approximately from $K'_{ZnL,Zn^{2+}} = 10^7$ to 10^{12} for a ligand concentration of 1 nmol/L. The first two vials were not spiked with Zn and were used as replicates for the starting point of the titration. The voltammetric conditions were differential pulse mode, 4 minutes N_2 gas

purge, 180 s deposition time at -0.6 V, 8 s equilibration time, and a negative scan from -0.75 to -1.2 V with a 0.05 V pulse amplitude, 0.02 s pulse time, 0.0047 V voltage step, 0.2 s step time, and 0.024 V/s sweep rate. One example of a titration is shown in Fig. 2-5.

2-3 Optimal conditions for voltammetric analysis

2-3-1 Deposition potentials

To increase the sensitivity, the optimal deposition potential and concentration of APDC were investigated. Although the maximum peak height was found at the -0.6 V of deposition potential (Fig. 2-6), the baseline of the voltammetric scan was not flat. At the -0.3 V of deposition potential, on the other hand, the baseline of the voltammetric scan was flat even though the sensitivity was relatively lower than that of -0.6 V. If the extremely lower Zn concentration is analyzed, flat baseline is necessary to calculate Zn concentration. Therefore in this study, both -0.3 V and -0.6 V of deposition potential were selected for Zn determination. The voltammetric scan conditions used in this study are listed in Table. 2-2.

2-3-2 APDC concentration

The dependence of the zinc reduction current on the APDC concentration was also studied by using UV irradiated seawater (UVSW) which containing 0.5 mol/L PIPES (pH 7) and 4 nmol/L of Zn. The zinc reduction current was increased with the APDC concentration up to ~ 100 $\mu\text{mol/L}$, beyond that the current was constant and

then decreased slightly at concentrations $\geq 100 \mu\text{mol/L}$ (Fig 2-7). In this study, $100 \mu\text{mol/L}$ APDC was selected.

2-3-3 Optimal pH for total dissolved Zn analysis

When Zn was determined at a pH higher than 8.5, there is a possibility that the linear calibration line can not be obtained because of adsorption of Zn to cell and electrode. Fig. 2-8a shows the change of sensitivities with time at pH 7 and pH 8.

At both pH 7 and pH 8, sensitivities were gradually decreased with time. At pH 7, sensitivities were relatively higher than those of pH 8. In this study, therefore, pH for total dissolved Zn analysis was adjusted around 7 by adding PIPES buffer solution and samples were determined immediately after adding samples and reagents into Teflon cell cup. The calibration curve at pH 7 is shown determined (Fig 2-8b).

2-3-4 UV irradiation method for sample pretreatment

In seawater, Zn is complexed by natural organic ligands (Van den Berg, 1985). For total dissolved Zn determinations by CSV, it is necessary to release Zn from the natural metal-organic complex prior to analysis. Furthermore, the removal of dissolved organic matter (DOM) from samples is often preferable as Zn determination might be interfered. UV digestion is a preferred approach for the breakdown of dissolved Zn-organic complexes and the removal of DOM prior to trace metal determination (Lohan et al., 2002). UV digestion is a clean sample pretreatment method, as the addition of large amounts of oxidants is not required.

In this study, seawater samples in PFA Teflon beakers with quartz lids were UV-irradiated using a high-pressure mercury vapor UV lamp (UM-453B-A, USHIO) (Fig. 2-3). The optimal irradiation time of UV digestion was investigated (Fig. 2-9). During the UV irradiation, the sample solution was maintained at a low temperature using ice to prevent water evaporation. Zn reduction current (concentration) increased as the UV irradiation time increased up to 30 minutes, beyond which the current remained constant. Even though there was no increase in the reduction current after 30 minutes of UV irradiation, seawater samples were UV-irradiated for over 40 minutes in this study to ensure the full break down of naturally occurring DOM.

2-4 Improvement of precise analysis for Zn

2-4-1 Procedure for preparing Zn-free seawater

To obtain a procedural blank value for Zn analysis, surface seawater was passed through a chelating resin column (NOBIAS CHELATE-PA1, Hitachi High-Tec). This procedure was processed in a class-1000 clean room. At the beginning of the procedure, the chelating resin column was rinsed by 0.1 mol/L HCl and MQW. The seawater was then passed through the chelating resin column where Zn was captured. Zn-removed seawater was stored into a Teflon beaker and acidified to pH < 1.8 with ultra-pure HCl (Tamapure AA-100, Tama Chemicals). After acidification, this Zn-removed seawater was UV-digested under a UV lamp over 2 h, and left in a class-1000 clean room overnight to eliminate the remaining oxidized species (e.g., bromine and chlorine). Thereafter, any Zn present in the Zn-removed seawater sample

was determined. The deposition potential was set at -0.3 V, and the deposition time was 360 s.

2-4-2 UV-irradiation time for Zn-free seawater

Sensitivity values corresponding to different UV irradiation times were studied by the standard addition method (Table 2-3). The original surface seawater, which had not been passed through the chelating resin column, was UV-irradiated for 40 min in the same manner as other seawater samples; in this case the sensitivity was 10.4 nA/(nmol/L). However, for Zn-removed samples that had been passed through the chelating resin column once and UV-irradiated for 40 min, the sensitivity was less (6.7 nA/(nmol/L)) than that for the original surface seawater. This indicates that the artificial ligand, which originated from the chelating resin column, remained in the Zn-removed seawater even after UV-irradiation. Therefore, to decompose the remaining artificial ligand completely, a longer UV irradiation time was required. After more than 2 h of UV irradiation, sensitivity (10.4 nA/(nmol/L)) similar to that of the original seawater was realized. Furthermore, the Zn-removed seawater that had been passed through the chelating resin column twice, also showed a similar sensitivity (10.1 nA/(nmol/L)) after 2 h of UV irradiation; thus indicating that the artificial ligand was fully decomposed.

2-4-3 Procedural blank value and detection limit

Zn-removed seawater was prepared by passing it through the chelating resin column. To confirm whether Zn in the original seawater (432 ± 18 pmol/L ($n = 2$))

was removed thoroughly, the seawater was passed once again through the column. In this case, the Zn concentration was calculated as 77 ± 6 pmol/L ($n = 2$), which is consistent with the Zn concentration (75 ± 3 pmol/L ($n = 7$)) of the seawater passed through the column once, and indicates that Zn in seawater could be thoroughly removed by passing through the column once only (Fig. 2-10). To investigate what caused the procedural blank value, Zn-removed seawater was determined with doubling reagents or buffer concentrations. However, no differences were observed for the procedural blank values. Furthermore, when Zn-removed seawater was analyzed at different deposition times (60–360 s) with -0.6 V deposition potential, almost identical reduction currents were obtained. Therefore, it was considered that the procedural blank value was not caused by any remaining Zn in the “Zn-removed” seawater.

Based on the results, the procedural blank value was calculated as 75 ± 3 pmol/L ($n = 7$) by taking measurements for the Zn-removed seawater obtained by passing seawater through the chelating resin column once. This obtained blank value was used for calculating the total dissolved Zn concentration, which was subtracted from the measured values. The detection limit (calculated as three times the standard deviation of measurements of blank values for purified seawater) was 28 pmol/L, and this was compared with the limits found in other analysis methods such as AAS, ICP-MS, CSV, anodic stripping voltammetry (ASV), flow injection analysis with fluorescence (FIA-FL), and sequential injection analysis laboratory-on-valve technique (SIA-LOV) (Table 2-1).

2-4-4 Intercalibration

Recently, accompanying with the advance in analytical and sampling methods, determination methods of many trace metals in seawater have been developed. Now, determining concentrations of trace metals in seawater precisely has become major issues. But, it was still very difficult to produce the reliable data because of differences among laboratories in analytical methods, sampling methods, clean techniques, etc. Therefore, international program named GEOTRACES has been started with a goal of determining full water column distributions of selected trace elements.

At the GEOTRACES program, among the laboratories that are participating in the program, intercalibration is conducted to compare obtained values of selected elements for the same seawater samples. In this study, Zn concentrations in SAFe samples S (SAFe Intercalibration North Pacific 2004, surface seawater), D1 and D2 (SAFe Intercalibration North Pacific 2004; 1000 m), GS (GEOTRACES Intercalibration North Atlantic BATS 2008; surface seawater), and GD (GEOTRACES Intercalibration North Atlantic BATS 2008; 2000m) were determined (Table 2-4). The results of this intercalibration are in good agreement with the results from other labs (<http://www.geotraces.org/science/intercalibration/322-standards-and-reference-materials>), which was the first certified data for Zn using CSV method. It indicates that precise Zn determination using CSV is accomplished.

Table 2-1. Dissolved Zn data from present study and other relevant studies.

AAV : Atomic Absorption Spectrometry; ICP-MS : Inductively Coupled Plasma-Mass Spectrometry; ASV : Anodic Stripping Voltammetry; CSV : Cathodic Stripping Voltammetry; FIA-FL : Flow Injection Analysis (coupled with fluorescence detection); SIA-LOV : Sequential Injection Analysis (coupled with laboratory-on-valve)

Method	Chemical forms measured	Blank (nmol/L)	Detection limit (nmol/L)	References
AAS	Zn total	0.31	0.31	(Yebra-Biurrun and Cespón-Romero, 2006)
	Dissolved and particulate Zn	0.007-0.011	0.031-0.052	(Coale et al., 2005)
	Total dissolved Zn	0.010-0.100	0.010-0.070	(Löschner, 1999)
ICP-MS	Dissolved Zn	0.09±0.02	0.062	(Ellwood, 2008)
		0.10±0.02	0.057	(Sohrin et al., 2008)
		0.12	0.09	(Jakuba et al., 2012)
ASV	Total dissolved Zn		0.006	(Ellwood, 2004)
CSV	Total dissolved Zn	0.025	0.02	(Lohan et al., 2002)
			0.30	(Achterberg and Van den Berg, 1996)
			0.028	This study
FIA-FL	Total dissolve Zn		<0.1	(Nolting et al., 2000)
			<0.006	(Gosnell et al., 2012)
SIA-LOV	Dissolved Zn		0.3	(Grand et al., 2011)

Table 2-2. Voltammetric conditions used in this study for CSV method.

Sample volume		10 mL
Reagents	0.5 mol/L PIPES	40 μ L
	10 mmol/L APDC	100 μ L
pH	After addition of PIPES	7
Temperature		20 $^{\circ}$ C
Operation parameters (HMDE, Linear sweep)	Differential pulse mode	
	Initial purge time	240 s
	Additional purge time	90 s
	Deposition potential	-0.6 V or -0.3 V
	Deposition time	60 - 300 s
	Equilibration time	8 s
	Start potential	-0.75 V
	End potential	-1.2 V
	Pulse amplitude	0.05 V
	Pulse time	0.02 s
	Voltage step	0.00473 V
	Voltage step time	0.2 s
	Sweep rate	0.0237 V/S
	Hg drop size	9
	Stirrer	2000 rpm

Table 2-3. Sensitivities of CSV analyses for added Zn in seawater.

Operation	UV-irradiation time	Sensitivity (nA / (nmol/L))
Raw surface seawater	40 minutes	10.4
Passing through the chelating resin once	40 minutes 120 minutes	6.7 10.4
Passing through the chelating resin twice	120 minutes	10.1

Table 2-4. Dissolved Zn concentrations of the intercalibration.

Intercalibration		Total zinc (nmol/L)	
Samples	Bottle No.	This study	Consensus value
GS	62	$0.033 \pm <0.000$	0.038 ± 0.011
	157	0.047 ± 0.003	
GD	39	1.63 ± 0.05	1.64 ± 0.22
	142	1.62 ± 0.14	
SAFe S	557	0.058 ± 0.003	0.064 ± 0.019
	227	0.071 ± 0.005	
SAFe D1	534	7.03 ± 0.13	7.1 ± 0.6
	535	7.24 ± 0.12	
SAFe D2	249	7.11 ± 0.38	7.2 ± 0.5
	445	7.25 ± 0.27	

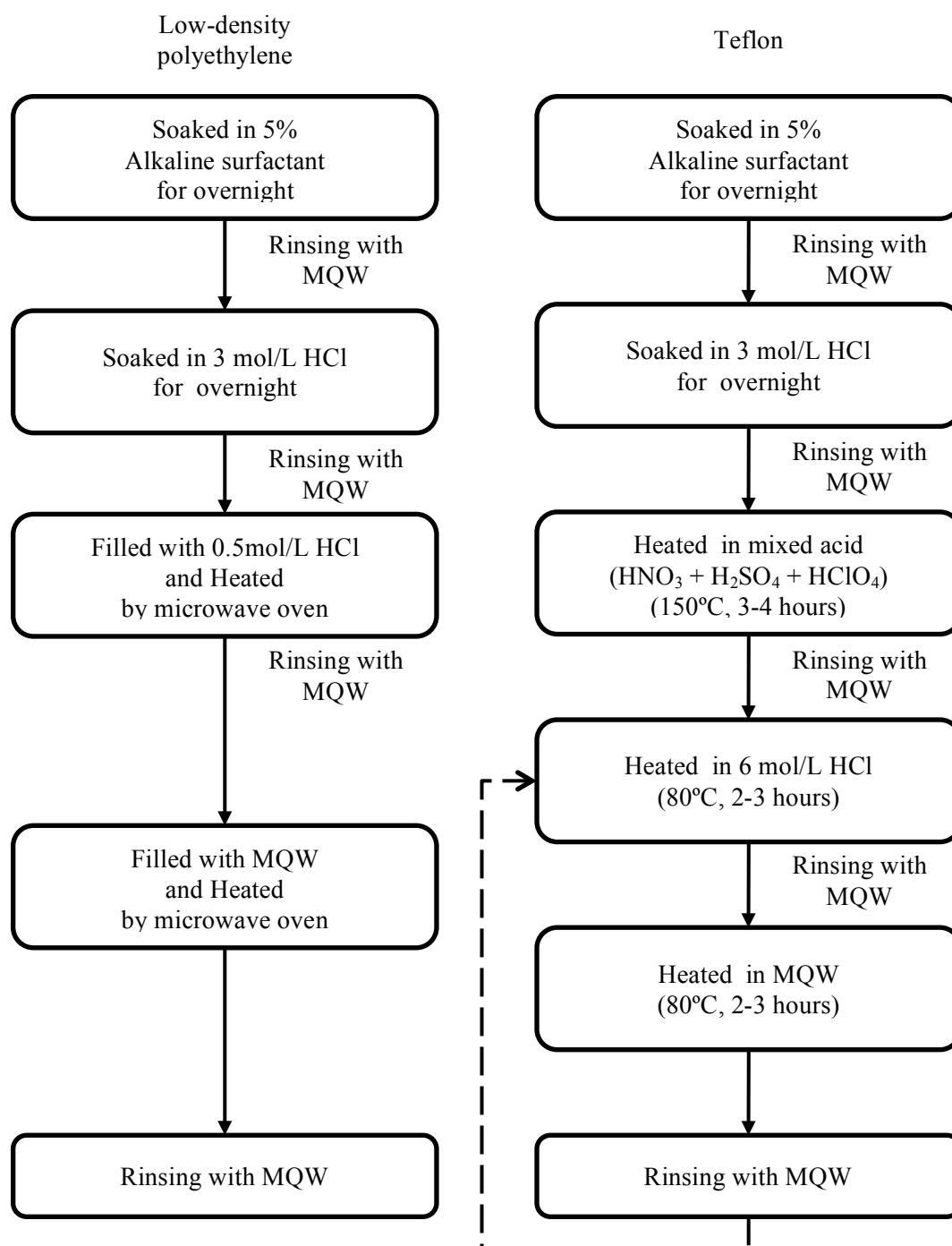


Fig. 2-1. Flow chart of cleaning procedures for low-density polyethylene bottles and Teflon materials.

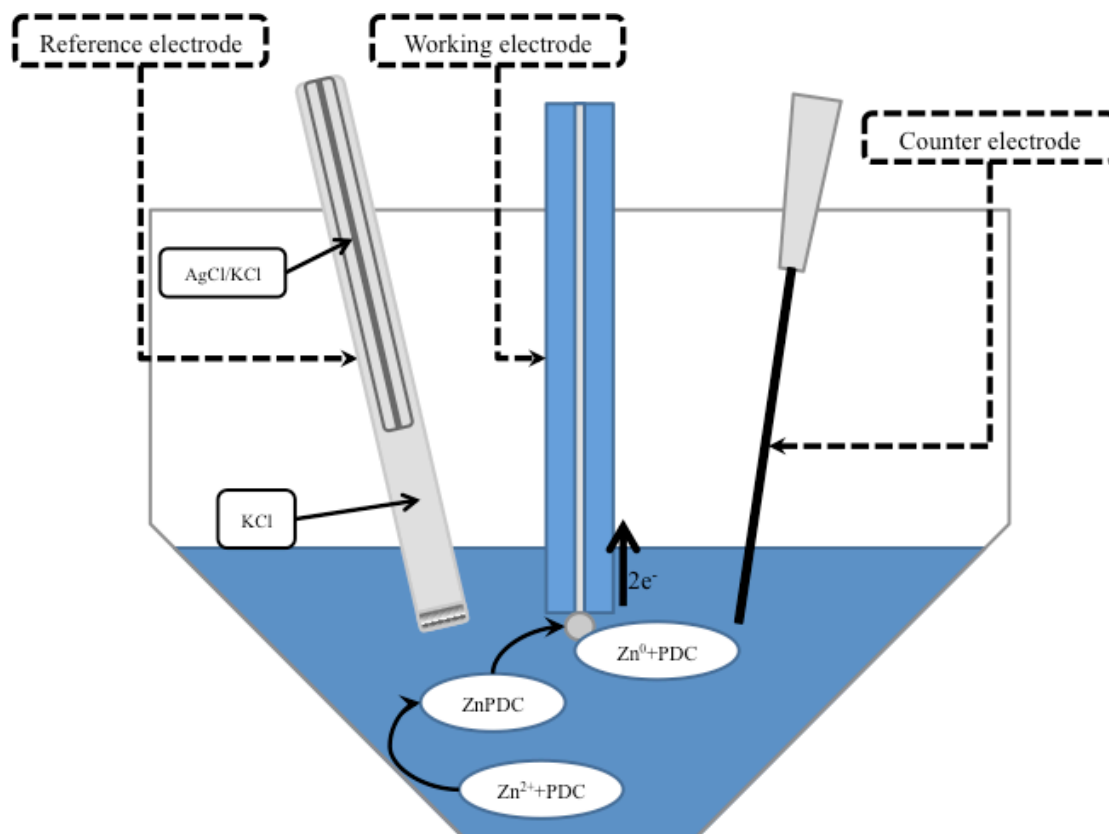


Fig. 2-2a. Concept scheme of CSV for Zn measurement.

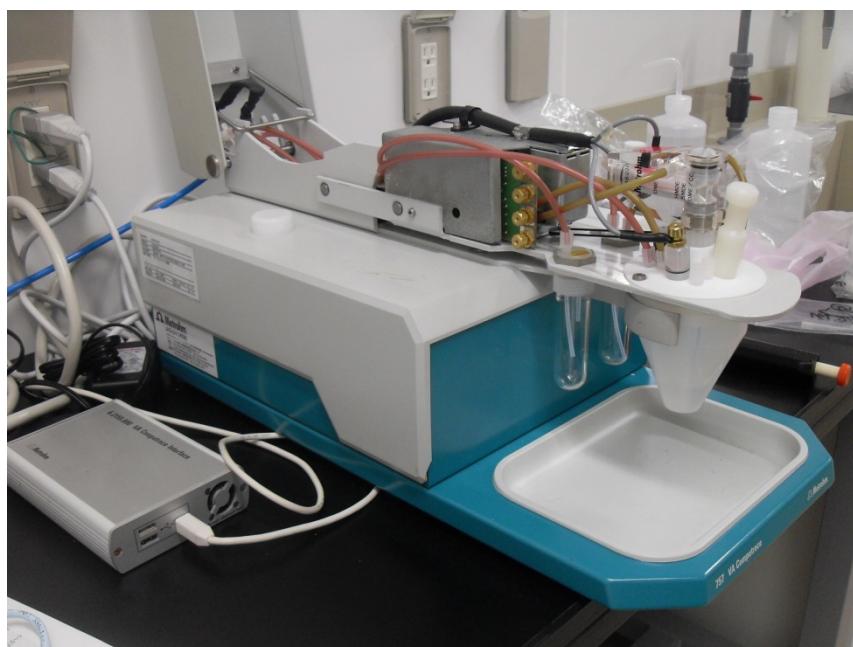


Fig. 2-2b. VA computrace Volatammetric system (Metrohm).

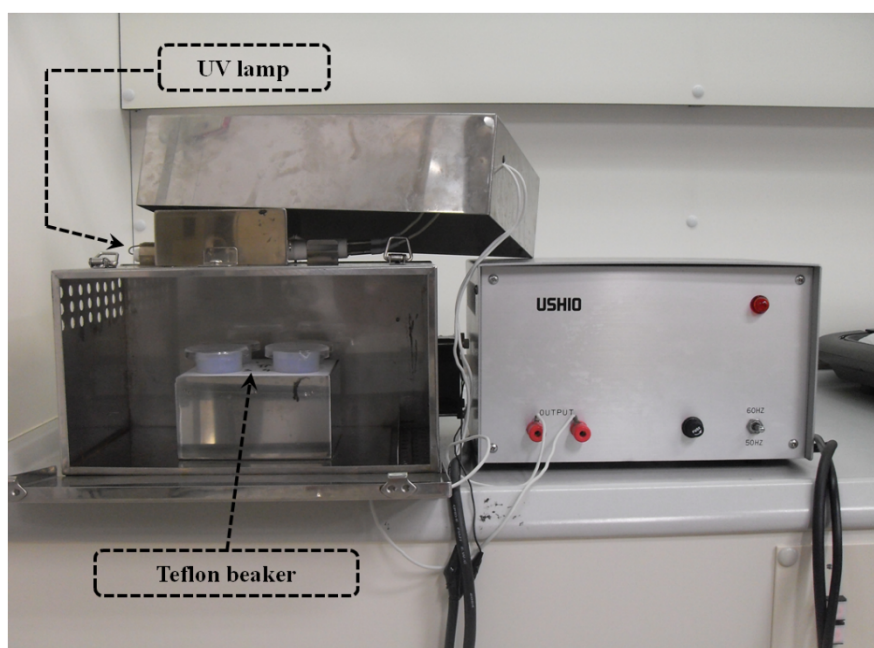


Fig. 2-3. UV irradiation system.

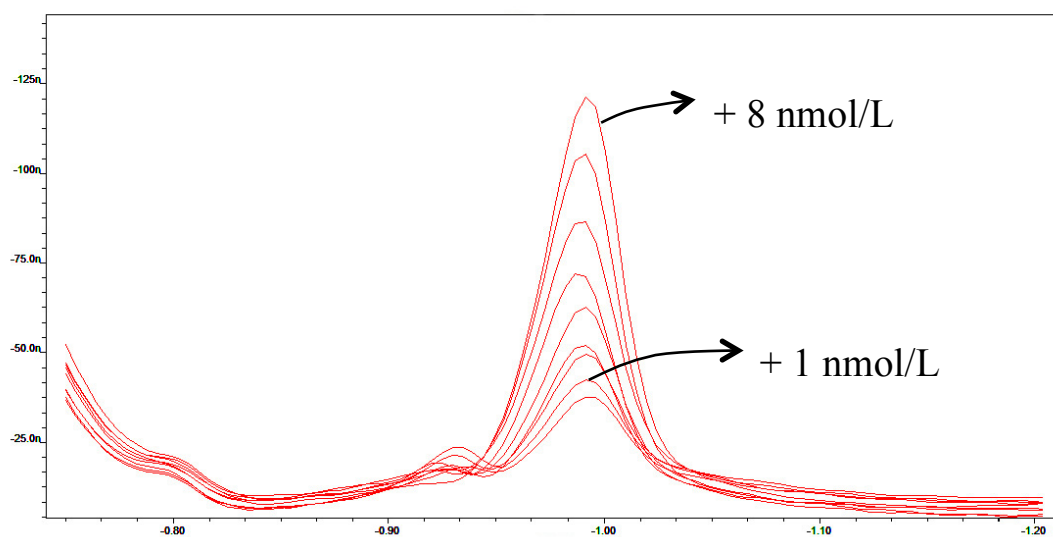


Fig. 2-4. Voltammetric scans for Zn in seawater. Concentrations of added Zn in seawater were from 1 nmol/L to 8 nmol/L.

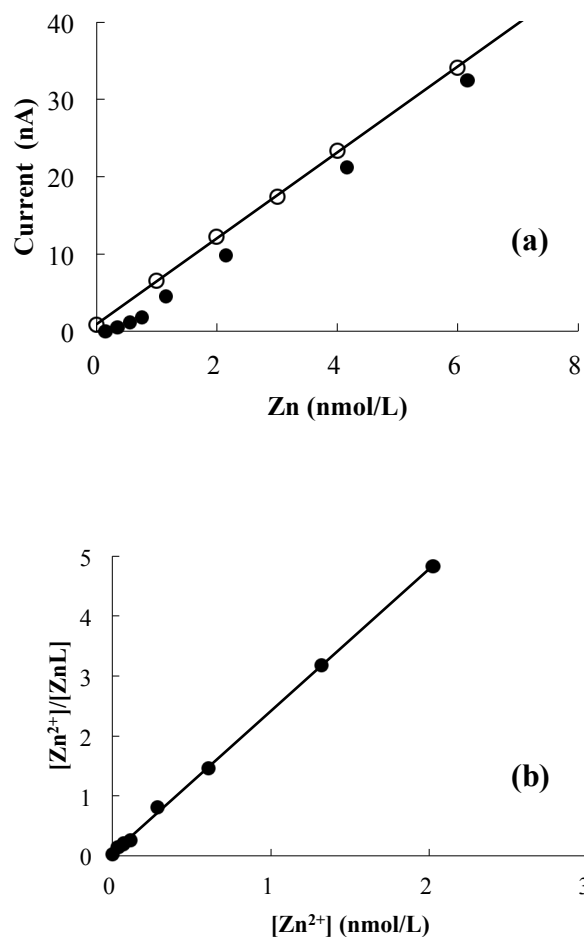


Fig. 2-5. Example of Zn titration data. (a) The response of Zn peak current with increasing Zn additions. The line and blank circles indicate the response of UV-irradiated seawater sample, which was used for Zn speciation calculation. (b) Linear relationship obtained by transforming the titration data. The calculated ligand concentration and $\log K'_{ZnL,Zn^{2+}}$ were 0.4 nmol/L and 10.8, respectively.

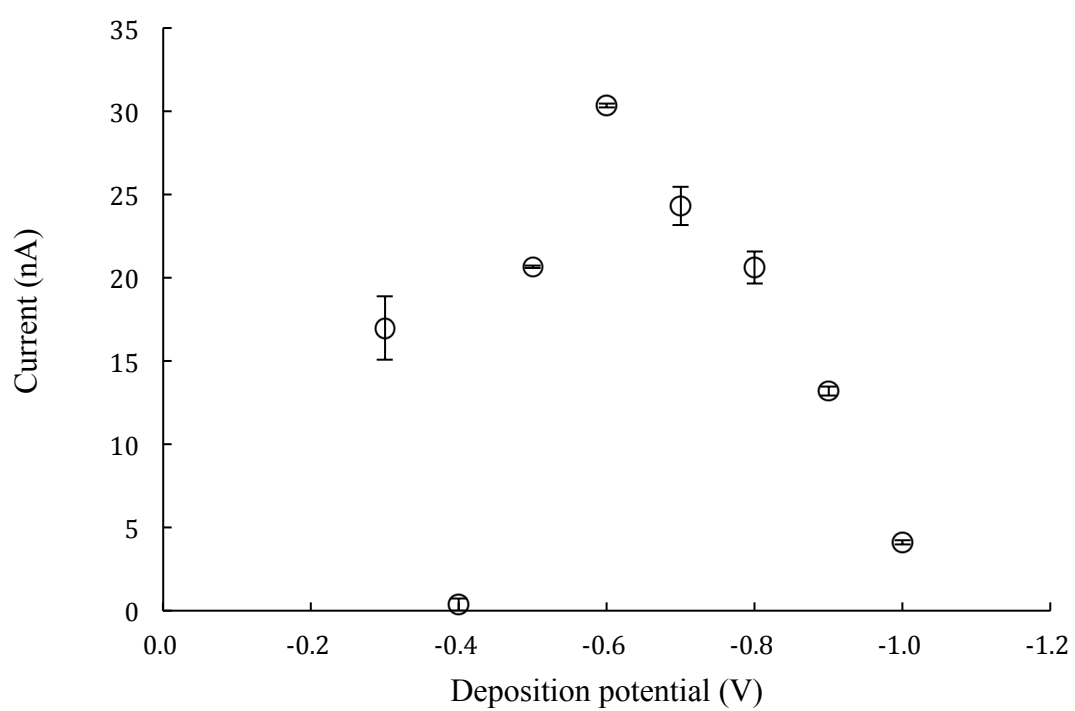


Fig. 2-6. Reduction current of Zn as a function of deposition potential.

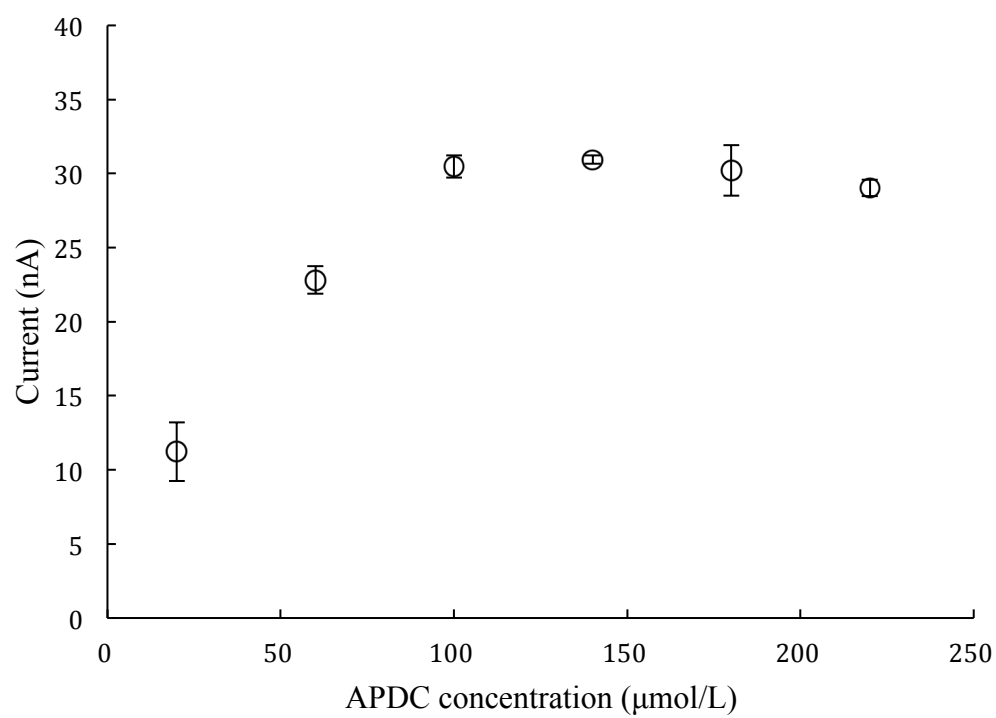


Fig. 2-7. Reduction current of Zn as a function of APDC concentration.

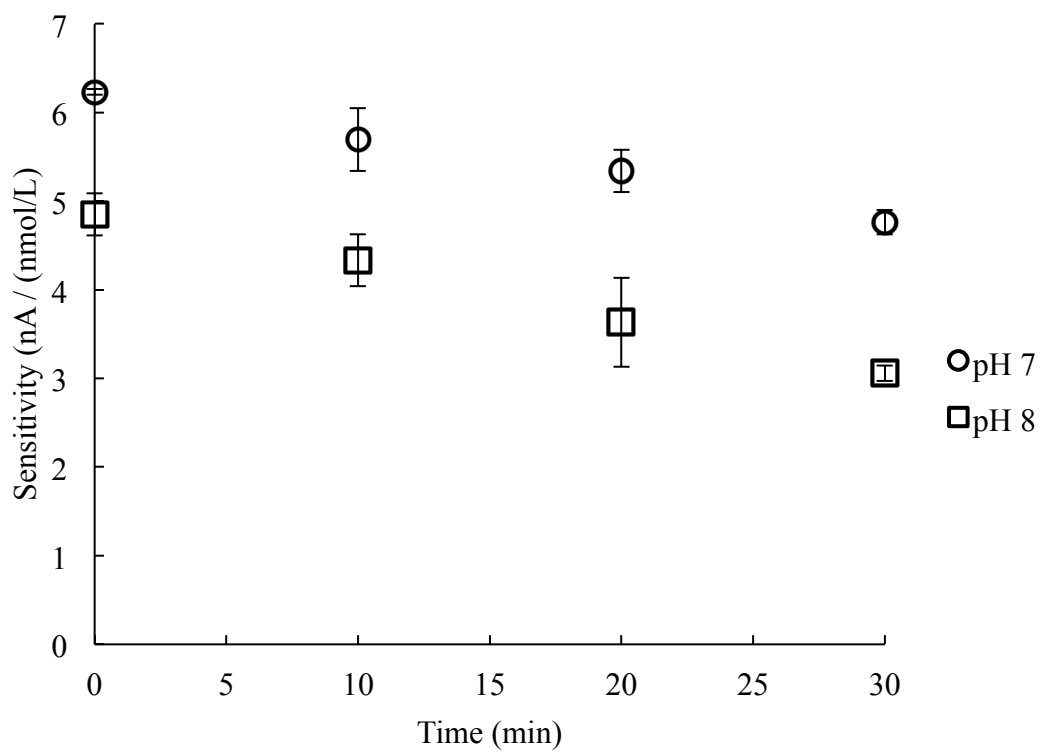


Fig. 2-8a. Changes of sensitivities with time at pH 7 and pH8.

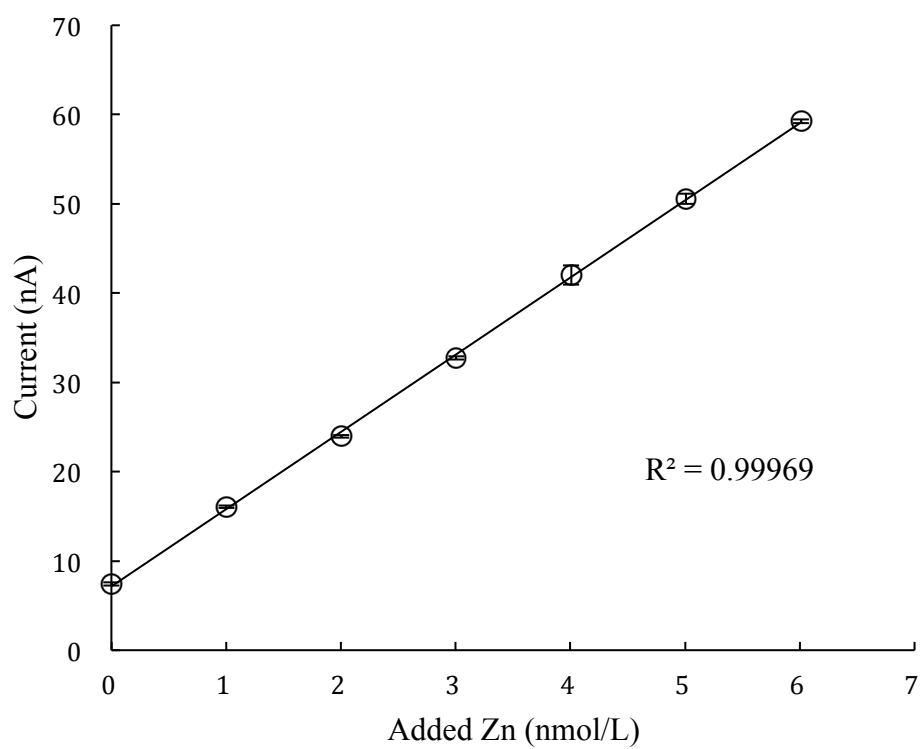


Fig. 2-8b. Reduction currents with addition of Zn standards at pH 7.

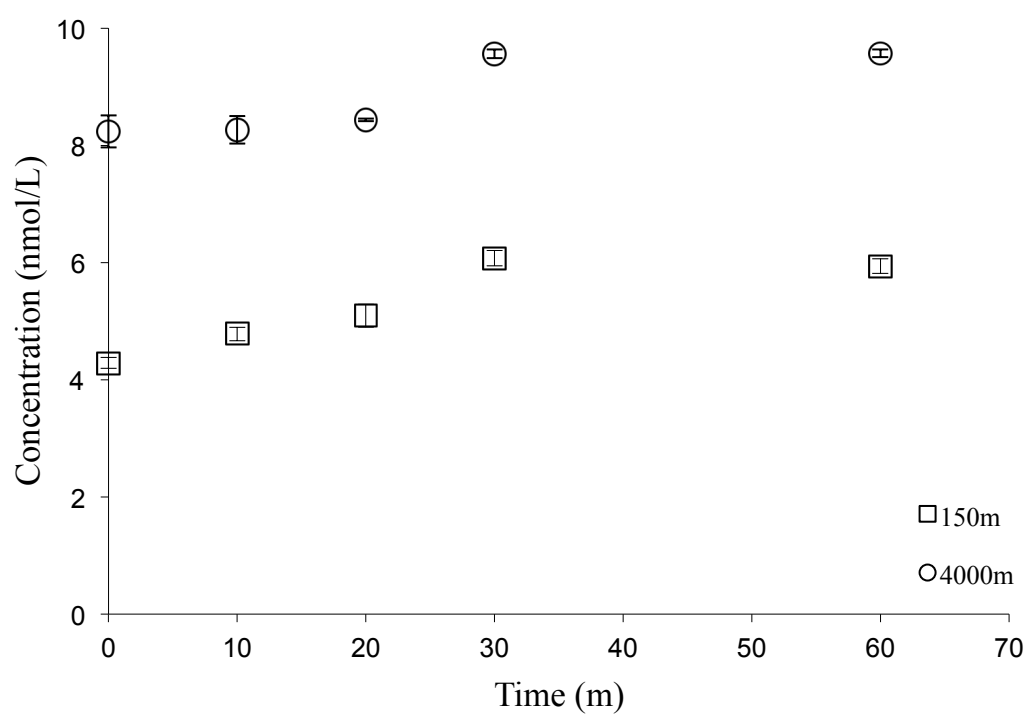


Fig. 2-9. Detected Zn concentrations in seawater with UV irradiation time (Seawater collected at depths 150m, 4000m).

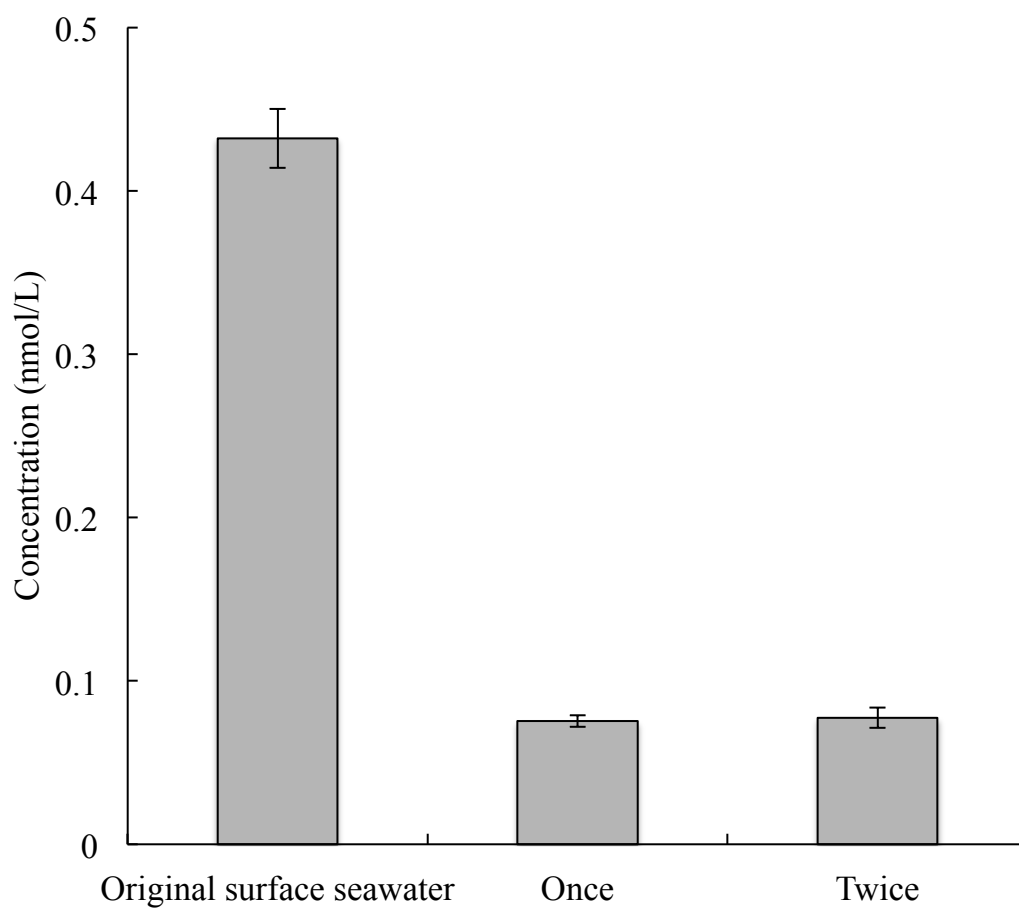


Fig. 2-10. Zn concentrations of surface seawater and those passed through the chelating resin column.

Chapter 3.

Establishment of clean seawater sampling

3-1 Introduction

Determination of trace concentrations of Zn is laden with complications caused by contamination (Fitzwater et al., 1982). Zn is ubiquitous in the laboratory and is typically used as sacrificial anodes in equipment deployed in seawater. This leads to severe contamination during the analytical and sampling processes of seawater. Therefore, it is important to develop clean sampling methods to collect seawater samples uncontaminated. Bruland et al. (1979) demonstrated one such method in which several internally Teflon-coated GO-FLO (General Oceanics) non-metallic water samplers were mounted on a Kevlar wire (a para-aramid synthetic fiber, having high strength and metal-free characteristics) triggered with plastic messengers. Soon, the first-ever reliable vertical distribution of Zn and their co-variances with major nutrients was reported (Bruland, 1980).

Although Kevlar wire hydrocast has become the standard sampling method for sampling trace metals in the oceans and has yielded high-quality clean data, mounting individual GO-FLO samplers one by one on Kevlar wire hydrocasts are very laborious and time-consuming. Recently, along with a Kevlar cable containing four embedded conductors, hydrographic sensors (pressure, conductivity, temperature, oxygen, beam transmittance, and fluorescence) were installed into the rosette frame to

obtain real-time hydrographic data during bottle closing (Measures et al., 2008); further, a Ti pressure case was applied for hydrographic sensors to eliminate potential sample contamination from Zn sacrificial anodes (Cutter and Bruland, 2012). An alternative custom-built rectangular Ti frame rosette system with a conducting Kevlar cable system called “TITAN” was specially built and deployed for the GEOTRACES program (de Baar et al., 2008); it can obtain samples that are not contaminated by Zn (Croot et al., 2011).

In the present study, all of the seawater samples were collected on R.V. Hakuho-maru (Japan Agency for Marine-earth Science and Technology, JAMSTEC), which is equipped with a Ti armoured cable (diameter 8.39 mm) of length ca. 9000 m for clean seawater sampling. This system has been used successfully for sampling of contamination-prone trace elements such as Fe and Al (Nishioka et al., 2013b; Obata et al., 1997; 2004).

This chapter describes the clean sampling techniques for determining trace levels of Zn in seawater. To confirm the reliability in sampling, I compared the results obtained by three different seawater sampling methods. Further, the vertical distributions of Zn in the subarctic North Pacific were obtained using the clean technique and CSV.

3-2 Sample collection and storage

Seawater samples were collected in the subtropical North and South Pacific during the KH-11-10 cruise of R/V Hakuho-maru (from December 2011 to January 2012, Stn. 2 and Stn.15) and in the subarctic North Pacific during the KH-12-4 cruise of R/V Hakuho-maru (from August to October 2012, stations BD-7, BD-11 and BD-

17). The stations are shown in Fig. 3-1. Seawater samples were collected using Teflon-coated Niskin-X samplers (General Oceanics), and all the sampling bottles used were cleaned according to procedures detailed in the GEOTRACES sample handling protocols (Cutter et al., 2010). The O-rings inside the Niskin samplers and the spigots were replaced with Viton and Teflon ones, respectively. Niskin-X samplers were cleaned using 1% alkaline surfactant (Extran MA01), 0.1 mol/L HCl (Special Grade, Wako Pure Chemical Industries) and Millipore Milli-Q water (MQW).

To compare the performance of trace metal clean sampling methods, seawater samples were collected using three different sampling methods, in which Niskin-X samplers were: (1) deployed on a conductivity-temperature-depth carousel multi-sampling system (CTD-CMS, SBE-911plus and SBE-32 water sampler, Sea Bird Electronics, Inc.) with Zn on the sacrificial anode replaced with Al to avoid the possibility of Zn contamination from the frame; (2) attached bottle by bottle to a Kevlar wire (6 mm diameter, Hikari-kogyo); and (3) attached bottle by bottle to a Ti wire equipped in R/V Hakuho-maru. The acid-cleaned Niskin-X samplers attached to both wires were closed with Teflon messengers. In this study, seawater sampling using CTD-CMS and Ti wire hydrocasts were performed on the starboard (right) side, while sampling using Kevlar wire hydrocasts took place on the stern side of the research vessel (Fig. 3-2).

For sub-sampling, the Niskin-X samplers were detached from the CTD-CMS frame or the Kevlar wire or the Ti wire, and carefully moved into a clean space (filled with air which was passed through an HEPA filter) in the onboard laboratory of the research vessel (Fig. 3-3). Seawater samples were filtered using an acid-cleaned 0.2 μm Acropak filter cartridge (PALL Co.) that was directly connected to the Niskin-X Teflon spigot, and the filtered samples were stored in acid-cleaned 500 mL low-

density polyethylene (LDPE) bottles (Nalgene Co., Ltd) after rinsing more than three times using filtered seawater. The seawater samples were subsequently acidified to achieve a $\text{pH} < 1.8$ by adding ultra-pure HCl (Tamapure AA-100), and were then stored.

3-3 Results

3-3-1 Comparing three sampling methods

At BD-7 (subarctic North Pacific) and Stn. 15 (subtropical South Pacific), I collected seawater samples using Niskin-X samplers with the CTD-CMS (method (1)) and those attached to the Kevlar wire (method (2)); I compared the dissolved Zn concentrations in these samples. Vertical Zn profiles obtained by both methods at Stn. 15 and BD-7 are shown in Fig. 3-4; the figure also shows the vertical Fe profiles (J. Nishioka, Kim et al., submitted) measured for the same samples at BD-7. At both stations, dissolved Zn concentrations in samples obtained by Kevlar wire hydrocasts (method (2)) were higher than those obtained by CTD-CMS (method (1)) in shallow waters. Hydrocast by Go-Flo samplers attached to a Kevlar wire has been recommended for trace-metal clean sampling for several years (Bruland et al., 1978). When I used Niskin-X samplers for the Kevlar hydrocast, Zn contamination seemed to occur for the shallow two samples, but little Fe contamination was accompanied (Fig. 3-4).

On the other hand, when the same samplers were attached to the Ti wire (method (3)), the dissolved Zn concentrations were consistent with those obtained by the method (1) (Fig.3-5). As described before, only the Kevlar hydrocast (method

(2)), among these sampling methods, was performed at the stern side of the main deck of R/V Hakuho-maru (Fig. 3-2), where the propellers might have affected Zn concentration in seawater during sampling. This is because Zn is used as a sacrificial anode for the propeller shaft and the pipes of cooling seawater system. Furthermore, the seawater used in the cooling system is also released in the vicinity of the propellers. In addition, the propellers are usually kept rotating in order to keep the same ship position during the sampling, which may have extended Zn-contaminated seawater around the stern zone. Because of such seawater from the cooling system of propellers, Zn contamination is thought to have occurred during seawater sampling using Kevlar wire hydrocasts.

Three different sampling methods: (1) Teflon-coated X-Niskin samplers fixed on CTD-CMS, (2) attached to a Kevlar wire, and (3) attached to a Ti wire were again compared at stations BD-11 and 17, and the results were shown in Fig. 3-6. During the sampling using Kevlar wire hydrocasts at these stations, I asked the ship to turn off the propellers to minimize the influence of seawater in contact with the Zn sacrificial anode. At both stations, I confirmed that Zn concentrations of samples obtained by the three different sampling methods are almost consistent within the limit of analytical error (Fig. 3-6).

3-4 Discussion

3-4-1 Operational conditions of sampling for trace Zn in seawater

When seawater sampling was performed on the starboard side of the R/V Hakuho-maru, Zn concentrations in samples collected by CTD-CMS were found to be

always consistent with those obtained by Ti wire hydrocasts (at Stn. 2, BD-11, and BD-17, Fig. 3-5, Fig. 3-6). Such consistency indicates that by performing water sampling on the starboard side of the R/V Hakuho-maru (Fig. 3-2), contamination from the research vessel was suppressed to an undetectable concentration level of Zn in seawater. Dissolved Zn concentrations in samples with Kevlar wire hydrocasts obtained from the stern side of the research vessel were higher than those in the samples obtained with CTD-CMS from the surface to a depth of 200 m (Fig. 3-4) at the usual conditions, and this was attributed to the cooling system used for propellers and to the Zn sacrificial anode of the research vessel. Seawater samples were collected using Niskin-X samplers opened onboard the research vessel. As Zn-contaminated seawater enters the sampler near the propellers, it is considered possible that some could remain inside the samplers up to a depth of 200 m. Although contamination sources of Zn exist on the research vessel, I succeeded in minimizing the influence of the Zn sacrificial anode during sampling by halting the propellers (Fig. 3-6). This examination showed that a severe contamination problem did not occur for Fe. Therefore, to avoid Zn contamination during sampling, it is necessary not only to understand the structure of the research vessel, (e.g., the locations of Zn sacrificial anodes), but to also examine the operational conditions of sampling.

3-4-2 Vertical distributions of North Pacific

Using the established technique of clean seawater sampling by CTD-CMS, seawater samples collected in the subarctic North Pacific (BD-7, BD-11, and BD-17) were measured (Fig. 3-7). Vertical distributions of Zn show nutrient-type vertical profiles, with a gradual increase in concentration from the surface to deep water.

Several studies have reported that the dissolved Zn profile is similar to that of Si in the North Pacific Ocean (Bruland et al., 1978; Donat and Bruland, 1990; Jakuba et al., 2012; Lohan et al., 2002). The Zn concentrations in the subarctic North Pacific (BD-7, BD-11, and BD-17) were compared with those of Si obtained in this study (Fig. 3-7), and Si concentrations were found to show a strong correlation with those of dissolved Zn, which is very consistent with the observations made in previous studies.

Dissolved Zn and Si concentrations show a clear trend at shallower depths from the surface to a depth of 700 m (Fig. 3-8). In this layer, the concentrations were highest in the western subarctic North Pacific (BD-7), and gradually decreased toward the eastern subarctic North Pacific (BD-17). Jakuba et al. (2012) also reported this trend in Zn concentration in the shallower waters of the subarctic North Pacific, showing common trends of dissolved Zn and Si in shallow waters. This consistency implies that the west–east gradient of Zn in the shallow waters of the subarctic North Pacific is controlled by the rates of diatom sinking and the simultaneous release of both Zn and Si from packaged sinking diatoms (Twining et al., 2014).

Station BD-7 is located within the western subarctic gyre of the North Pacific. The western subarctic North Pacific is known as a mixed water region (MWR) where the new North Pacific Intermediate Water (NPIW) is formed (Talley, 1997). This region is extremely active, and is an area of upwelling; wintertime cooling can make the water quite dense at the surface. Thus, the strong vertical mixing in the western subarctic Pacific region brings seawater with higher Si concentration up into the thermocline at the depth of the NPIW as well as above it (Sarmiento et al., 2004). Furthermore, the upward transport process from intermediate water to the surface mixing at the Kuril Straits, and entrainment by winter deep vertical mixing are considered to be important mechanisms involved in the supply of Fe and nutrients in

the western subarctic North Pacific (Nishioka et al., 2013a). It is therefore considered likely that the extensive vertical mixing could introduce not only remineralized Zn, but also Si to the surface, which could then spread eastward.

3-5 Conclusions

Since Zn is ubiquitously used in the research vessel, it is still a big challenge to determine dissolved Zn in seawater without the risk of contamination. By using a shipboard analytical method and comparing clean sampling methods, I have identified the cause of Zn contamination; amounts of which are dependent on the structure of the research vessel. To minimize Zn contamination, it is therefore necessary to understand the structure of the ship and find the optimal operational conditions for seawater sampling on board the research vessel.

Based on established analytical and sampling methods, I obtained detailed vertical distributions of Zn in the subarctic North Pacific (KH-12-4). Differences between Zn concentrations in shallow waters were greater-clarified in the subarctic North Pacific Ocean. The west–east decreasing trend of dissolved Zn is very similar to that of Si, suggesting that surface Zn distribution is controlled by biogeochemical processes, such as vertical transport, uptake by diatoms, and regeneration or dissolution of biogenic particles. It is thus considered, that by studying the detailed distribution of Zn and Si in the subarctic North Pacific, the biogeochemical cycles of both elements could be further clarified.

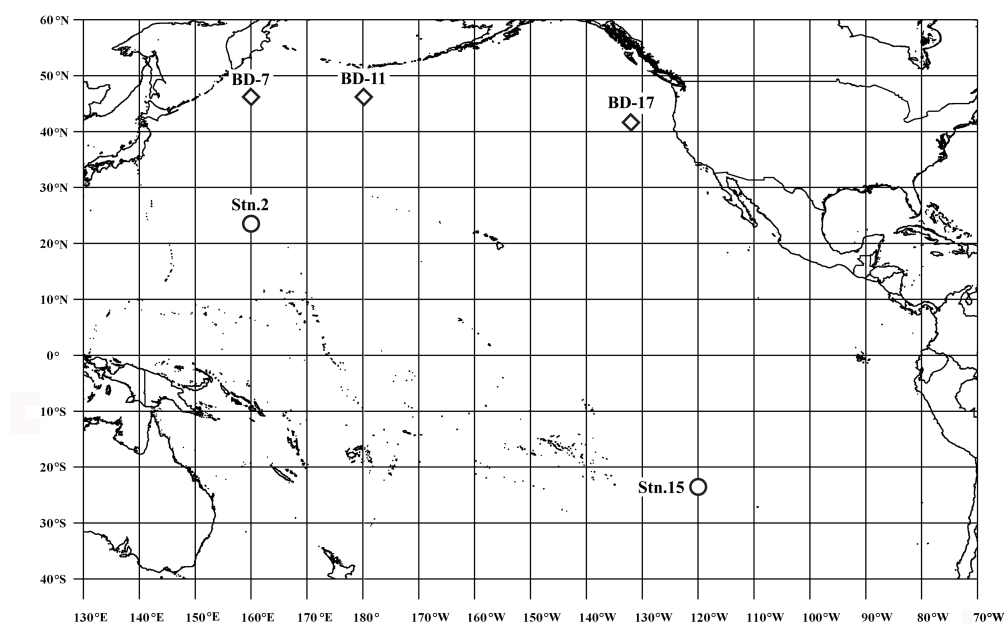


Fig. 3-1. Location of sampling stations. Seawater samples for subtropical North (Stn.2) and South Pacific (Stn.15) were collected as part of KH-11-10 R/V Hakuho-maru cruise (Dec 2011-Jan 2012). In the subarctic North Pacific (BD-7, BD-11 and BD-17), samples were collected as part of GEOTRACES GP 02 cruise (KH-12-4 R/V Hakuho-maru cruise, Aug-Oct 2012).

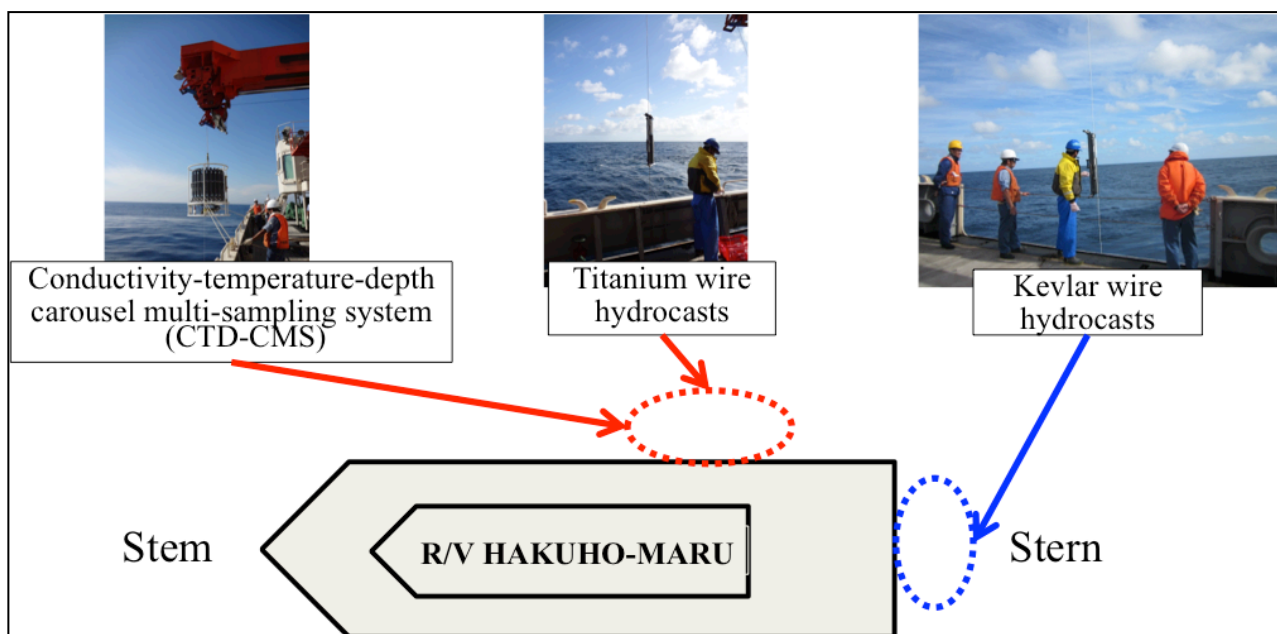


Fig. 3-2. Sampling positions where sampling method were examined.

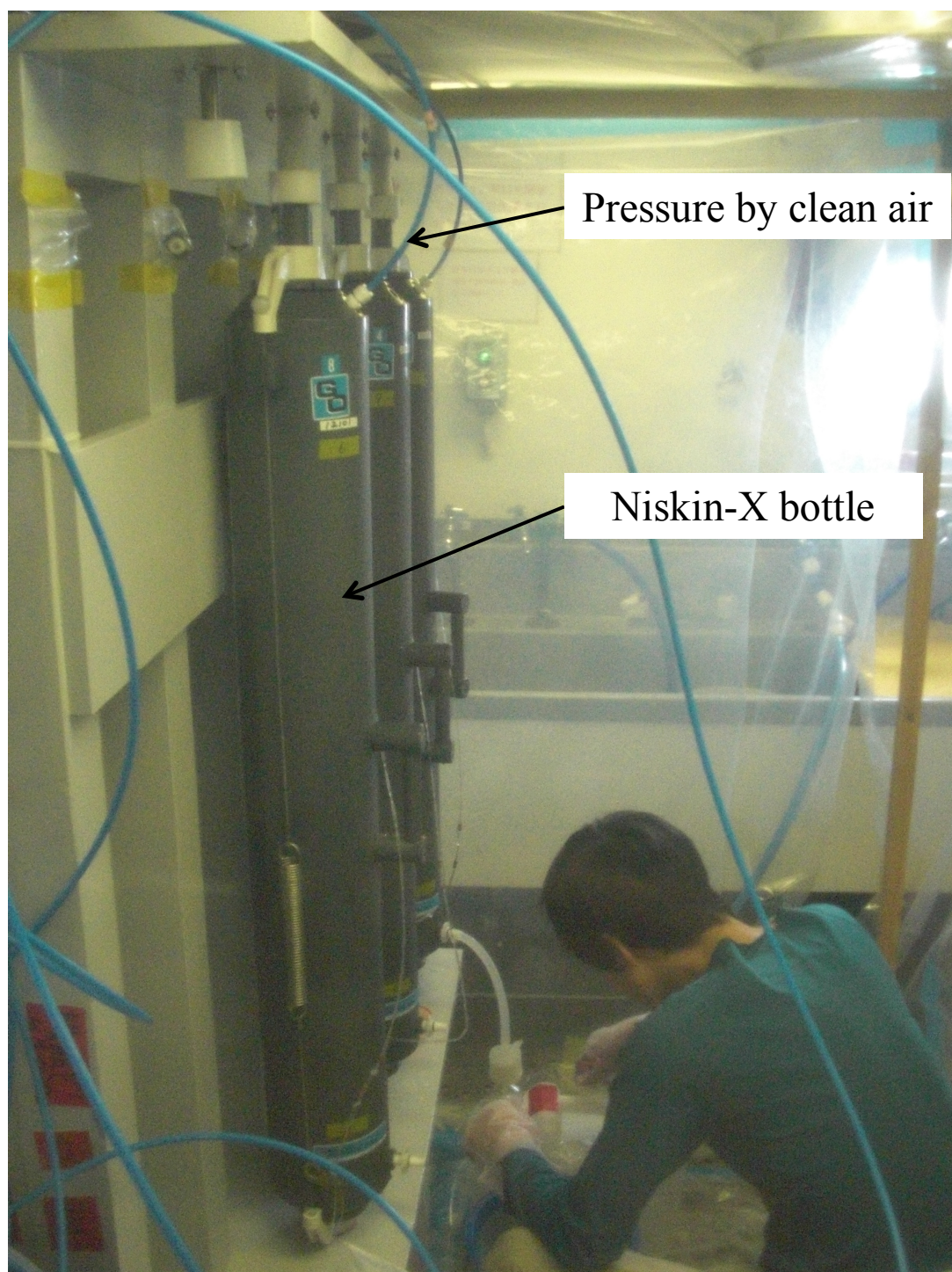


Fig. 3-3. Clean space (completely surrounded by transparent plastic sheets and filled with air which was passed through an HEPA filter) in the onboard laboratory of the research vessel.

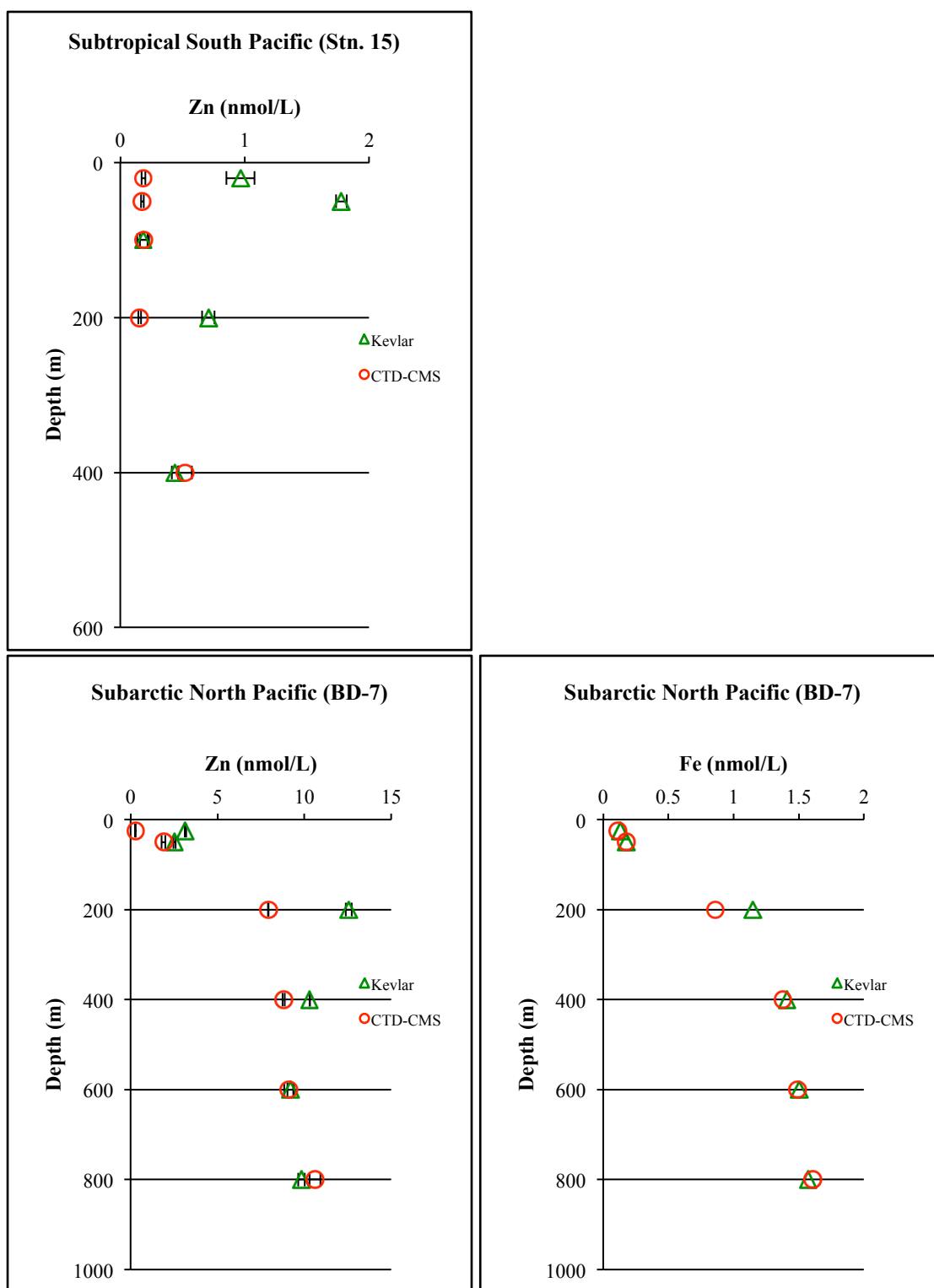


Fig. 3-4. Comparison of dissolved Zn (Stn. 15 and BD-7) and Fe (BD-7, data from J.Nishioka) concentrations in seawater collected with Niskin-X samplers deployed onto Kevlar wire hydrocasts and CTD-CMS.

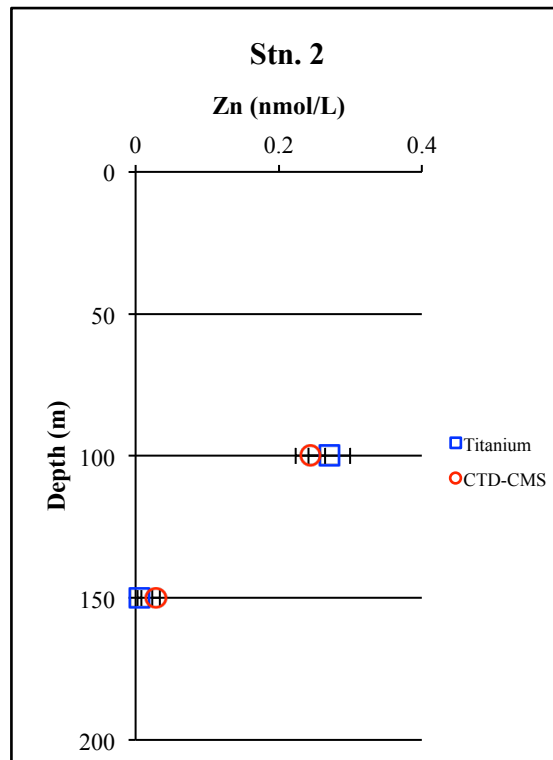


Fig. 3-5. Comparison of dissolved Zn concentrations in seawater collected with Niskin-X samplers deployed onto titanium wire and CTD-CMS at Stn. 2 in subtropical North Pacific (KH-11-10).

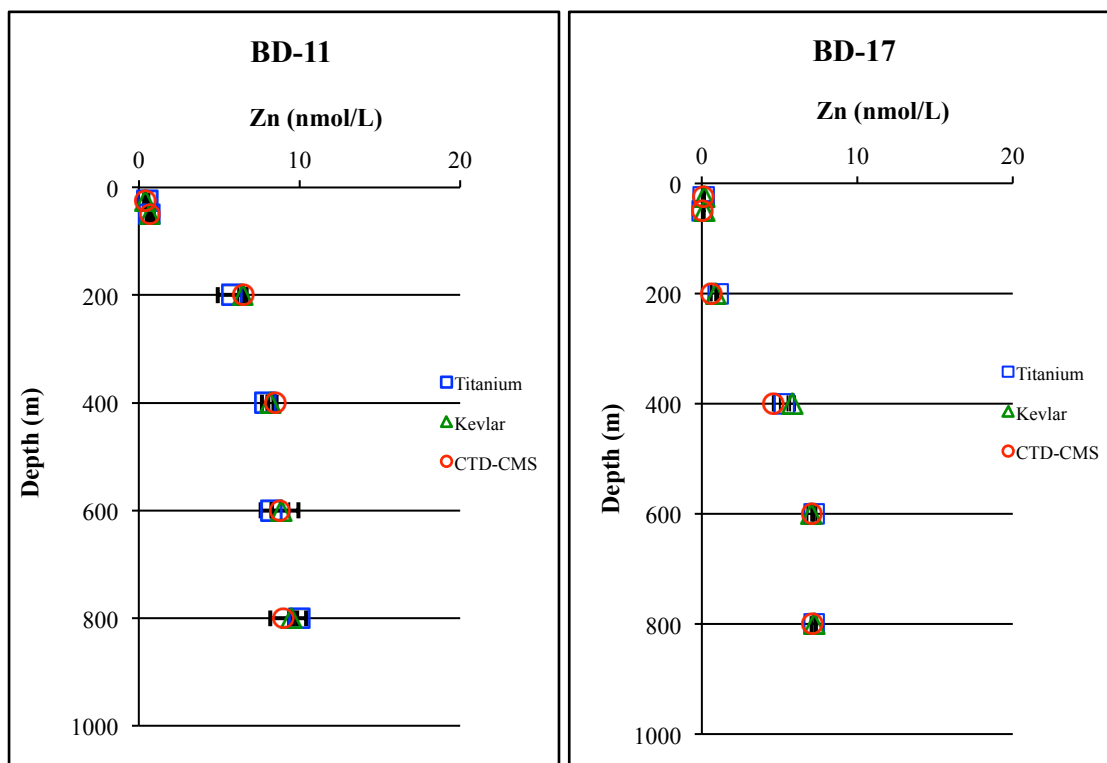


Fig. 3-6. Comparison of dissolved Zn concentrations in seawater collected with three different sampling methods, Niskin-X samplers with titanium wire hydrocasts, Kevlar wire hydrocasts and CTD-CMS at BD-11 and BD-17 in subarctic North Pacific (KH-12-4).

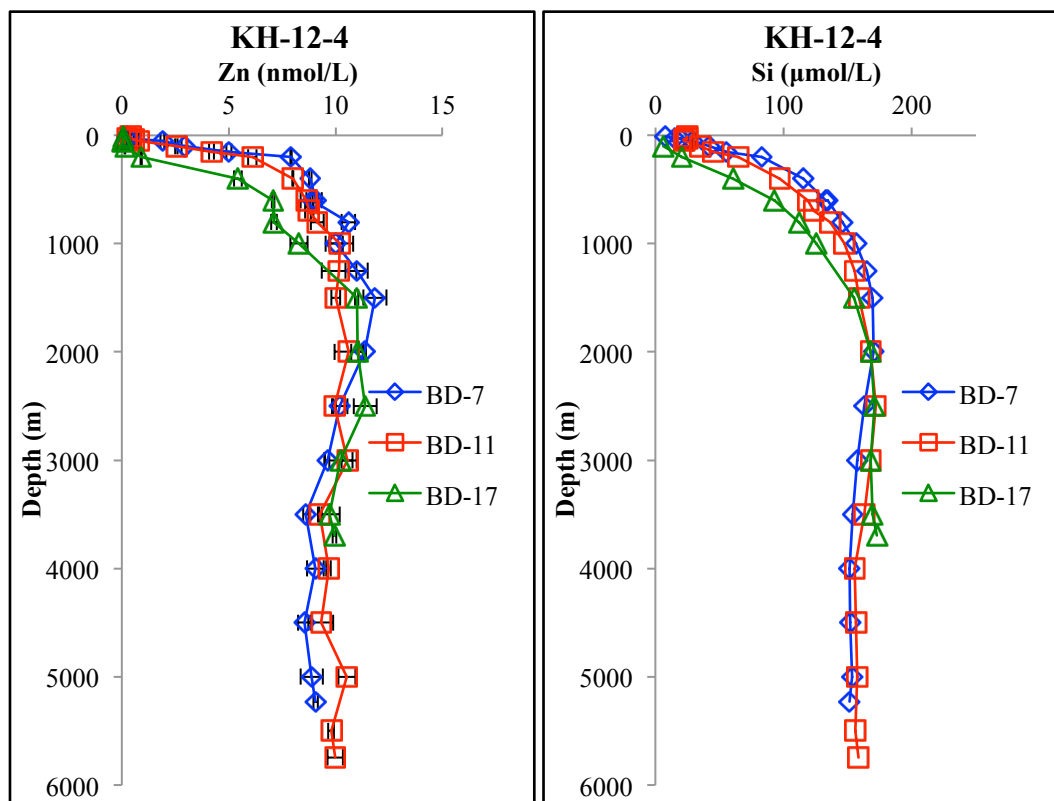


Fig. 3-7. Vertical distributions of dissolved Zn and Si at subarctic North Pacific (KH-12-4).

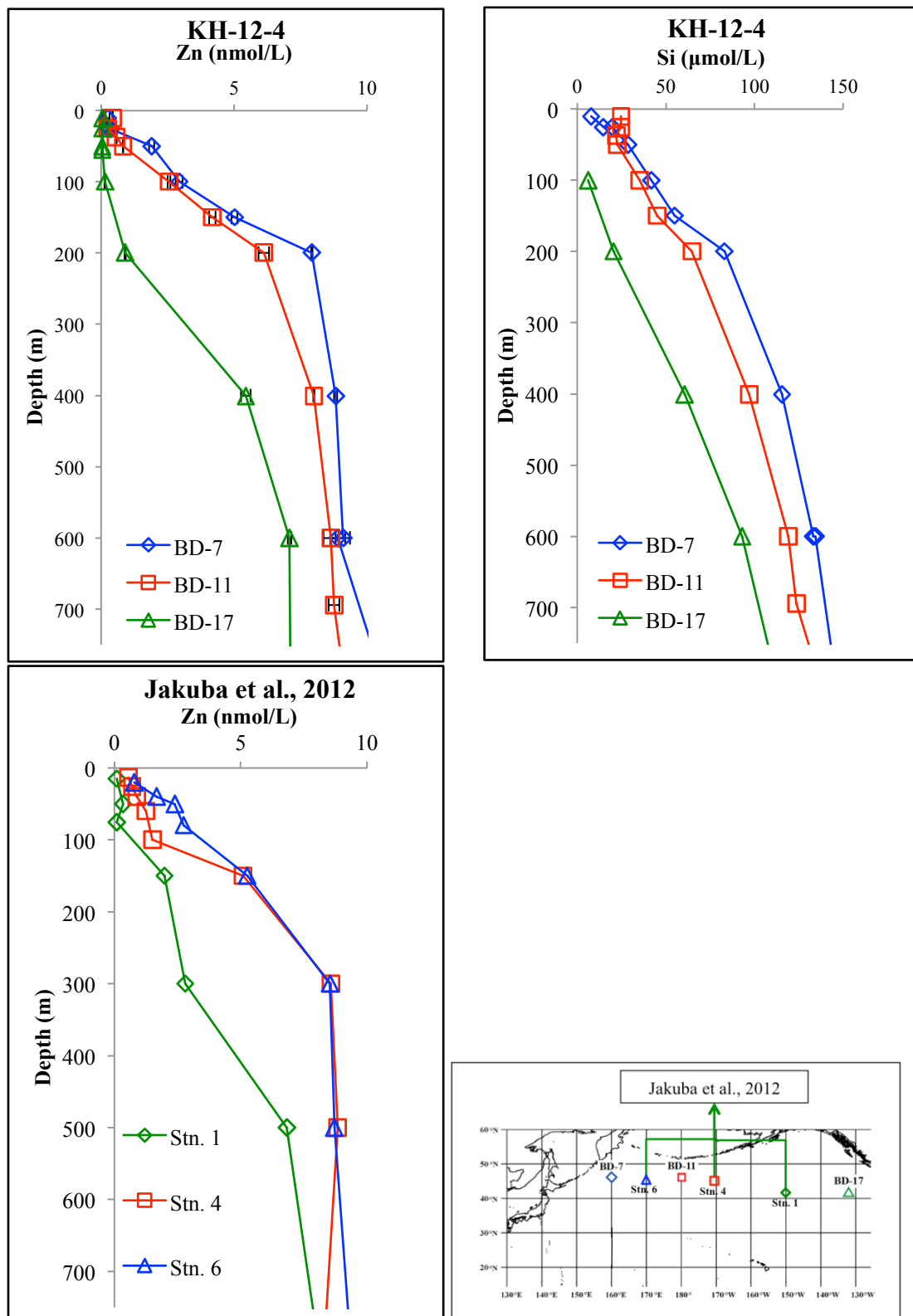


Fig. 3-8. Vertical distributions of dissolved Zn and Si between surfaces to 700m in subarctic North Pacific (KH-12-4), dissolved Zn data from previous study from Jakuba et al. (2012) (map shows sampling area).

Chapter 4.

Distribution and speciation of dissolved zinc in the western North Pacific and its adjacent seas

4-1 Introduction

This chapter presents total dissolved Zn concentrations and Zn speciation in the western subtropical and subarctic North Pacific and the adjacent Sea of Okhotsk and Sea of Japan (East Sea)¹. At the western North Pacific, this is the first data of the whole vertical profile for total dissolved Zn, while Jakuba et al. (2012) reported vertical profiles of Zn only upper 3000 m depths at western subarctic North Pacific. At the Sea of Okhotsk and Sea of Japan, there are no previous studies of total dissolved Zn and its speciation in the Sea of Okhotsk and Sea of Japan. Zn speciation was determined using competitive ligand equilibration—adsorptive cathodic stripping voltammetry (CLE-ACSV)—to investigate organic Zn complexing ligands and their influence on the chemical speciation of Zn.

4-2 Sample collection and storage

¹ Sea of Japan is also called as East Sea in Korea. In this study, by following the international standard, I used the term of Sea of Japan.

Seawater samples were collected in the western subarctic North Pacific, Sea of Okhotsk and Sea of Japan during R/V Hakuho-maru research cruises KH-10-2 (in June and July 2010, at stations CR-27, CR-30 and CR-47) and R/V Hakuho-maru research cruise KH-12-4 (from August to October 2012, at stations BD-7 and BD-11). Additional samples were collected in the western subtropical North Pacific (in December 2011, at Stn. 2) during R/V Hakuho-maru research cruise KH-11-10. Locations of the sampling stations are indicated in Fig. 4-1.

Detailed sampling methods are described elsewhere (see chapter 3). Briefly, seawater samples were collected using acid-cleaned Teflon-coated X-type Niskin samplers. O-rings, inside the Niskin samplers, and spigots were replaced with Viton ones and Teflon ones, respectively. X-type Niskin bottles were cleaned using a 1% alkaline surfactant (Extran MA01), 0.1 mol/L HCl (Special Grade, Wako Pure Chemical Industries) and Millipore Milli-Q water (MQW). The acid-cleaned Teflon-coated X-type Niskin bottles were then deployed on a conductivity-temperature-depth carousel multi-sampling system (CTD-CMS, SBE-911plus and SBE-32 water sampler, Sea Bird Electronics, Inc.), on which a Zn sacrificial anode was replaced with an Al one to avoid the possibility of Zn contamination from the frame. For sub-sampling from the Niskin-X bottles, the bottles were detached from the CTD-CMS frame and carefully moved into a clean space (filled with air that had been passed through a HEPA filter) in the onboard laboratory of the research vessel. Seawater samples were filtered using an acid-cleaned (0.1 mol/L HCl) 0.2- μ m Acropak filter cartridge (PALL Co.) directly connected to the Niskin-X Teflon spigot. Filtered samples were stored in acid-cleaned 500-mL low-density polyethylene (LDPE) bottles (Nalgene Co., Ltd) after rinsing at least three times with filtered seawater. Samples were then acidified to achieve a pH < 1.8 by using ultra pure HCl (Tamapure AA-100, Tama chemicals) and

then placed in storage for later measurement of total dissolved Zn concentration. The filtered samples for Zn speciation analysis were frozen immediately after collection and kept frozen until just before analysis.

4-3 Results

4-3-1 Hydrography

A potential temperature-salinity diagram is shown in Fig. 4-2. North Pacific Deep Water (NPDW) is observed at water depths below 2000 m at both Stn. 2 and CR-27. North Pacific Intermediate Water (NPIW), characterized by a salinity minimum layer (Yasuda, 1997), is present at depths of around 600 m in the western subtropical North Pacific (Stn. 2). In the surface layer of the western subtropical North Pacific (Stn. 2), warm high-salinity surface water exists in this region. The North Pacific Subtropical Gyre (NPSG) is characterized by warm surface waters with low macronutrient concentrations (Karl, 1999). The euphotic zone of the NPSG has been described as a two-layered system: an uppermost, light-saturated, nutrient-limited layer accounting for most of the primary production, and a lower, light-limited, nutrient-saturated layer (Small et al., 1987). On the other hand, in the western subarctic North Pacific (CR-27), salinity minima occur at the surface (Fig. 4-2). Western Subarctic Gyre (WSG) is characterized by low surface salinity due to low evaporation rate and a small rate of flow into and out of the near surface layer (Warren, 1983). Furthermore, the sharp halocline is present at the bottom of the layer at about 200 m depth. The halocline prohibits the deepening of the upper layer due to winter cooling because the surface water is too fresh (Reid, 1973).

The Sea of Okhotsk (CR-30), a marginal sea on the northwest rim of the Pacific Ocean, is characterized by low temperatures throughout the water column, with the exception of surface water and low salinities in shallower waters (Fig. 4-2). Those low salinities in shallower waters are influenced by the fluvial discharge from the Amur River (Ohshima et al., 2002), which also brings additional dissolved metals and suspended matters (Shulkin and Bogdanova, 2003). The Sea of Okhotsk has the lowest-latitude seasonal sea ice in the world (Kimura and Wakatsuchi, 2012). The water temperature between 50 and 148 m was below 0°C; this layer is referred to as “dicothermal layer” (Kitani, 1973). Deep water below the dicothermal layer is considerably warmer with its temperature slowly increasing with depth to between 2°C and 3°C.

The Sea of Japan (CR-47) is a semi-closed marginal sea with a maximum depth of >3700 m. The Sea of Japan is bounded by the Asian continent and Japanese islands, and is connected to neighboring seas through just four straits: the East China Sea through the Tsushima/Korea Strait, the North Pacific through the Tsugaru Strait, and the Sea of Okhotsk through the Soya and Mamiya (Tatar) Straits, all with maximum depths of less than 130 m. Exchange of waters between the Sea of Japan and other seas is not pronounced, and about 90% of the entire water body is occupied by homogeneous water (Kawabe, 1982). In the surface layer, water inflows by the Tsushima Current through the Tsushima/Korea Strait and outflows through the Tsugaru Strait. The Sea of Japan has been regarded as the miniature of the global ocean because it has its own deep convection system (thermohaline conveyor belt); cold weather in winter is thought to make surface seawater in the northern area dense enough to sink toward the bottom (Gamo and Horibe, 1983; Kim et al., 2001). The potential temperature-salinity diagram for the Sea of Japan is therefore characterized

by an almost invariant salinity, which means that its deep waters have unique and different properties (Fig. 4-2). These waters have been termed the “Japan Sea Proper Water” (Sudo, 1986).

4-3-2 Silicate and Chl *a* concentrations

Vertical distributions of dissolved Si are shown in Fig. 4-3. In the Sea of Okhotsk and Sea of Japan, Si concentrations show similar profiles to those in the North Pacific. The lowest concentrations (0.5 to 3.8 $\mu\text{mol/L}$) were found near the surface (< 200 m) in the subtropical North Pacific. Conversely, in the western subarctic North Pacific (CR-27), Si concentrations (33.8–93.1 $\mu\text{mol/L}$) near the surface are one order of magnitude higher than those of the subtropical North Pacific. Si concentrations at shallower depths in the Sea of Okhotsk (CR-30) and Sea of Japan (CR-47) ranged from 17.8 to 49.4 $\mu\text{mol/L}$ and from 15.9 to 21.0 $\mu\text{mol/L}$, respectively.

In this study, Chl *a* contents were obtained at depths < 200 m. In the subtropical North Pacific, the maximum Chl *a* concentration was 0.17 (99 m) $\mu\text{g/L}$, which is much lower than in the subarctic North Pacific where concentration was 1.06 (21 m) $\mu\text{g/L}$. The maximum Chl *a* content was 0.20 (20 m) $\mu\text{g/L}$ in the Sea of Okhotsk and 1.18 (50 m) $\mu\text{g/L}$ in the Sea of Japan.

4-3-3 Total dissolved Zinc

Total dissolved Zn profiles are nutrient type at all stations in this study (Fig. 4-3), which is consistent with previous studies in the North Pacific (Bruland, 1980; Bruland et al., 1979; 1978; 1994; Cutter and Bruland, 2012; Lohan et al., 2002),

South Atlantic (Wyatt et al., 2014), southern Indian Ocean (Gosnell et al., 2012), and Southern Ocean (Croot et al., 2011). In the subtropical North Pacific (Stn. 2), total dissolved Zn concentrations in the shallow waters (6–198 m) ranged from 0.1 to 0.2 nmol/L. These values are clearly lower than those in the subarctic North Pacific (CR-27, 1.9–7.5 nmol/L), Sea of Okhotsk (CR-30, 1.0–2.7 nmol/L) and Sea of Japan (CR-47, 0.9–6.0 nmol/L). In the Sea of Okhotsk (CR-30), the total dissolved Zn concentration in the surface exceeded 2 nmol/L, higher than those in the North Pacific. This result may indicate that there are additional sources of Zn in the Sea of Okhotsk. Total dissolved Zn in the subarctic North Pacific (CR-27) at intermediate depths (1484–2926 m) ranged from 10.9 to 13.9 nmol/L, which is higher than those in the subtropical North Pacific (Stn. 2, 8.6–10.4 nmol/L). Below the intermediate depth (> 2962 m), total dissolved Zn concentrations in the subarctic North Pacific (CR-27) ranged from 9.0 to 10.3 nmol/L, which is slightly higher than concentrations in the subtropical North Pacific (Stn. 2, 8.4 – 9.6 nmol/L).

In the Sea of Okhotsk (CR-30), total dissolved Zn almost doubles between 593 m (5.5 nmol/L) and 790 m (10.0 nmol/L), then remains relatively constant at greater depths (Fig. 4-3). In the Sea of Japan (CR-47), Zn concentrations obtained from 50 to 600 m are relatively high compared with those of the Sea of Okhotsk, even though those of the surface samples (20 m depth) were lower (0.9 nmol/L) than those of the Sea of Okhotsk (2.1 nmol/L).

The location of CR-27 is almost identical to that of BD-7. However, the total dissolved Zn concentration in CR-27 was 1.9 nmol/L (20 m), whereas in BD-7 it ranged from 0.3 to 0.4 nmol/L at < 27 m (Kim et al., submitted). Additionally, total dissolved Zn in BD-7 was drastically increased below 27 m (1.9 nmol/L, 51 m). The same trend has been observed in Si concentrations. The Si concentration in CR-27

was 34.0 $\mu\text{mol/L}$ at a depth of 20 m, whereas BD-7 ranged from 7.9 to 19.8 $\mu\text{mol/L}$ at < 27 m (Kim et al., submitted). Mixed layer depths, defined as the depth at which a change in the surface seawater density (σ_θ) of 0.125 occurs (Suga et al., 2004), were 6 m at BD-7 and 30 m at CR-27, which correspond with the temporal change of total dissolved Zn and Si concentrations. The temporal surface mixing affects total Zn concentrations and Si in this area.

4-3-4 Labile Zn concentrations

The Zn titrations of most samples in the subarctic North Pacific (CR-27, n=9), Sea of Okhotsk (CR-30, n=6) and Sea of Japan (CR-47, n=9) showed a linear increase with added Zn^{2+} , indicating that all of the natural ligands in the sample were already saturated with Zn. To understand Zn speciation in the saturated samples, I show the vertical profiles of labile Zn ($\text{Zn}_{\text{labile}}$) (Fig. 4-3). In the Southern Ocean, vertical profiles of $\text{Zn}_{\text{labile}}$ were reported by using anodic stripping voltammetry (ASV) (Baars and Croot, 2011). Here, by using CSV under the same conditions as those of titration (pH 8.2, addition of 25 $\mu\text{mol/L}$ APDC, 12 hour equilibration time), $\text{Zn}_{\text{labile}}$ was determined for natural samples without adding Zn and with no UV-irradiation. In this study, $\text{Zn}_{\text{labile}}$ would include not only Zn^{2+} and inorganic Zn, but also the part of Zn weakly complexed with organic ligands. The labile Zn ($\text{Zn}_{\text{labile}}$) concentrations in CR-27 ranged from 1.1 to 7.2 nmol/L (21–4915 m) (Fig. 4-3). At intermediate depths (992–1487 m), higher $\text{Zn}_{\text{labile}}$ values (5.5–7.2 nmol/L) have been obtained relative to those of deep waters (3941–4915 m, 3.8–3.9 nmol/L). $\text{Zn}_{\text{labile}}$ concentrations in the Sea of Okhotsk (CR-30) and Sea of Japan (CR-47) ranged from 0.4 to 4.6 nmol/L (20–2699 m) and 0.7 to 4.4 nmol/L (20–3413 m), respectively (Fig. 4-3).

4-3-5 Total ligand concentrations and conditional stability constants

In this study, total ligand concentrations (C_L) and $\log K'_{ZnL,Zn^{2+}}$ were obtained for the samples, in which titration curvatures were observed, between 6 and 198 m in the subtropical North Pacific (Stn. 2, $n=6$) (Fig. 4-4) and at a depth of 20 m in the Sea of Okhotsk and Sea of Japan (Table 4-1). At CR-27, all of the natural ligands in the sample were already saturated with Zn. Thus, no estimate of $\log K'_{ZnL,Zn^{2+}}$ could be obtained from the titration data. To understand Zn speciation in the subarctic North Pacific, I also analyzed the samples that were collected in the western subarctic North Pacific during another cruise (BD-7, 47°00'N, 160°05'E and BD-11, 47°00'N, 179°59'W). The C_L and $\log K'_{ZnL,Zn^{2+}}$ in the subarctic North Pacific (BD-7, BD-11) have been obtained between 10 and 27 m at BD-7 ($n=3$) and between 10 and 37 m at BD-11 ($n=3$) (Table 4-1).

The total ligand concentrations, which ranged from 0.2 to 0.5 nmol/L in the subtropical North Pacific (Stn. 2), were relatively low compared with those of the subarctic North Pacific (BD-7, BD-11), which ranged from 0.7 to 1.2 nmol/L. $\log K'_{ZnL,Zn^{2+}}$ values in the subtropical North Pacific ranged from 9.5 to 10.8, which is similar to those of the subarctic North Pacific (9.7–10.2). $\log K'_{ZnL,Zn^{2+}}$ values in the Sea of Okhotsk (9.5) and Sea of Japan (9.1) were relatively low compared with those of the open oceans (Table 4-1).

The highest C_L was observed at 20 m in the Sea of Okhotsk (CR-30, 2.6 nmol/L). The Sea of Okhotsk and Sea of Japan showed relatively high total ligand concentrations in comparison to those of open oceans (Table 4-1). The resultant free Zn^{2+} concentrations were lower in the subtropical North Pacific (0.004–0.069 nmol/L)

than in the subarctic North Pacific (0.017–0.106 nmol/L). The free Zn^{2+} concentrations in the Sea of Okhotsk and Sea of Japan were much higher at 0.234 and 0.257 nmol/L, respectively (Table 4-1).

4-4 Discussion

4-4-1 Total dissolved Zinc

In the subtropical North Pacific (Stn. 2), the average of the total dissolved Zn within the upper 200 m was 0.14 ± 0.07 nmol/L ($n=6$), which is consistent with previous studies in the eastern subtropical North Pacific (Bruland et al., 1994). Both sampling stations were located in the North Pacific Subtropical Gyre (NPSG), which is characterized by warm surface waters with low macronutrient concentrations (Karl, 1999). Total dissolved Zn concentrations at western subtropical North Pacific (Stn. 2) were strongly correlated with those of silicate (Fig. 4-5). Previous studies also showed that distributions of total dissolved Zn are similar to silicate distributions in the eastern subtropical North Pacific (Bruland, 1980; Bruland et al., 1994; 1978), which suggests that total dissolved Zn is governed by an internal cycle with rapid removal from surface waters coupled with almost complete remineralization in deep waters (e.g., Bruland et al., 1994). Therefore, total dissolved Zn in the western subtropical North Pacific could be influenced by internal biogeochemical cycles rather than by external sources.

In the subarctic North Pacific (CR-27), much higher total dissolved Zn concentrations have been obtained not only in surface waters, but also in deeper water, in comparison to concentrations observed in the subtropical North Pacific (Stn. 2).

Such high concentrations of total dissolved Zn have also been reported in previous studies in the subarctic North Pacific (Jakuba et al., 2012; Kim et al., submitted). There could be additional input of dissolved Zn in the western subarctic North Pacific from sources such as atmospheric dust (Duce and Tindale, 1991; Jickells et al., 2005; Meskhidze et al., 2003; Tanaka and Chiba, 2006). Indeed, Thuróczy et al. (2010) suggests that the solubility of Zn in the natural soil (a loess similar to Saharan soil) is high (16%) in seawater. Therefore, the additional input of Zn could be reasons for the high concentrations of total dissolved Zn (1.9–7.5 nmol/L) in the near surface (21–200 m) of the subarctic North Pacific (CR-27), when compared with the subtropical North Pacific (Stn. 2, 0.1–0.2 nmol/L). Furthermore, below the thermocline (299 – 992 m), total dissolved Zn concentrations were increased (9.4–11.9 nmol/L) while the concentrations of dissolved oxygen were very low (14.2–21.4 $\mu\text{mol/kg}$)(Fig. 4-3), which is probably caused by sinking of biogenic particles and their subsequent remineralization. Those remineralization of biogenic particles and additional dust input could cause the high concentrations of total dissolved Zn at intermediate depths (1484–2926 m) in the subarctic North Pacific (CR-27)(Fig. 4-3). Below intermediate depths (> 2962 m), where the NPDW is present, total dissolved Zn concentrations were nearly homogeneous in both the subarctic North Pacific (CR-27, 9.0–10.3 nmol/L) and subtropical North Pacific (Stn. 2, 8.4–9.6 nmol/L), but slightly higher in the subarctic North Pacific when compared with those of the subtropical North Pacific (Fig. 4-3). This might indicate the accumulation of remineralized Zn in deep waters as they move northward (Jakuba et al., 2012).

Total dissolved Zn concentrations in the shallow waters (< 200 m) of the subarctic North Pacific (CR-27) and marginal seas (CR-30 and CR-47) are clearly higher than those of the mid-latitude station in the subtropical North Pacific (Stn. 2).

The Sea of Okhotsk and Sea of Japan are marginal seas where atmospheric and river inputs can have a large influence on surface Zn distributions when compared with the open ocean. For example, in the Sea of Okhotsk (CR-30), the total dissolved Zn concentration at the surface exceeds 2 nmol/L, which is higher than that of the subarctic North Pacific.

In the Sea of Okhotsk, inputs such as atmospheric dust (Jickells et al., 2005; Tanaka and Chiba, 2006) and fluvial discharge (Shulkin and Bogdanova, 2003) have been reported. Furthermore, Sea of Okhotsk is known as an area of strong vertical mixing (Talley, 1991). High input of Zn and enhanced vertical mixing could be the reason for high concentrations of total dissolved Zn in the Sea of Okhotsk not only in the near surface, but also in deep water.

Total dissolved Zn in the Sea of Japan (CR-47) obtained from depths of 50 to 600 m were relatively high compared with those measured in the Sea of Okhotsk, although Zn concentration in the uppermost water were lower (0.9 nmol/L). As mentioned, the Sea of Japan is known as a semi-closed marginal sea with different water masses than those of the Sea of Okhotsk (Fig. 4-2). In the Sea of Japan (CR-47), relatively high concentrations of dissolved oxygen and low concentrations of Si have been observed in the deep water, in comparison to the other stations (Fig. 4-3). Previous work has also reported the relatively high dissolved oxygen and low Si concentrations and suggested the injection of oxygen-rich and nutrient-poor surface waters directly to the bottom (Kim et al., 2002). Furthermore, water inflow via the Tsushima Current from the East China Sea through the Tsushima/Korea Strait could be a source of Zn in the Sea of Japan (Guo et al., 2006). The external inputs and the subsequent internal cycles in the water column could affect to the vertical distribution of Zn in the Sea of Japan.

4-4-2 Relationship between total dissolved Zn and silicate

In the eastern North Pacific, a strong correlation between Zn and Si has been reported ($[C_{Zn}](\text{nmol/L}) = 0.054 [\text{Si}](\mu\text{mol/L}) + 0.15$), suggesting that Zn is taken up and regenerated similarly to $\text{Si}(\text{OH})_4$ (Bruland, 1980; Bruland et al., 1978). Therefore, I compared the concentrations of total dissolved Zn and Si obtained in this study (Fig. 4-5).

From separate linear regression analyses of data from these different regions, total dissolved Zn concentrations at western subtropical North Pacific (Stn. 2) were strongly correlated with those of silicate ($R^2 = 0.993$). The Zn:Si slope value was calculated as 0.059, in good agreement with previous studies in the eastern tropical North Pacific (0.054) (Fig. 4-5a) (Bruland, 1980; Bruland et al., 1978).

In the subarctic North Pacific (CR-27), the slope value was relatively high (0.065) and weakly correlated with Si ($R^2 = 0.847$) when compared with that of subtropical North Pacific (Fig. 4-5b). However, this trend was in good agreement with previous research in the subarctic North Pacific (Zn:Si slope = 0.067) (Martin et al., 1989). The data obtained during this study indicate that Zn is slightly enriched in intermediate waters compared with the linear relationship. Previous studies also showed this trend in the subarctic North Pacific (Jakuba et al., 2012; Martin et al., 1989). This trend could be derived from the additional input of dissolved Zn from sources, e.g. atmospheric dust (Duce and Tindale, 1991; Jickells et al., 2005; Meskhidze et al., 2003; Tanaka and Chiba, 2006). Therefore, an additional dust input in the near surface and remineralization of biogenic particles cause relatively high concentrations of total dissolved Zn compared with those of Si at intermediate depths.

The Zn:Si slopes in the Sea of Okhotsk (CR-30) have relatively similar values (0.070, $R^2 = 0.929$) to those of the subarctic North Pacific (Fig 4-5c). The communication of deep water through Kuril Straits (e.g., Bussol' Strait, sill depth 2318 m) from the subarctic North Pacific might be a reason why the slope value is similar to that of the subarctic North Pacific (CR-27). Conversely, in the Sea of Japan (CR-47), a lower slope value was calculated (0.034) and poor correlation was found ($R^2 = 0.298$, Fig 4-5c) because Zn was elevated above 600 m compared with the Sea of Okhotsk. The Sea of Japan is characterized by high dissolved oxygen and low Si concentrations (Fig. 4-3), which suggested the injection of oxygen-rich and nutrient-poor surface waters directly to the bottom (Kim et al., 2002). These low Si and high input of Zn into the Sea of Japan might result in a poor correlation between Zn and Si.

4-4-3 Zn complexation in the western North Pacific and its marginal seas

The $\log K'_{\text{ZnL,Zn}^{2+}}$ of natural organic ligands in the shallow waters of the subtropical and subarctic North Pacific were observed to range from 9.5 to 10.8 and from 9.7 to 10.2, respectively. When I compare these $\log K'_{\text{ZnL,Zn}^{2+}}$ values to those of previous studies in which the Zn ligand was determined using CSV, the $\log K'_{\text{ZnL,Zn}^{2+}}$ values are in good agreement with previous studies in the eastern North Pacific (10.1–10.5) (Donat and Bruland, 1990; Lohan et al., 2005) and South Pacific (10.6) (Ellwood, 2004), respectively (Table 4-2). In the marginal seas, relatively low $\log K'_{\text{ZnL,Zn}^{2+}}$ values were obtained in the Sea of Okhotsk (9.5) and Sea of Japan (9.2) for each 20 m depth in this study. Even though previous studies in which the Zn ligand was determined used ASV, the $K'_{\text{ZnL,Zn}^{2+}}$ value in the Sea of Okhotsk is in good agreement with previous result from the Bering Sea (9.6) (Jakuba et al., 2012),

but slightly lower than those of the Black Sea (9.7–10.7) (Muller et al., 2001). There have been no other reports regarding Zn speciation in the marginal seas, but further study is required to evaluate whether this trend is ubiquitous in the marginal seas.

In this study, a clear positive correlation can be seen relating total ligand concentrations to total dissolved Zn, as reported in the previous study from the subarctic North Pacific (Jakuba et al., 2012) (Fig. 4-6). Total ligand concentrations in the North Pacific ranged from 0.2 to 1.2 nmol/L, which is slightly lower than in previous studies in the open ocean (Table 4-2). In the subtropical North Pacific, total Zn concentrations were low enough to detect total ligand concentrations. However, in contrast, total ligand concentrations were undetectable in the subarctic North Pacific where total Zn concentrations were relatively high. At the same location, I could detect total ligand concentrations for Zn in the shallow layer (< 27 m) of BD-7 where total Zn concentrations were 0.25–0.36 nmol/L, but could not detect ligands at a depth of 20 m in CR-27 (total Zn concentration was 1.86 nmol/L). Previous studies have also described that Zn complexed ligands were saturated in high-latitude regions because of high total Zn concentrations (Baars and Croot, 2011; Bruland, 1989).

In the marginal seas, total ligand concentrations were found to increase in the Sea of Okhotsk (2.6 nmol/L) and Sea of Japan (1.3 nmol/L) for each 20 m depth. A previous study showed much higher total ligand concentrations (7.8–16.6 nmol/L, 10 m) in the Black Sea (Muller et al., 2001). In the Bering Sea, Jakuba et al. (2012) showed relatively high total ligand concentrations (3.6 nmol/L, 20 m) compared with those in this study. Although there are few studies of Zn speciation at present, total ligand concentrations near surface in the marginal seas might be higher than those in the open ocean.

To understand the Zn speciation in Zn-saturated waters, I also examined the vertical profiles of labile Zn (Zn_{labile}) (Fig. 4-3). In CR-27, the fractions of labile Zn (Zn_{labile}) to total dissolved Zn at intermediate depths (992–1487 m) ranged between 53 and 55%, whereas in each deep water sample (3941 and 4915 m), the fraction was 42 % (Fig. 4-3). The variable fractions of Zn_{labile} observed between intermediate depth and deep waters seem to have been affected by different water masses: the intermediate water and NPDW. In the marginal seas, the fractions of Zn_{labile} in the Sea of Okhotsk (CR-30) and Sea of Japan (CR-47) ranged from 34 to 38% (> 1479 m) and 32 to 56% (> 988 m), respectively. Since I determined labile Zn in the samples without acidification, some of the Zn in seawater may have been adsorbed onto the walls of the storage bottles and voltammetric cells. To calculate sensitivity during the titration, I also determined labile Zn in UV-irradiated seawaters without acidification (C_{UVSW}). The fraction of C_{UVSW} to total dissolved Zn (C_{Zn}) ranged from 85 to 94%, indicating that the differences between C_{Zn} and C_{UVSW} were partially caused by adsorption to the walls of bottles and cells. Even if the adsorption is taken into account, non-labile fractions of Zn still exist through the water columns of the western subarctic Pacific, the Sea of Okhotsk and the Sea of Japan. The non-labile fractions of Zn probably include inert Zn-organic complexes at specific conditions, as suggested in the previous study (Baars and Croot, 2011). It is assumable that large non-labile fractions were present in the Sea of Okhotsk (CR-30), where a relatively low fraction of Zn_{labile} existed (34–38%, > 1479 m), in comparison to not only those of the CR-27 (42–55%, > 992 m), but also those of the Sea of Japan (CR-47) (32–56%, > 988 m) (Fig. 4-3). These large non-labile fractions of Zn might indicate the presence of high Zn complexing ligands in the deep waters of the Sea of Okhotsk

(CR-30), where the highest total ligand concentration (2.6 nmol/L) was obtained in the near surface (20 m).

4-4-4 Probable sources of Zn complexing ligands

The origin of Zn complexing ligands is still under debate, although humic substances (Campbell et al., 2002), phytoplankton and bacteria-excreted organic substances (Bruland, 1989) and substances from pore waters via estuarine marine sediments (Skrabal et al., 2006) are thought to act as the ligands. Fig. 4-7 shows the relationship between Chl *a* and the total ligand concentrations (C_L) obtained in this study. Those relationships have been divided into three groups: (1) relatively low values of both Chl *a* and C_L in the western subtropical North Pacific (Stn. 2), (2) relatively high values of Chl *a* and C_L in the western subarctic North Pacific (BD-7 and BD-11), and (3) low Chl *a* and high C_L that can be observed in the marginal seas (CR-30 and CR-47) (Fig. 4-7).

In the western North Pacific (Stn. 2, BD-7 and BD-11), total ligand concentrations are positively correlated with Chl *a* concentrations ($R^2 = 0.814$). An earlier study by Bruland (1989) suggested that Zn complexing ligands might be derived from bacteria and phytoplankton in the central subtropical North Pacific. In high productivity regions, organic substances excreted by abundant phytoplankton and bacteria are possibly the main source of Zn ligands in the surface water of the open ocean.

In the marginal sea regions (Sea of Okhotsk (CR-30) and Sea of Japan (CR-47)), relatively high concentrations of the ligands exist in low-Chl *a* waters (Fig. 4-7). Previous study in the Bering Sea indicated a high total ligand concentration (3.6

nmol/L) with high Chl *a* (0.86 µg/L) content and postulated that Zn complexed ligands are derived from phytoplankton (Jakuba et al., 2012). The high total ligand concentrations in the Sea of Okhotsk and Sea of Japan accompanied by low-Chl *a* contents might suggest different sources of Zn complexing ligands from those in the Bering Sea.

The Sea of Okhotsk is affected by discharge from the Amur River, where large volumes of humic substances are carried (Nakatsuka et al., 2004). Furthermore, the lower surface salinity in the Sea of Okhotsk found in this study (32.7, 20 m) indicates that surface water is affected by fresh water discharge. Therefore, humic substances from fresh water might be a source of Zn complexed ligands in the Sea of Okhotsk.

In the Sea of Japan (CR-47), Zn complexing ligands are also high compared with those of the open ocean. The Tsushima Warm Current from the East China Sea has a large impact on surface circulation in the Sea of Japan (Guo et al., 2006), which could affect the presence of Zn complexing ligands in the Sea of Japan. Since I obtained few data from the marginal seas, I will need more detailed data to identify the source of the ligands.

4-5 Conclusions

In this chapter, I reported the first obtained data of the whole vertical profiles for total dissolved Zn and its speciation in the western North Pacific and its adjacent seas. In high-latitude areas such as the subarctic North Pacific, the Sea of Okhotsk and the Sea of Japan, Zn complexing ligands in most samples were saturated with zinc because of the high dissolved Zn concentration. Relatively high total ligand

concentrations and low $K'_{ZnL,Zn^{2+}}$ have been obtained in the surface waters of marginal seas, relative to those from the open ocean. In high latitude areas such as the subarctic North Pacific, the Sea of Okhotsk and the Sea of Japan, I observed relatively high concentrations of total dissolved Zn and ligands compared with those seen in the subtropical North Pacific. Furthermore, there is a relatively small fraction of Zn_{labile} in the deep waters of the Sea of Okhotsk, compared not only with those of the subarctic North Pacific, but also with those of the Sea of Japan, which might indicate the presence of high Zn complexing ligands in deep waters of the Sea of Okhotsk. Although few data were obtained, the source of ligands would be different between the open ocean and marginal seas. They suggest that Zn complexing ligands in the North Pacific might be derived from bacteria and phytoplankton, whereas there are different sources in the Sea of Okhotsk and Sea of Japan.

Table 4-1. Dissolved Zn and its speciation data.

Region	Station	Depth (m)	[C _{Zn}] (nmol/L)	[C _L] (nmol/L)	Log K' _{ZnL,Zn²⁺}	[Zn ²⁺] (nmol/L)
Subtropical North Pacific	Stn. 2 (23°00'N, 159°59'E)	6	0.2	0.2	9.7	0.056
		19	0.3	0.3	9.1	0.020
		51	0.4	0.4	10.8	0.008
		99	0.5	0.5	10.6	0.011
		149	0.3	0.3	10.4	0.004
		198	0.3	0.3	9.8	0.069
Subarctic North Pacific	BD-7 (47°00'N, 160°05'E)	10	1.1	1.1	10.2	0.017
		25	1.1	1.1	10.2	0.024
		27	1.2	1.2	9.9	0.037
	BD-11 (47°00'N, 179°59'W)	10	1.2	1.2	9.6	0.067
		25	0.9	0.9	9.8	0.048
		37	0.7	0.7	9.9	0.106
	CR-27 (47°02'N, 160°00'E)	21	-	-	-	-
Sea of Okhotsk	CR-30 (45°15'N, 145°04'E)	20	2.6	2.6	9.5	0.234
Sea of Japan	CR-47 (42°43'N, 138°13'E)	20	1.3	1.3	9.1	0.257

Table 4-2. Zn complexation data from other relevant seawater studies. The values of $\log K'_{\text{ZnL,Zn}^{2+}}$ for ASV method are calculated by the equation ($K'_{\text{ZnL,Zn}^{2+}} = K'_{\text{ZnL,Zn}'} \times \alpha_{\text{Zn}'}).$

Region	Sample	[C _L] (nmol/L)	Log $K'_{\text{ZnL,Zn}^{2+}}$	Method	Reference
Open Ocean					
Central subtropical North Pacific	Profile	1.0-1.5	10.7-11.3	ASV	(Bruland, 1989)
Eastern North Pacific	60 and 150 m	1.7-2.3	11.0-11.5	ASV	(Donat and Bruland, 1990)
	60 and 150 m	1.6-2.3	10.1-10.5	CSV	(Donat and Bruland, 1990)
Eastern subarctic North Pacific	15 m	0.7	10.5	CSV	(Lohan et al., 2005)
	15-150 m	1.1-2.6	10.3-11.3	ASV	(Jakuba et al., 2012)
Western subarctic North Pacific	25-40 m	2.3-2.7	10.1-10.8	ASV	(Jakuba et al., 2012)
South Pacific	20 m	1.3	10.2	ASV	(Ellwood, 2004)
	20 m	1.2	10.6	CSV	(Ellwood, 2004)
Northeastern Atlantic	Surface	0.4-2.5	10.0-10.5	CSV	(Ellwood and Van den Berg, 2000)
Central North Atlantic	Profile	0.9-1.5	9.8-10.5	ASV	(Jakuba et al., 2008)
	Profile	0.6-1.3	9.8-11.3	CSV	(Jakuba et al., 2008)
Southern Ocean	10-200 m	1.4-5.3	9.1-10.4	ASV	(Baars and Croot, 2011)
Western subtropical North Pacific	6-198 m	0.2-0.5	9.5-10.8	CSV	This study
Western subarctic North Pacific	10-37 m	0.7-1.2	9.7-10.2	CSV	This study
Marginal Sea					
Sea of Okhotsk	20 m	2.6	9.5	CSV	This study
Sea of Japan	20 m	1.3	9.2	CSV	This study
Bering Sea	20 m	3.6	9.6	ASV	(Jakuba et al., 2012)
Black Sea	10 m	7.8-16.6	9.7-10.7	ASV	(Muller et al., 2001)

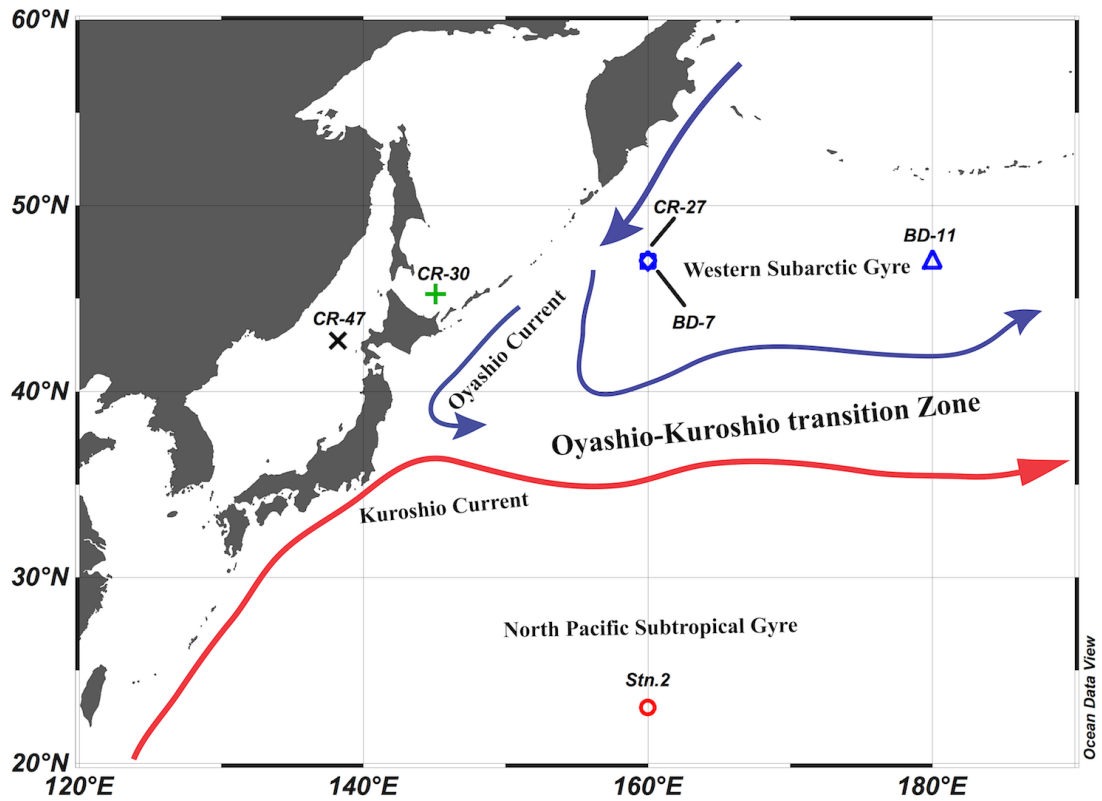


Fig. 4-1. Location of sampling stations in the subtropical North Pacific (Stn. 2, red), subarctic North Pacific (CR-27, BD-7 and BD-11, blue), Sea of Okhotsk (CR-30, green) and Sea of Japan (CR-47, black). Schematic illustration of the near-surface current is described based on Yasuda (2003).

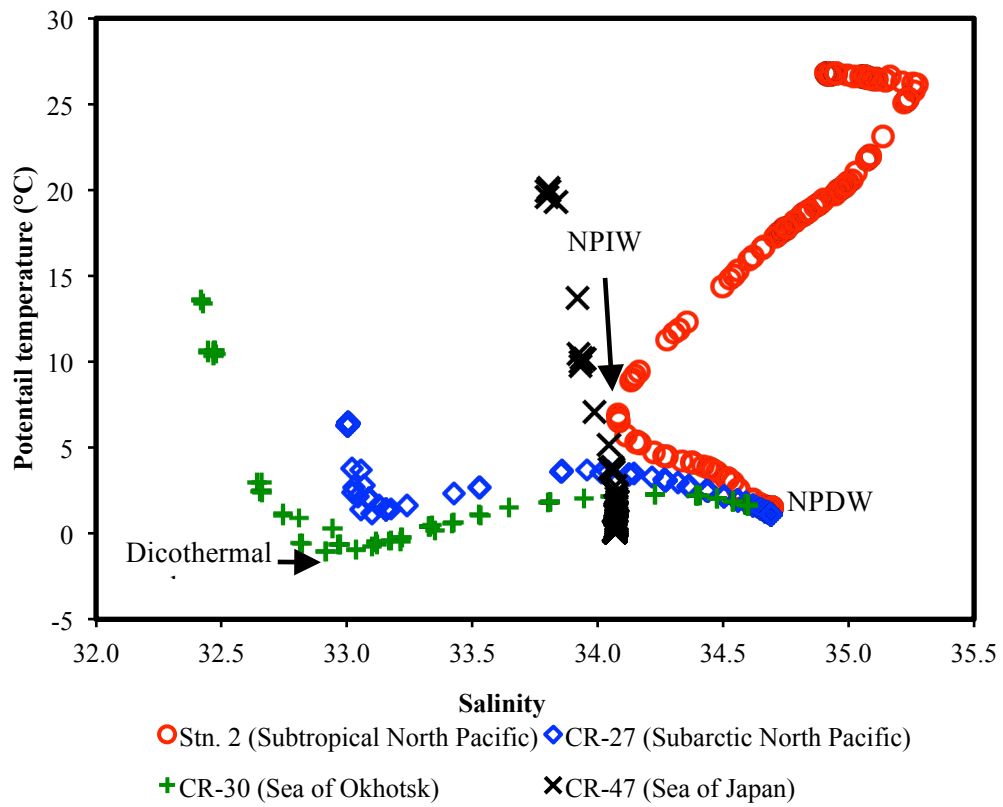


Fig. 4-2. Potential temperature versus salinity diagram in the subtropical North Pacific (red), subarctic North Pacific (blue), Sea of Okhotsk (green) and Sea of Japan (black), respectively.

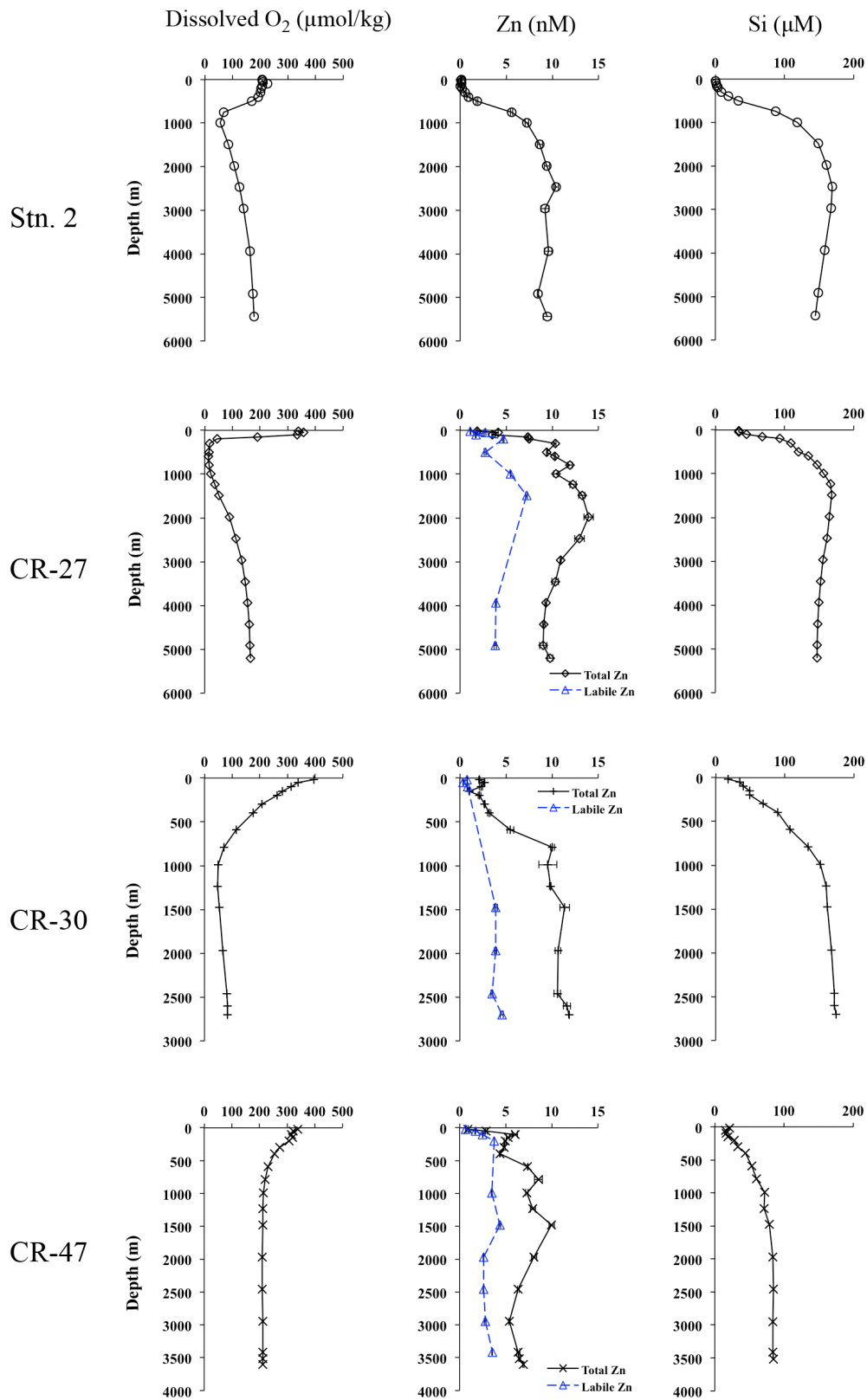


Fig. 4-3. Vertical distributions of dissolved oxygen, Zn (total Zn and labile Zn) and silicate in the subtropical North Pacific (Stn. 2), subarctic North Pacific (CR-27) and marginal seas such as the Sea of Okhotsk (CR-30) and the Sea of Japan (CR-47). See Fig. 4-4 for Zn speciation in Stn. 2.

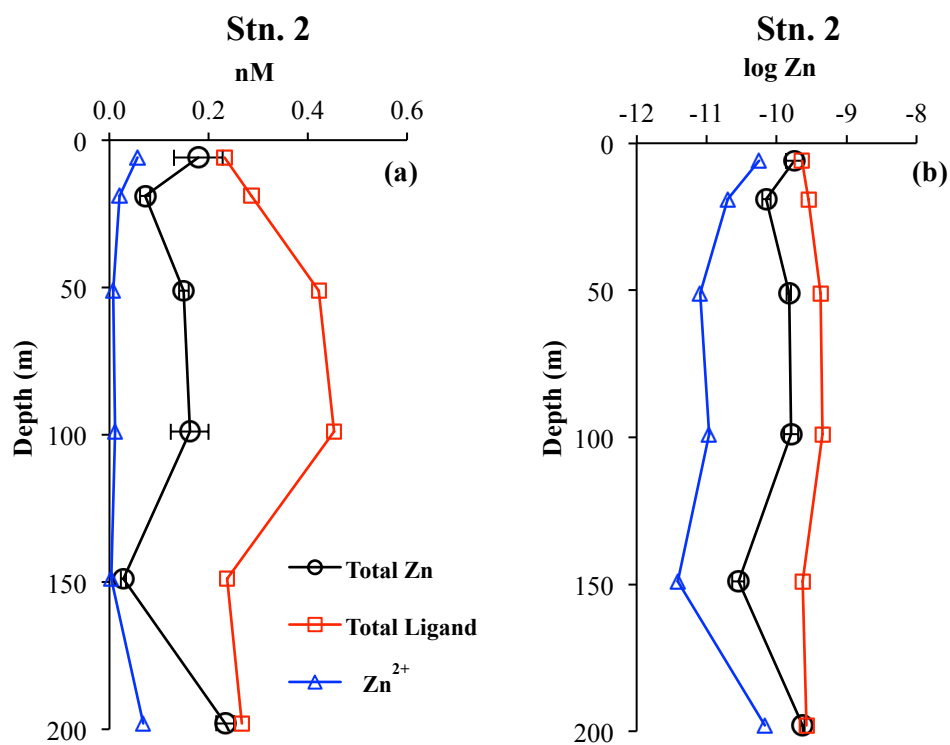


Fig. 4-4. Zn speciation in the subtropical North Pacific (Stn. 2). (a) Vertical distribution of total dissolved Zn (black), total ligand (red) and Zn^{2+} (blue). (b) Vertical distribution of Zn speciation converted to log scales.

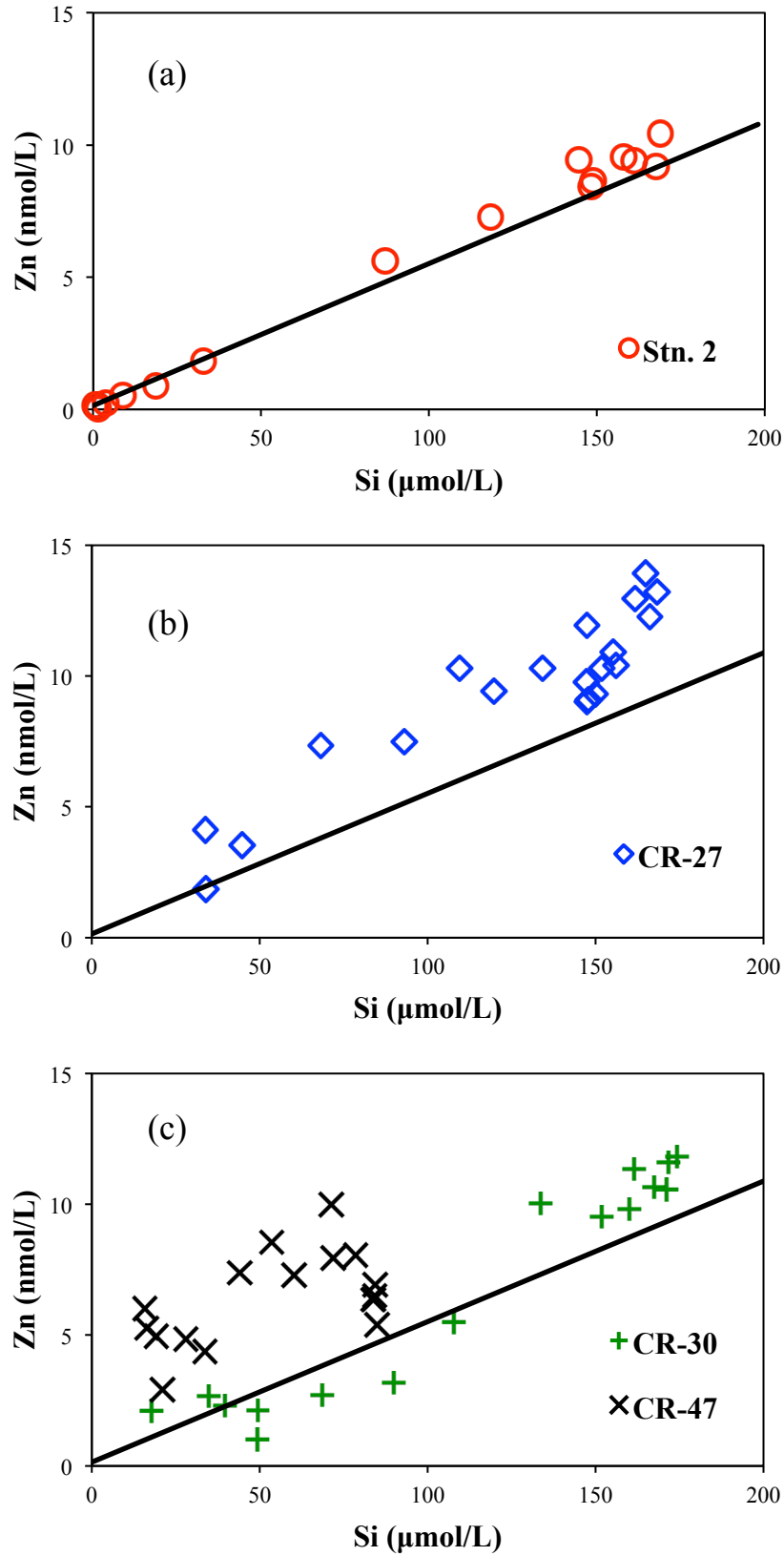


Fig. 4-5. Relationship between total dissolved Zn and Si in seawater obtained in this study. The black line indicates the relationship ($[C_{Zn}](\text{nmol/L}) = 0.054 [Si](\mu\text{mol/L}) + 0.15$) previously obtained in the eastern North Pacific (Bruland, 1980; Bruland et al., 1978).

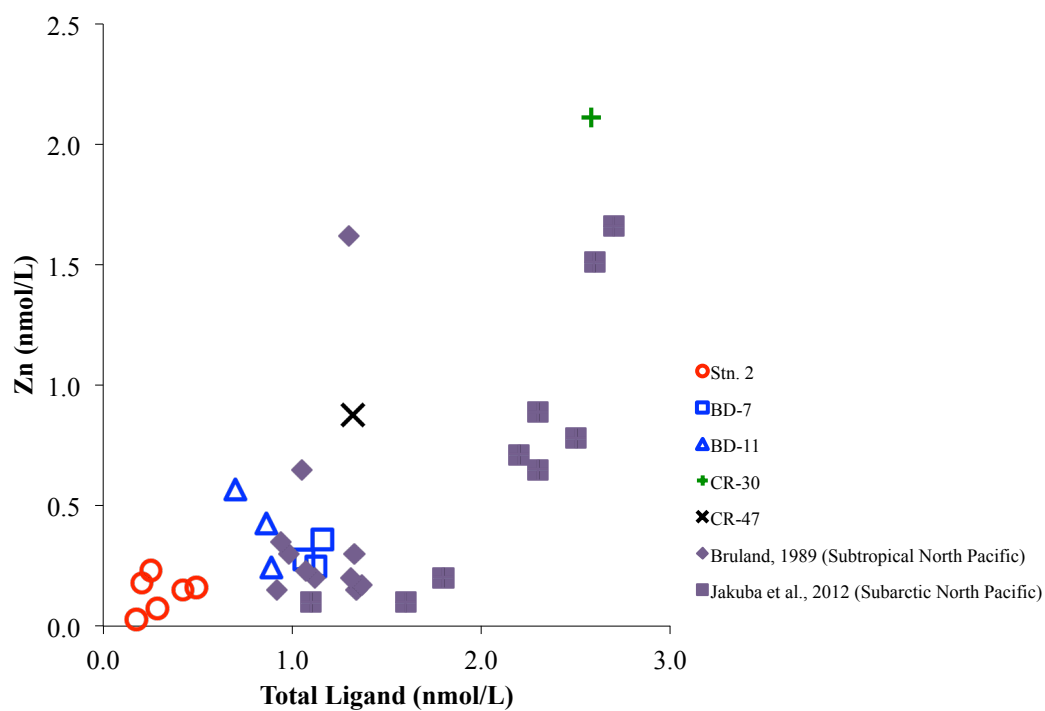


Fig. 4-6. Comparison of total ligand concentrations and total dissolved Zn concentrations. Filled diamonds (Bruland, 1989) and filled squares (Jakuba et al., 2012) indicate previous studies in the central subtropical North Pacific and subarctic North Pacific, respectively.

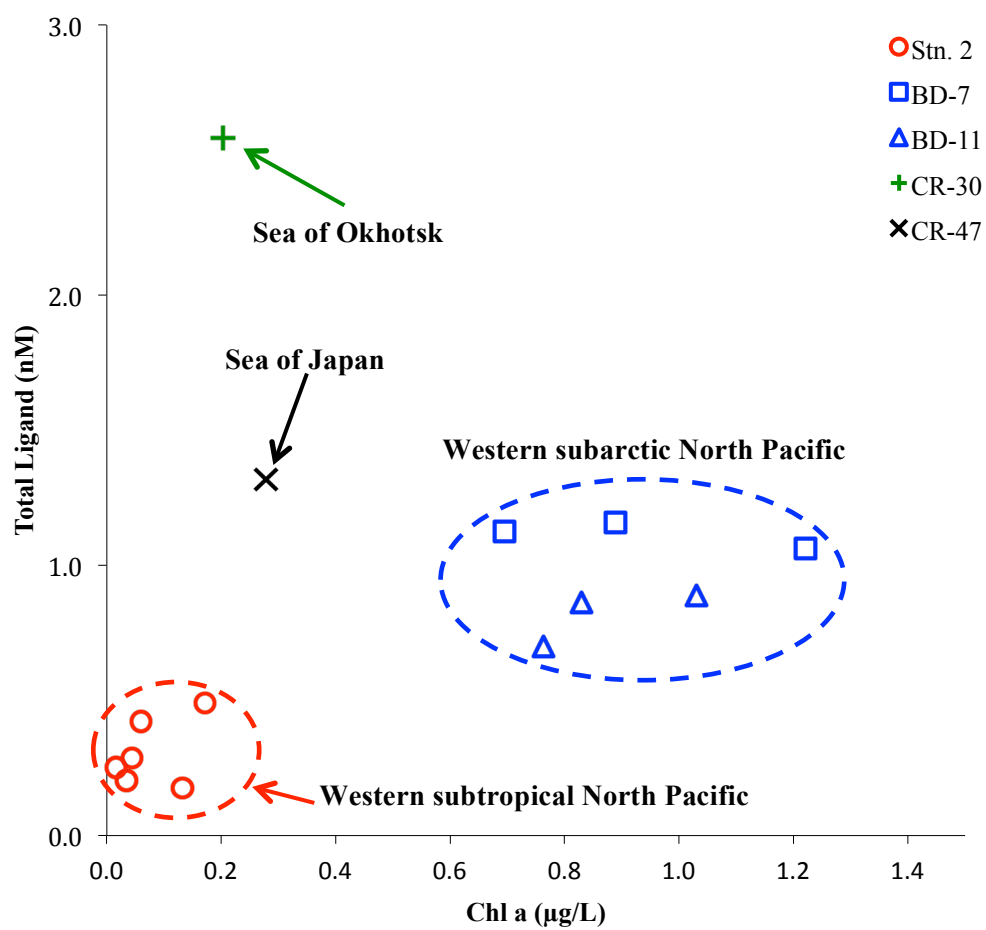


Fig. 4-7. Comparison of Chl *a* and total ligand concentrations.

Chapter 5.

Dissolved zinc and its speciation in the northeastern Indian Ocean and Andaman Sea

5-1 Introduction

The northeastern Indian Ocean, including the Bay of Bengal and the Andaman Sea (Fig. 5-1), is characterized by the surface current systems with strong monsoon seasonality (Shankar et al., 2002), and the biogeochemical cycles in the basins are significantly influenced by fluvial dissolved and suspended sediment loads (Milliman and Meade, 1983; Robinson et al., 2007). The Ganges and Brahmaputra rivers carry a large amount of terrestrial debris ($\sim 2 \times 10^{12}$ kg / yr) into the northern part of the Bay of Bengal, which corresponds to ~ 10 % of the world's sediment discharge (Milliman and Meade, 1983). In the Bay of Bengal, this high sediment discharge built a broad submarine alluvial Bengal Fan and affected the entire sea bottom topography.

The Andaman Sea is a marginal sea of the Indian Ocean that extends between the Malayan Peninsula on the east and the Andaman and Nicobar islands on the west. The Andaman Sea forms the far eastern part of the northern Indian Ocean, separated from the western Bay of Bengal by the Andaman–Nicobar island chain. In the northern region, the submarine delta has been built by the outflow of the Irrawaddy and Salween rivers, which is connected to the eastern shallow shelves along the Malay Peninsula and the Malacca Strait (Robinson et al., 2007). From the shelves, the

sea bottom drops off sharply into a large central and two smaller basins deeper than 2000 m extending along the north-south island arc. The maximum depth is 4180 m located at the south end of the central basin. Because the sill depths of channels across the Andaman-Nicobar Ridge are shallower than 1800 m, the deep water of the Andaman Sea Basin is isolated from the Bay of Bengal and its water temperature difference between two was about 2 °C, the deep waters of the Andaman Sea were consistently warmer than those of the Bay of Bengal. (Sarma and Narvekar, 2001). Previous studies reported the vertical profiles of dissolved rare earth elements (REEs) (Nozaki and Alibo, 2003), ^{230}Th (Okubo et al., 2004) and Al, In and Ce (Obata et al., 2004). Total dissolved Zn and its speciation in the Andaman Sea, however, have not been reported yet.

In this chapter, I describe the distributions of total dissolved Zn concentrations and Zn speciation in the northeastern Indian Ocean and Andaman Sea to characterize organic Zn complexing ligands and their influence on the chemical speciation of Zn.

5-2 Sample collection and storage

Seawater samples were collected in the northeastern Indian Ocean (station NR-1) and Andaman Sea (stations MY-1 to MY-6) during R/V Hakuho-maru KH-13-4 research cruise (in July and August 2013). Locations of the sampling stations are indicated in Fig. 5-1.

Sampling methods were the same as those described in the previous chapters. Seawater samples were collected using acid-cleaned Teflon-coated X-type Niskin samplers. The acid-cleaned Teflon-coated X-type Niskin bottles were then fixed on a conductivity-temperature-depth carousel multi-sampling system (CTD-CMS, SBE-

911plus and SBE-32 water sampler, Sea Bird Electronics, Inc.). Detailed sampling methods for total dissolved Zn and Zn speciation are described in chapter 3 and chapter 4.

5-3 Results

5-3-1 Hydrography

Table 5-1 shows the data obtained in this study. The surface salinities (< 50 m) in the Andaman Sea (28.769 – 33.864) were relatively lower than those of the northeastern Indian Ocean (NR-1, 34.226 – 34.228). Additionally, surface salinities in the Andaman Sea were gradually increased from north to south (Table 5-1).

Fig. 5-2 shows the vertical profiles of salinity, potential temperature and dissolved oxygen in both northeastern Indian Ocean (NR-1) and Andaman Sea (MY-1 and MY-3). The deep water of the northeastern Indian Ocean is most simply characterized as a tongue of circumpolar water extending north from the south (Wyrski, 1971). In the Southern Ocean, the original circumpolar deep water has relatively high in salinity and oxygen due to the presence of North Atlantic Deep Water. Although dissolved oxygen is consumed by respiration during the northward transport of the deep water, the salinity in the northeastern Indian Ocean (NR-1) and Andaman Sea (MY-1 and MY-3) remains high as indicated by the salinity maximum (> 35.0) at 275 – 596 m (Fig. 5-2).

The potential temperature in both northeastern Indian Ocean and Andaman Sea are comparable to a depth of 1000 m (Fig. 5-2), but deep waters below 1500 m of the Andaman Sea are consistently warmer than those of the northeastern Indian Ocean.

Below 1500 m, potential temperatures in the Andaman Sea are relatively uniform (4.99 – 5.32 °C), where those of northeastern Indian Ocean were decreased from 4.55°C to 1.80°C (Fig. 5-2).

Dissolved oxygen decreases sharply from the surface to the minimum of < 20 µmol/L between 298 – 396 m for NR-1, 198 – 249 m for MY-1 and 239 m for MY-3, respectively. In deep layers, dissolved oxygen concentrations increase toward the bottom. The high dissolved oxygen concentrations near the bottom at station NR-1 suggests that the bottom and the deep waters are fed from the south. The sharp decrease of dissolved oxygen from the surface with increasing depth is ascribable to high productivity, and the shallow depths of the oxygen minimum and the salinity maximum are indicative of the upwelling regime of the northeastern Indian Ocean, consistent with the general circulation pattern (Schmitz, 1995). The profiles of the northeastern Indian Ocean and the Andaman Sea for dissolved oxygen diverge below 1000 m, consistent with those of temperature. The deep water of Andaman Sea has uniform concentrations for dissolved oxygen, while it increases with depth in northeastern Indian Ocean (Fig. 5-2).

The deep water of the Andaman Sea is isolated from the Bay of Bengal by the Andaman-Nicobar Ridge. Since the Preparis Channel is shallower than 250 m, exchange of deep water can occur mainly through the Ten Degree Channel with a maximum sill depth of approximately 800 m and the Great Passage with a maximum depth of approximately 1800 m (Nozaki and Alibo, 2003).

5-3-2 Total dissolved Zn and silicate concentrations

The first Zn profiles in the Andaman Sea are shown in Fig. 5-3. In this study, total dissolved Zn profiles are nutrient type, which is consistent with previous studies in the North Pacific (Bruland, 1980; Bruland et al., 1994; 1979; 1978; Cutter and Bruland, 2012; Lohan et al., 2002), South Atlantic (Wyatt et al., 2014), southern Indian Ocean (Gosnell et al., 2012), and Southern Ocean (Croot et al., 2011). Si concentrations were strongly correlated with total dissolved Zn (Fig. 5-3). Vertical profiles in the northeastern Indian Ocean (NR-1) and Andaman Sea (MY-1 and MY-3) also showed similar features that both total dissolved Zn and Si diverge below 1000 m.

In the Andaman Sea, total dissolved Zn at near surface (< 50 m) was ranged from 0.33 to 1.14 nmol/L at the southernmost station (MY-1), which was an order of magnitude higher than those of the northernmost station (MY-6, 0.03 – 0.22 nmol/L) (Table 5-1, Fig. 5-4). On the other hand, Si concentration in the near surface, the highest Si concentration has obtained in the northernmost station of the Andaman Sea (MY-6, 16.3 $\mu\text{mol/L}$), where is close from the estuaries of Irrawaddy-Salween rivers.

5-3-3 Zn speciation

In this study, total ligand concentrations (C_L) and $\log K'_{\text{ZnL,Zn}^{2+}}$ were obtained at 17 m in the northeastern Indian Ocean and between 5 and 74 m in the Andaman Sea (Table 5-1). In the northeastern Indian Ocean (NR-1), the Zn titration for most of the samples showed a linear increase with added Zn^{2+} , indicating that all of the natural ligands in the sample were already saturated with Zn. Thus, only one estimate of $\log K'_{\text{ZnL,Zn}^{2+}}$ could be obtained from the titration data for NR-1.

The total ligand concentration in the northeastern Indian Ocean (NR-1, 0.5 nmol/L) was relatively low compared with those of the Andaman Sea (from MY-1 to MY-6), which ranged from 0.4 to 0.9 nmol/L. Log $K'_{ZnL,Zn^{2+}}$ value in the northeastern Indian Ocean was 10.0, which is relatively low compared with those of the Andaman Sea, which ranged from 9.6 to 11.4 (Table 5-1). In the Andaman Sea, the highest C_L was 0.9 nmol/L, which observed at both MY-1 and MY-6 (Table 5-1). The resultant free Zn^{2+} concentrations in the northeastern Indian Ocean and Andaman Sea were 0.047 nmol/L and 0.002 – 0.066 nmol/L, respectively (Table 5-1).

5-4. Discussion

5-4-1 Total dissolved Zinc

In the northeastern Indian Ocean (NR-1), the average of the total dissolved Zn within the upper 200m was 0.94 ± 0.37 nmol/L ($n=6$), which is slightly lower than the previous study in the northwestern Indian Ocean (~ 5.00 nmol/L) (Saager et al., 1992). From the surface to 1000 m depth, vertical distributions of total dissolved Zn in the northeastern Indian Ocean (NR-1) and Andaman Sea (MY-1 and MY-3) show almost identical concentrations. Below 1000 m depth, total dissolved Zn in the northeastern Indian Ocean increased almost twice, whereas the concentrations remain nearly constant with depth in the Andaman Sea (Fig. 5-3). Nozaki and Alibo (2003) mentioned that the deep waters of the northeastern Indian Ocean and Andaman Sea could communicate through both the Ten Degree Channel (< 800 m sill depth) and the Great Passage (< 1800 m sill depth). Okubo et al. (2004) estimated the residence time in deep water (< 1250 m) to be 6 years using the scavenging-mixing model with

vertical distribution of ^{230}Th , suggesting that the deep water appears to be rapidly replaced by the incoming waters from the northeastern Indian Ocean across the sills and then homogenized by vertical mixing.

The distributions of total dissolved Zn from surface to 200 m in the Andaman Sea are shown in Fig. 5-4. Very low total dissolved Zn ($< 0.3 \text{ nmol/L}$) at northern stations (MY-5 and MY-6) was observed $< 50 \text{ m}$ depth, where the salinities are also low (28.769 – 33.393), which indicates the riverine input (Irrawaddy-Salween rivers) affects the northern area of the Andaman Sea.

Fig. 5-5 shows the total dissolved Zn and Si concentrations as function of salinity. Both total dissolved Zn and Si showed a trend that the very low concentrations in the lower salinity (< 34.000) at northern Andaman Sea. During the sampling period, southwest monsoon from June to November, the outflows of freshwater from the Irrawaddy-Salween rivers increase more than an order of magnitude compared to northeastern monsoon in winter (Robinson et al., 2007), which is the third largest contributor of sediment load after Amazon and Ganges-Brahmaputra rivers (Robinson et al., 2007). This high level of sediment load from Irrawaddy-Salween rivers may contribute to lower total dissolved Zn concentrations.

Flocculation of humic acids may lead to removal of trace metals, however total dissolved Zn will be less affected because Zn appears to exist predominantly as inorganic forms (primarily with chloride) in river water (Hart and Davies, 1981; Kuwabara et al., 1989). Sediment resuspension also could be affect to removal of trace metals by porewater infusion. For instance, porewater infusion is a major source of Fe to the water column in the high turbidity zone. Once released into the water column, dissolved Fe is rapidly oxidized and precipitated (Zwolsman, 1994). The in situ formation of Fe (oxy) hydroxides, which are effective scavengers of dissolved Zn

(Johnson, 1986), will lead to removal of dissolved Zn in the low salinity, high turbidity area.

This study found that the low concentrations of total dissolved Zn in northern stations (MY-5 and MY-6) are also correlated with high Chl *a* contents (Fig. 5-4). In MY-6, the northernmost station, the highest Chl *a* content has obtained (2.59 µg/L, 5 m), which is much higher than not only in the northeastern Indian Ocean, but also southern station of the Andaman Sea (Table 5-1). This result may indicate removal of total dissolved Zn in the northern Andaman Sea by biological uptake. The involvement of trace metals in biological cycles is a well-established fact for the open ocean (Bruland et al., 1991), but similar processes have been reported to operate in estuaries as well (Kuwabara et al., 1989; Shiller and Boyle, 1991; Zwolsman et al., 1997).

In southern stations (MY-1, MY-2 and MY-3) of the Andaman Sea, total dissolved Zn < 50 m showed similar range (0.13 – 1.14 nmol/L) comparing with those of the northeastern Indian Ocean (0.26 – 1.03 nmol/L). During the summer, the Summer Monsoon Current flows eastward as a continuous current from the western Arabian Sea to the northeastern Indian Ocean (Shankar et al., 2002). Therefore, the Summer Monsoon Current, by intruding into the Andaman Sea, may influence surface Zn concentrations in both northeastern Indian Ocean and Andaman Sea.

5-4-2 Relationship between total dissolved Zn and silicate

This study demonstrated that the total dissolved Zn profiles are similar to that of silicate (Si) (Fig. 5-3) as was already observed in the Pacific Ocean (see chapter 3).

As similar with total dissolved Zn, Si concentrations show similar feature that diverged below 1000 m depth (Fig. 5-3).

In the Andaman Sea, Si concentrations (< 50 m) in MY-5 (4.3 – 5.8 $\mu\text{mol/L}$) and MY-6 (5.4 – 16.3 $\mu\text{mol/L}$), in contrast to total dissolved Zn, were relatively higher than those in the northeastern Indian Ocean (NR-1, 1.4 – 1.6 $\mu\text{mol/L}$) and southern stations (e.g., MY-1, 2.4 – 2.9 $\mu\text{mol/L}$) (Table 5-1). It indicates that Si is influenced by Irrawaddy-Salween rivers. Fig. 5-6 shows the relationship between total dissolved Zn and Si concentrations obtained in this study. In the Andaman Sea, the slope value was relatively lower (0.043) in comparison to that of northeastern Indian Ocean (0.052) (Fig. 5-6). This lower slope value of the Andaman Sea results from the relatively high Si concentrations and lower total dissolved Zn in the Andaman Sea, where total dissolved Zn decreased to near-zero concentrations while silicate concentrations were remain high (Fig. 5-6). As I mentioned above, high Chl *a* contents are obtained at northern areas of the Andaman Sea, suggesting that Zn might be depleted by phytoplankton uptake.

In the northeastern Indian Ocean (NR-1), total dissolved Zn were strongly correlated with those of silicate ($R^2 = 0.913$) and the Zn:Si slope value was calculated as 0.052 (Fig. 5-6). The obtained slope value in the northeastern Indian Ocean is in a good agreement with previous study in the southwestern Indian Ocean (0.049) (Morley et al., 1993), but slightly lower than the previous studies in the northwestern Indian Ocean (0.062) (Saager et al., 1992), southern Indian Ocean (0.059) (Gosnell et al., 2012) and northern and southern Indian Ocean (0.064) (Vu and Sohrin, 2013). The result of this study seems to indicate that Zn is relatively depleted comparing to Si in the northeastern Indian Ocean. Additionally, if I subtracted Zn:Si data of deep water (> 1500 m) in the northeastern Indian Ocean (NR-1), the slope value is

calculated as 0.038, which is almost identical with that of Andaman Sea (0.043). This similarity of slope values also support that both northeastern Indian Ocean and Andaman Sea could communicate at depths above 1500 m.

5-4-3 Zn complexation in the Andaman Sea

Total ligand concentrations (C_L) in the Andaman Sea ranged from 0.4 to 0.9 nmol/L, which is lower than those in the other marginal seas (Table 5-2). Previous researches showed much higher total ligand concentrations in the Black Sea (7.8–16.6 nmol/L) (Muller et al., 2001), Bering Sea (3.6 nmol/L) (Jakuba et al., 2012), Sea of Okhotsk (2.6 nmol/L) and Sea of Japan (1.3 nmol/L) (Kim et al., in press). Total ligand concentrations in northeastern Indian Ocean (NR-1) was calculated as 0.5 nmol/L and relatively lower than those of the Andaman Sea, which might suggest some specific sources of ligand in Andaman Sea. Total ligand concentrations generally correlate positively with total dissolved Zn in this study (Fig. 5-7). Contrastingly, total ligand concentrations were poorly correlated to total dissolved Zn in MY-6, northernmost station of the Andaman Sea.

The $\log K'_{ZnL,Zn^{2+}}$ of natural organic ligands in the Andaman Sea ranged from 9.6 to 11.5. This range was higher than previous studies in the marginal seas (Table 5-2). The $\log K'_{ZnL,Zn^{2+}}$ values in the northeastern Indian Ocean was 10.0, which was comparable with previous studies in the open oceans, such as western subtropical North Pacific (9.5 – 10.8) and western subarctic North Pacific (9.7 – 10.2) (Kim et al., in press), eastern subarctic North Pacific (10.5) (Lohan et al., 2005), South Pacific (10.6) (Ellwood, 2004) and North Atlantic (10.0 – 10.5) (Ellwood and Van den Berg, 2000).

5-4-4 Probable sources of Zn complexing ligands

The origin of Zn complexing ligands is still under debate, although humic substances (Campbell et al., 2002), substances from pore waters via estuarine marine sediments (Skrabal et al., 2006) and phytoplankton and bacteria-excreted organic substances (Bruland, 1989) are thought to act as the ligands (see chapter 4). In the marginal seas, Muller et al. (2001) mentioned that Zn complexing ligands in the Black Sea could be derived from the bacterial breakdown of particulate organic matter.

In the Andaman Sea (from MY-1 to MY-6), which is influenced by Irrawaddy-Salween rivers, the third largest contributor of sediment load influenced (Robinson et al., 2007), Zn complexing ligands could be derived from various sources. In the northernmost station (MY-6), total ligand concentrations are correlated with Chl *a* contents ($n=6$, $R^2 = 0.651$) (Fig. 5-8), which might support that phytoplankton and bacteria-excreted organic substances were dominant source for Zn complexing ligands in MY-6. However, clear correlation was not observed in other stations in the Andaman Sea, suggesting that different sources of Zn complexing ligands might be dominant at those stations. Based on the first total dissolved Zn and its speciation data obtained in this study for the northeastern Indian Ocean and Andaman Sea, further works are needed to clarify the sources of Zn complexing ligands in these regions.

5-5 Conclusions

I determined total dissolved Zn and its speciation in the northwestern Indian Ocean and Andaman Sea, which were the first time to be reported. These results show

that there are high influence of fluvial discharge from Irrawaddy and Salween rivers. In the northern Andaman Sea, where high Chl *a* content has obtained, total dissolved Zn were relatively lower than those of southern Andaman Sea, indicating removal of total dissolved Zn in the northern Andaman Sea by biological uptake. Vertical distributions of total dissolved Zn in the northeastern Indian Ocean (NR-1) and Andaman Sea (MY-1 and MY-3) are shown to be almost similar from the surface to 1000 m depth. Below 1000 m depth, total dissolved Zn was diverged to indicate that the deep water in the Andaman Sea appears to be rapidly replaced by the incoming waters from the northeastern Indian Ocean across the sills and then homogenized by vertical mixing.

In the northernmost station of the Andaman Sea (MY-6), total ligand concentrations are correlated with Chl *a* contents, which supports that phytoplankton and bacteria-excreted organic substances were dominant source for Zn complexing ligands. Other stations in the Andaman Sea, however, showed poor correlations suggesting that different sources of Zn complexing ligands were dominant at those stations.

Table 5-1. Seawater analysis results and associated hydrographic data obtained in this study.

Station	Depth m	Potential temperature °C	Salinity PSU	O ₂ μmol/L	Si μmol/L	C _{zn} nmol/L	Chl <i>a</i> μg/L	C _L nmol/L	K' _{ZnL,Zn²⁺}	Zn ²⁺ nmol/L
NR-1 (6°29'N,89°57'E)	5	28.18	34.226	205.43	1.5	0.75	0.58			
	10	28.18	34.226	168.20	1.4	1.03	0.61			
	17	28.19	34.226	196.33	1.4	0.26	0.61	0.5	10.0	0.047
	19	28.19	34.226	204.47	1.6	0.94	0.67			
	49	28.19	34.228	175.85	1.5	0.92	0.58			
	100	25.22	34.414	110.11	10.0	0.74	0.15			
	199	14.87	34.913	29.63	29.8	2.00	0.01			
	274	12.41	35.043	22.42	31.6	3.14	0.01			
	298	11.65	35.055	16.76	33.9	2.14	0.01			
	396	10.71	35.049	18.93	37.8	2.36	0.01			
	596	9.07	35.008	27.41	57.5	2.54	0.01			
	794	7.85	34.969	40.79	73.4	4.14				
	993	6.72	34.933	52.76	83.6	3.54				
	1486	4.55	34.852		107.3	4.83				
	2176	2.30	34.757	137.86	130.0	8.61				
	2842	1.80	34.736	158.17	135.2	8.43				
MY-1 (10°00'N,96°00'E)	5	27.98	32.185	210.98	2.5	0.69	0.37	0.9	10.4	0.063
	20	28.00	32.201	205.22	2.4	1.14	0.39			
	50	28.28	32.664	191.27	2.9	0.33	0.59			
	53	28.35	32.818	191.12	2.9	0.35	0.54	0.7	10.9	0.011
	100	23.80	34.293	81.04	13.9	0.54	0.11	0.8	11.4	0.008
	198	12.94	34.964	16.05	35.7	1.98	0.01			
	249	11.87	35.010	19.42	35.5	1.41	0.01			
	397	10.59	35.025	25.87	43.9	2.06	0.01			
	595	9.17	35.002	34.58	56.9	2.77	0.01			
	793	7.77	34.960	46.42	72.6	3.84	0.01			
	992	6.86	34.928	56.37	74.5	3.67	0.01			
	1486	5.32	34.876	75.24	101.8	4.28				
	1907	5.00	34.864	82.95	101.4	4.48				
MY-2 (11°00'N,96°00'E)	5	28.10	32.411	213.57	2.6	0.13	0.26	0.5	10.7	0.007
MY-3 (12°00'N,96°00'E)	5	28.09	31.777	205.70	4.1	0.22	0.23	0.4	10.3	0.039
	20	28.11	31.865	212.00	4.2	0.52	0.29			
	35	28.03	33.292	164.59	5.9	0.31	0.82	0.8	9.8	0.061
	50	26.48	33.864	124.03	8.9	0.57	0.46			
	100	22.88	34.360	50.66	17.9	1.45	0.04			
	199	13.79	34.947	31.59	32.9	1.71	0.01			
	239	12.45	34.989	16.40		1.77	0.01			
	398	10.53	35.026	25.06	44.2	2.33	0.01			
	596	9.12	34.998	37.34	54.9	2.64	0.01			
	794	7.62	34.954	47.35	76.8	2.79	0.01			
	992	6.64	34.921	58.53	88.2	3.72	0.01			
	1486	5.25	34.872	71.78	104.2	4.31				
	1978	5.01	34.862	83.86	104.8	4.31				
	2367	4.99	34.861	80.48	107.5	4.69				
MY-4 (12°36'N,96°00'E)	5	28.00	30.443	212.09	3.9	0.39	0.79			
MY-5 (13°36'N,95°36'E)	5	27.78	28.771	224.08	4.3	0.23	1.24	0.6	10.0	0.036
	20	28.24	31.474	202.60	3.9	0.14	0.52			
	45	28.63		175.99		0.14		0.4	10.4	0.013
	50	28.13	33.393	121.97	5.8	0.10	0.14	0.5	10.2	0.011
	74	26.37	33.782	129.18	9.6	0.43	0.16	0.7	10.2	0.052
	101	24.05	34.053	76.46	15.6	0.97	0.19			
	122	20.48	34.702	7.24	22.7	1.28	0.04			
	149	14.34	34.878	21.40	34.3	1.91	0.09			
	194	13.24	34.702	7.25	38.0	1.80	0.01			
MY-6 (15°00'N,96°00'E)	5	27.88	28.769	221.63	16.3	0.12	2.59	0.7	9.6	0.023
	10	27.94	29.487	216.67	11.1	0.11	2.50			
	15	28.33	30.811	200.38	5.4	0.03	2.17	0.9	10.1	0.002
	20	28.37	31.054	197.17	5.4	0.07	1.90	0.6	9.7	0.013
	31	28.66	31.533	183.21	6.2	0.07	1.00	0.7	10.2	0.006
	40	28.82	32.397	150.83	8.1	0.22	0.16			
	51	28.45	32.654	126.46	10.3	0.20	0.12	0.5	10.4	0.019
	59	27.72	33.076	106.75	10.9	0.21	0.06	0.5	10.2	0.030

Table 5-2. Zn complexation data from other relevant seawater studies. The values of $\log K'_{\text{ZnL,Zn}^{2+}}$ for ASV method are calculated by the equation ($K'_{\text{ZnL,Zn}^{2+}} = K'_{\text{ZnL,Zn}'} \times \alpha_{\text{Zn}'}$).

Region	Sample	C _L (nmol/L)	logK' _{ZnL,Zn²⁺}	Method	Reference	
Open Ocean						
	Northeastern Indian Ocean	17 m	0.5	10.0	CSV	This study
Marginal Sea						
	Andaman Sea	Profile	0.4-0.9	9.6-11.4	CSV	This study
	Sea of Okhotsk	20 m	2.6	9.5	CSV	Kim et al. (in press)
	Sea of Japan	20 m	1.3	9.2	CSV	Kim et al. (in press)
	Bering Sea	20 m	3.6	9.6	ASV	Jakuba et al. (2012)
	Black Sea	10 m	7.8-16.6	9.7-10.7	ASV	Muller et al. (2001)

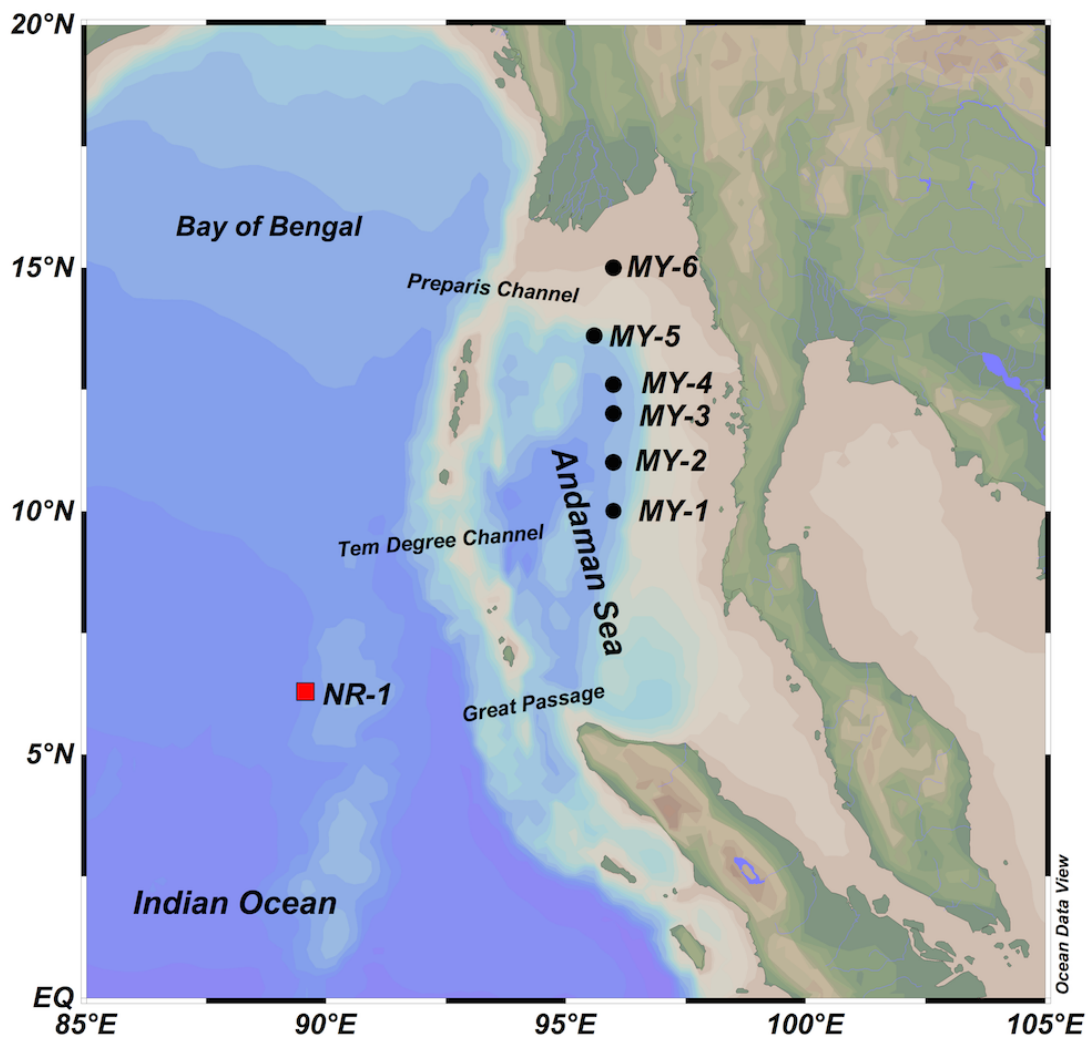


Fig. 5-1. Location of sampling stations in the northeastern Indian Ocean (NR-1, red) and the Andaman Sea (MY-1 - MY-6, black).

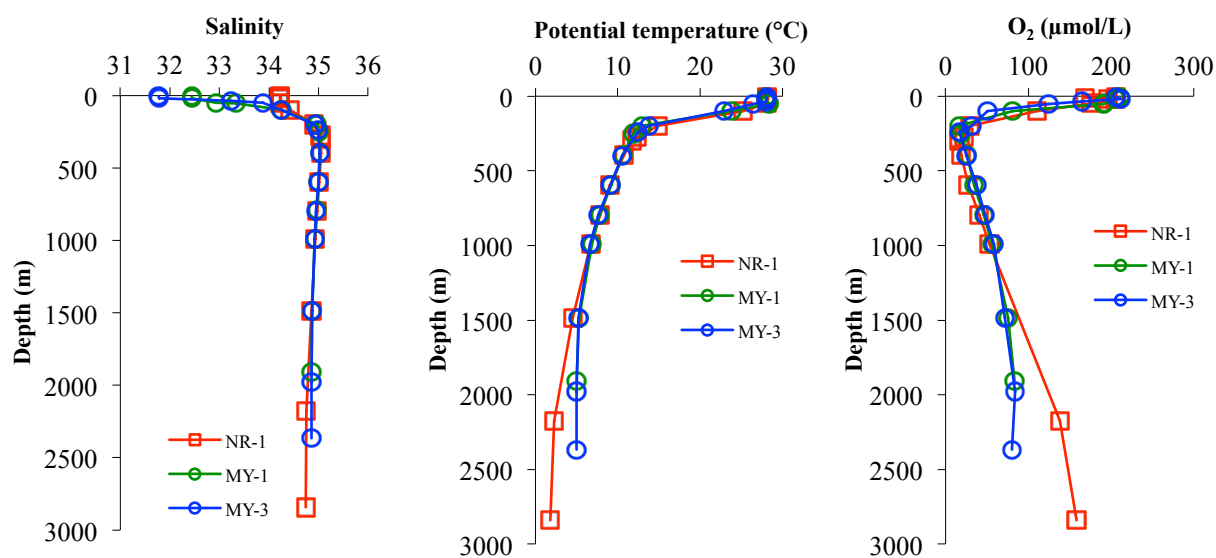


Fig. 5-2. The vertical profiles of salinity, potential temperature and dissolved oxygen in the northeastern Indian Ocean (NR-1) and the Andaman Sea (MY-1 and MY-3).

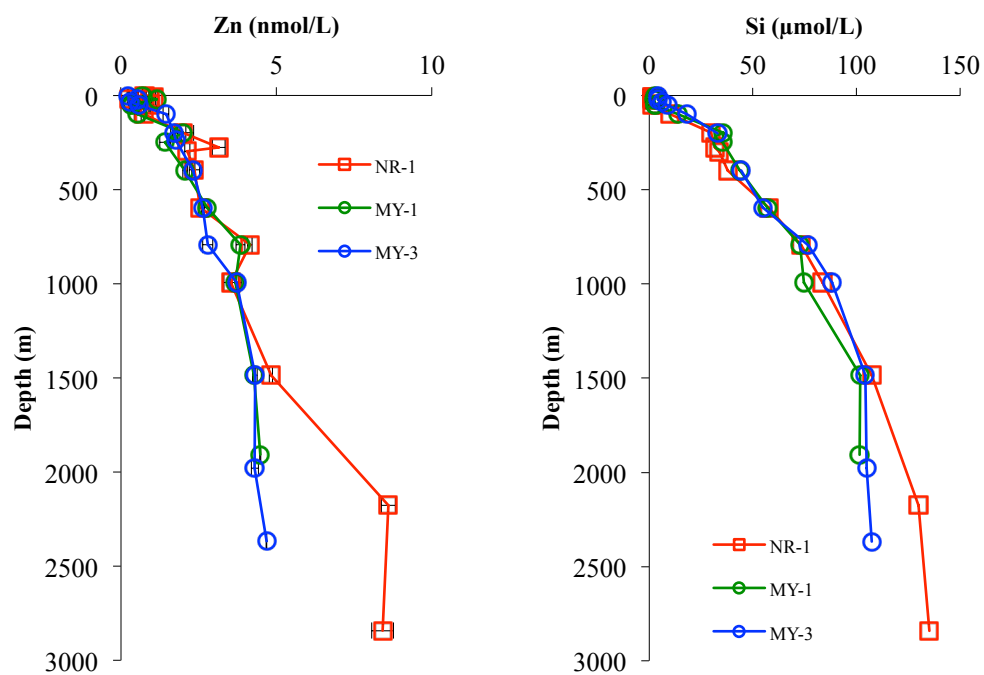


Fig. 5-3. The vertical profiles of total dissolved Zn and silicate in the northeastern Indian Ocean (NR-1) and the Andaman Sea (MY-1 and MY-3).

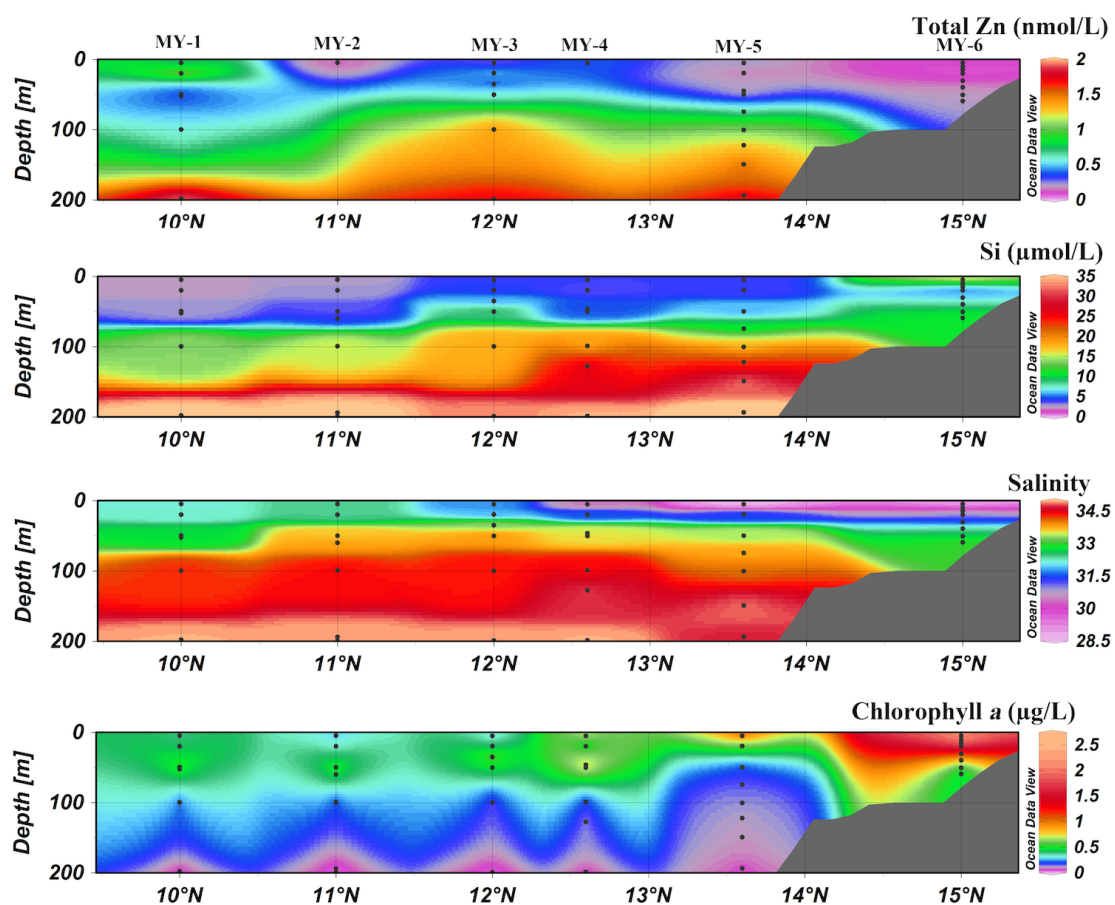


Fig. 5-4. Distributions of total dissolved Zn, silicate, salinity, and chlorophyll *a* in the Andaman Sea (MY-1 to MY-6).

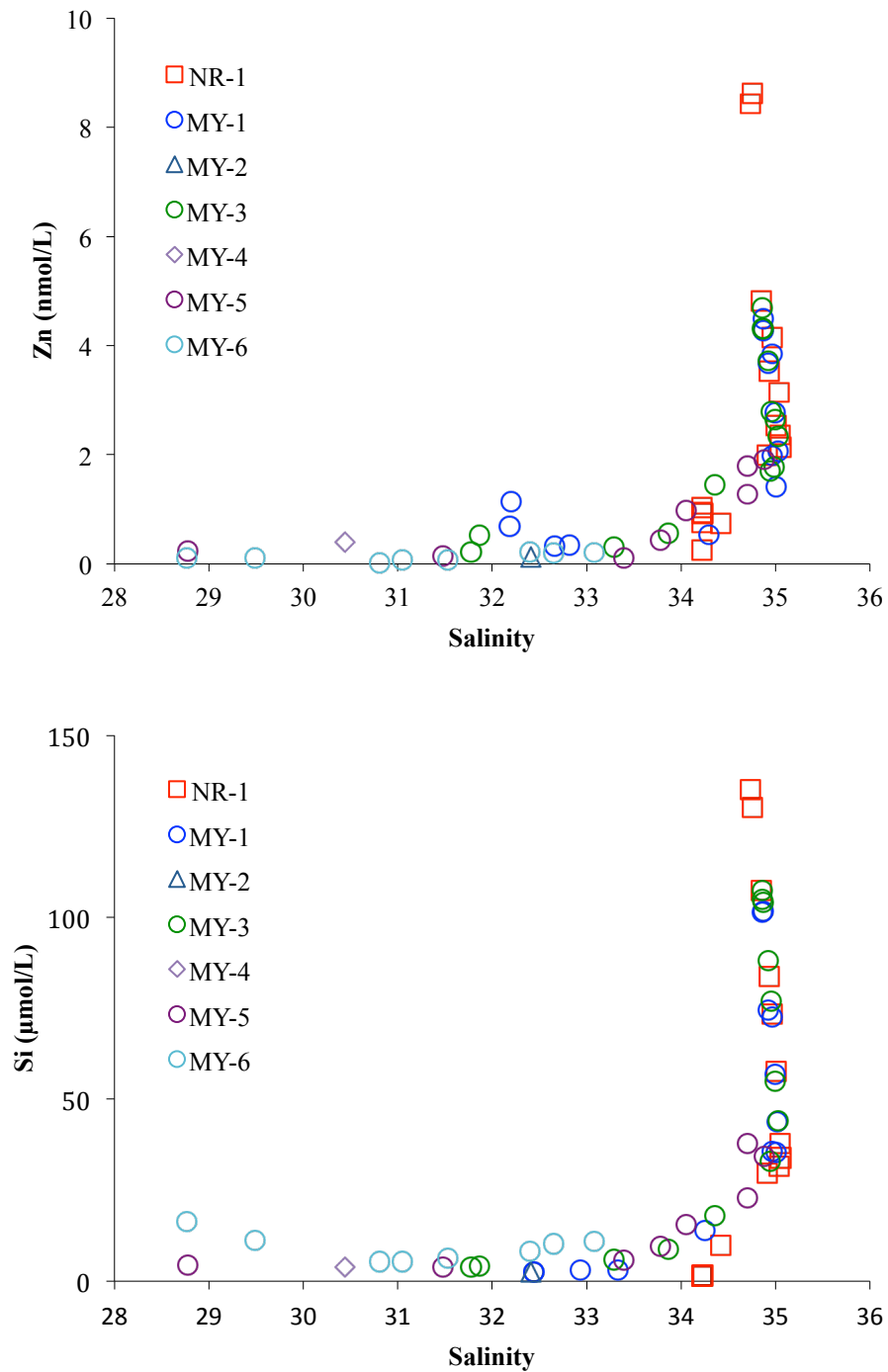


Fig. 5-5. Comparison of practical salinity with Zn and Si in the northeastern Indian Ocean (NR-1) and Andaman Sea (MY-1 to MY-6).

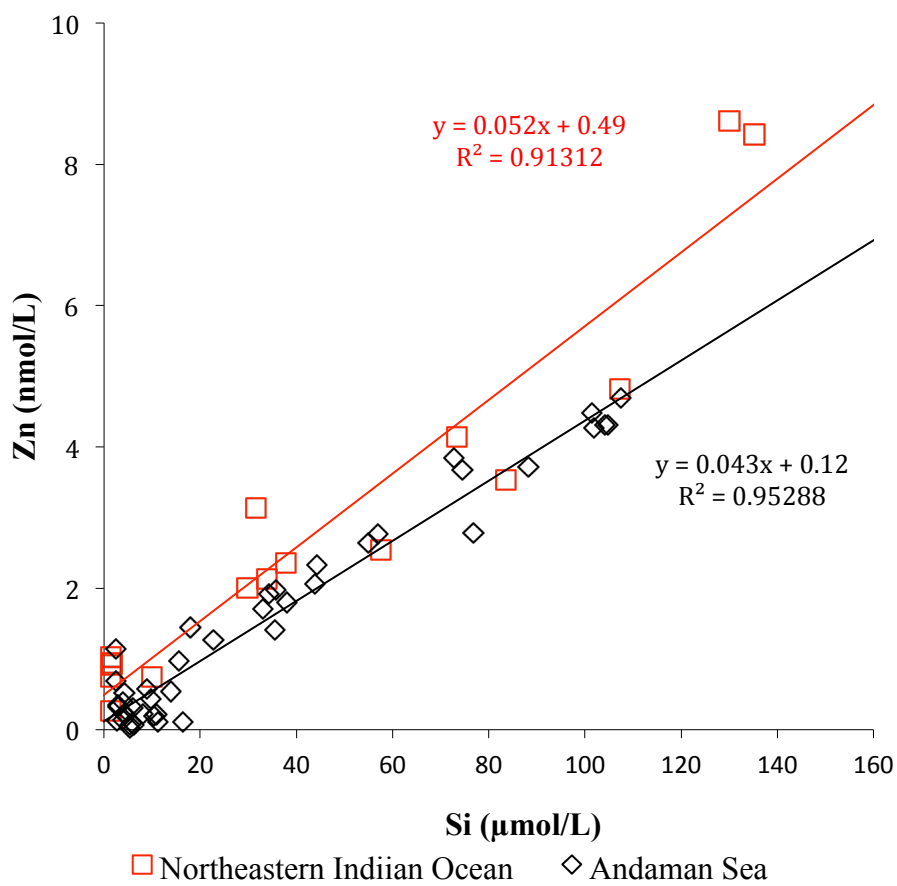


Fig. 5-6. Relationship between total dissolved Zn and Si in seawater obtained in the northeastern Indian Ocean and Andaman Sea.

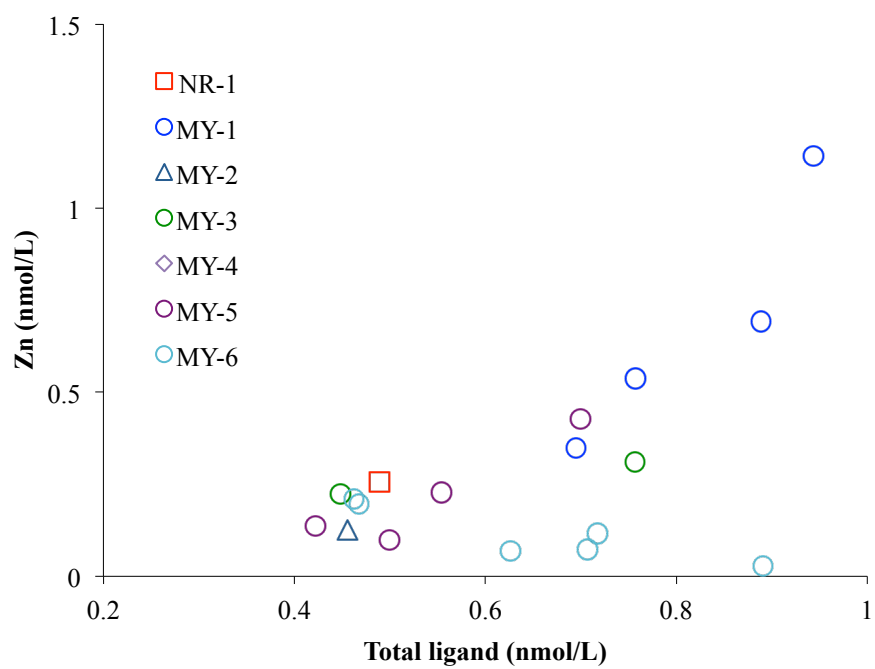


Fig. 5-7. Comparison of total ligand concentrations and total dissolved Zn in the northeastern Indian Ocean (NR-1) and Andaman Sea (MY-1 to MY-6).

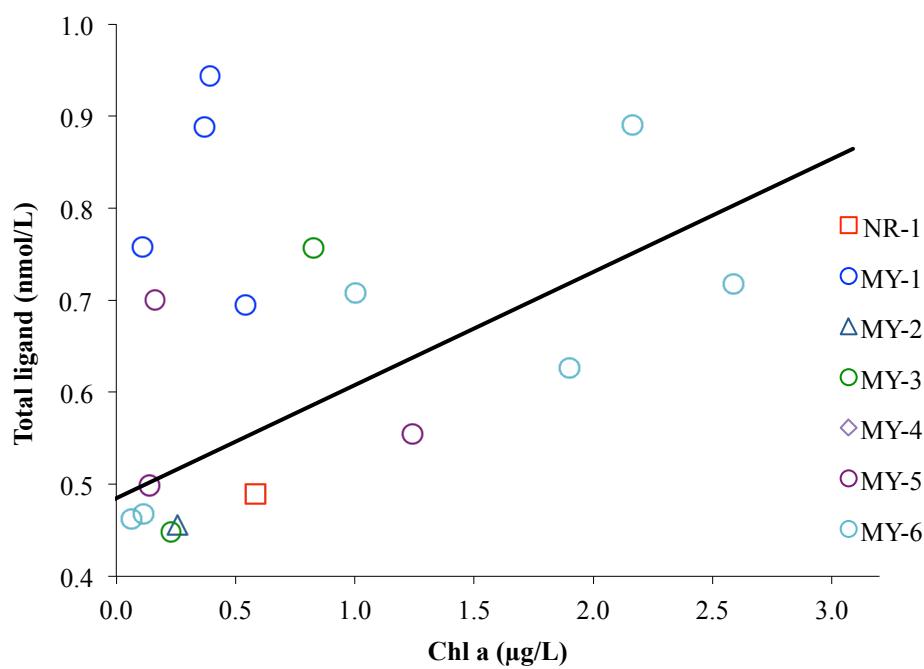


Fig. 5-8. Comparison of Chl *a* and total ligand concentrations. Black line indicates the relationship between Chl *a* and total ligand concentrations in the MY-6 ($R^2=0.651$)

Chapter 6.

General conclusion

Zinc is known as an essential micronutrient for bacteria and phytoplankton in the ocean. It was suggested that Zn availability is an important selective force acting on phytoplankton and ultimately affecting the structure of phytoplankton communities. However, this bioavailability is still under debate. Because of the low concentration of Zn in the surface water and complicated contamination problems, precise determination of dissolved Zn in the seawater has been a big challenge. Therefore, to understand biogeochemical cycles of Zn in the ocean, it is necessary to improve not only analytical method, but also clean seawater sampling technique.

In this study, I established a precise determination method for picomolar level of Zn in seawater without any contamination using cathodic stripping voltammetry (CSV). The results of this intercalibration are in good agreement with the results from other labs, which was the first certified data for Zn using CSV method. Furthermore, I clarified the cause of contamination during the seawater sampling by Kevlar wire hydrocasts on oboard R/V Hakuho-maru and confirmed that using a conductivity-temperature-depth carousel multi-sampling system (CTD-CMS) connected with the Ti-armoured cable of R/V Hakuho-maru is appropriate for clean seawater sampling for Zn analysis.

Using these established methods, the differences between Zn concentrations in shallow waters were clarified in the subarctic North Pacific Ocean in much more detail than the previous studies. The west–east decreasing trend of dissolved Zn was

shown to be quite similar to that of Si, suggesting that surface Zn distributions are controlled by biogeochemical processes, such as vertical transport, uptake by diatoms, and regeneration or dissolution of biogenic particles.

This study demonstrates that the source of Zn ligands should vary between the open ocean and marginal seas. Both the subtropical and subarctic North Pacific showed a positive relationship between total ligand concentrations and Chl *a*, but the data from the Sea of Okhotsk and Sea of Japan were found to significantly deviate from the North Pacific relationship. The data suggests that Zn complexing ligands in the North Pacific might be derived from bacteria and phytoplankton, whereas there are different sources in the Sea of Okhotsk and Sea of Japan.

Only in the northernmost station of the Andaman Sea (MY-6), where exists the high influence of fluvial discharge from Irrawaddy and Salween rivers, total ligand concentrations are found to correlate with Chl *a* contents. This supports that phytoplankton and bacteria-excreted organic substances were dominant sources for Zn complexing ligands. Other stations in the Andaman Sea, however, poorer correlations have been obtained, which might suggest different sources of Zn complexing ligands at those stations.

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