



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

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### Abstract

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. 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, which could limit the growth of some phytoplankton species. Therefore, the study of Zn speciation is important for understanding biogeochemical cycling of Zn in the ocean. 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. 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.

### 1. 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). However, (Ellwood and Hunter, 2000) showed that only a few percent of the cellular Zn in diatoms is associated with their frustules. The correlation between Zn and Si is therefore, likely attributed to sinking rates of diatoms and the simultaneous release of both elements from diatoms packaged with other detrital materials (Twining et al., 2014). A recent study has shown that in deep waters of the South Atlantic, the relationship between Zn and Si is due to the movement of water masses (Wyatt et al., 2014).

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., 2003; 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

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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). In the marginal seas, although there are few studies, relatively high total ligand concentrations and relatively low conditional stability constants ( $K'_{ZnL, Zn^{2+}}$ ) 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, more Zn speciation studies are needed to unravel sources and identities of Zn ligands.

The determination of trace concentrations of Zn in seawater is difficult because of complicated contamination problems (Fitzwater et al., 1982). The use of Zn is ubiquitous in the laboratory, and it is typically used as sacrificial anodes in equipment deployed in seawater. Therefore, this leads to severe contamination during the analytical and sampling processes of seawater. Clean seawater sampling methods using Go-Flo type (or external-spring Niskin type) samplers have been well-described in the GEOTRACES sampling and sample handling protocols (Cutter et al. 2010). However, although these protocols are well established, it is still possible that Zn in the seawater samples can be contaminated during research cruises, and a recent study pointed out that seawater samples for Zn at shallower depths could be affected by contamination from sources such as paint or the sacrificial Zn anodes of the research vessels (Cutter and Bruland, 2012).

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. Using these data, the geochemical processes of Zn in the ocean were investigated.

## 2. Analytical methods

### 2-1. Determination of total dissolved Zn in seawater using cathodic stripping voltammetry

Total dissolved Zn in seawater was determined using CSV (Kim et al., 2015); 757 VA Computrace (Metrohm) as the voltammetric system. The reference electrode was Ag/saturated AgCl, 3 M KCl, the counter electrode was made of glassy carbon, and the working electrode was a hanging mercury drop electrode (HMDE).

To decompose interfering dissolved organic matter (DOM) and metal-complexing organic ligands, which occur naturally in seawater (van den Berg, 1985), a UV irradiation system was used (Kim et al., 2015). Following UV-irradiation, 10 mL of an acidified sample was added into a Teflon cell with ammonium 1-pyrrolidinedithiocarbamate (APDC) and buffer

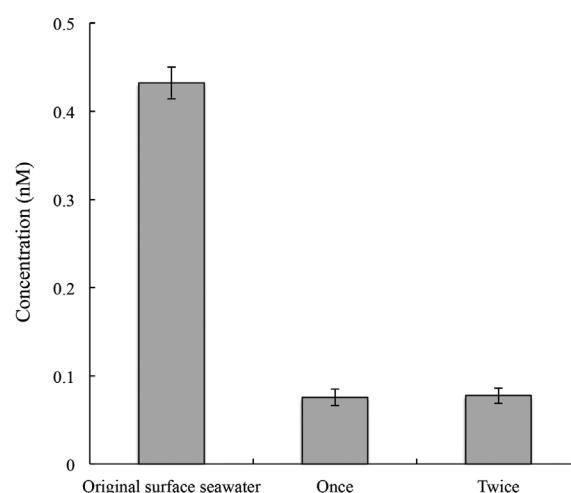
solution (PIPES), and the pH values of the samples were then adjusted to 7.0 by the ammonia solution. After addition of the ammonia solution, analysis of the seawater samples was immediately conducted. In the Teflon cell, Zn was complexed with APDC (Zn-PDC) and absorbed onto the hanging mercury drop electrode (HMDE). The potential of the electrode was scanned in the negative direction. The reduction current peak of  $Zn^{2+}$  in the adsorbed Zn-PDC complexes was seen to occur at around -1.1 V. Concentrations of Zn in seawater were then calibrated using a standard addition method (Lohan et al., 2002).

#### 2-2. 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 (Tmapure 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.

#### 2-3. Procedural blank value, detection limit, and reference seawater for Zn analysis

Zn-removed seawater was prepared by passing it through the chelating resin column. To confirm whether Zn in the original seawater ( $432 \pm 18$  pM ( $n = 2$ )) was removed thoroughly, the seawater was passed once again through the column. In this case, the Zn concentration was calculated as  $77 \pm 9$  pM ( $n = 2$ ), which is consistent with the Zn concentration ( $75 \pm 9$  pM ( $n = 7$ )) 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. 1). To investigate what caused the procedural blank value, Zn-removed seawater was analyzed with double additions of reagents and buffer. 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.



**Fig. 1.** Zn concentrations of surface seawater and those passed through the chelating resin column. Error bars represent the standard deviations between measurements.

Based on the results, the procedural blank value was calculated as  $75 \pm 9$  pM ( $n = 7$ ) by taking measurements for the Zn-removed seawater obtained by passing seawater through the chelating resin column once. Total dissolved Zn concentrations in seawater samples were determined by subtracting the obtained blank value from total measured values. The detection limit (calculated as three times the standard deviation of measurements of blank values for purified seawater) was 28 pM.

As a check on the CSV method and to compare to other laboratories, I determined 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) (Table 1). The results using CSV are considered to be in good agreement with those from other laboratories that used several other analytical methods such as AAS, ICP-MS and FIA-FL (<http://www.geotraces.org/science/intercalibration/322-standards-and-reference-materials>).

**Table 1.** Dissolved Zn concentrations of the intercalibration.

Intercalibration		Total zinc (nmol/kg)	
Samples	Bottle No.	This study	Consensus value
GS	62	$0.034 \pm < 0.001$	$0.041 \pm 0.007$
	157	$0.045 \pm 0.003$	
GD	39	$1.67 \pm 0.07$	$1.71 \pm 0.12$
	142	$1.66 \pm 0.14$	
SAFe S	557	$0.060 \pm 0.004$	$0.069 \pm 0.010$
	227	$0.073 \pm 0.005$	
SAFe D1	534	$7.21 \pm 0.13$	$7.40 \pm 0.35$
	535	$7.43 \pm 0.13$	
SAFe D2	249	$7.29 \pm 0.39$	$7.43 \pm 0.25$
	445	$7.43 \pm 0.27$	

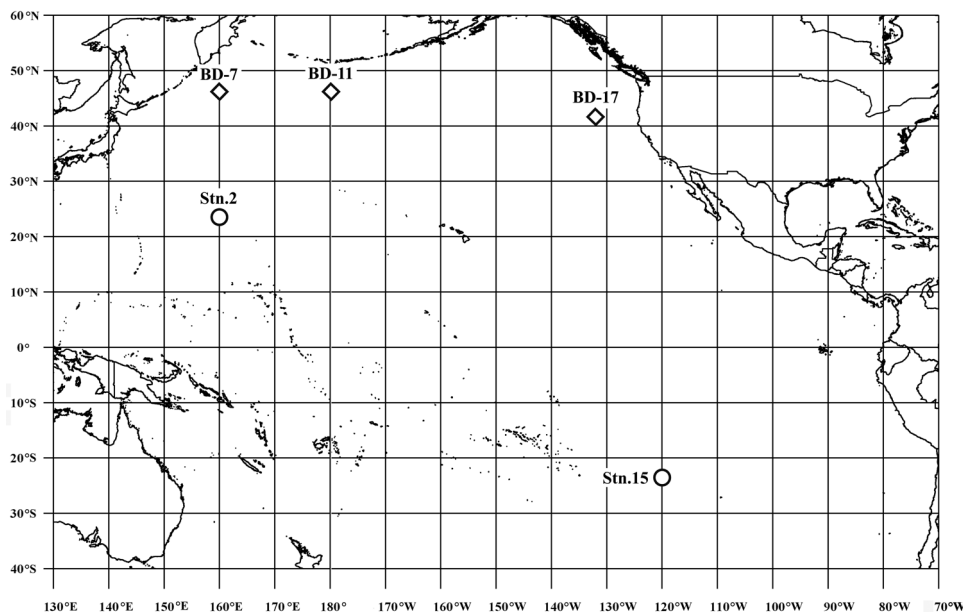
## 2-4. Zn speciation analysis

Zn speciation was determined through titration by using competitive ligand equilibrium/adsorptive cathodic stripping voltammetry (CLE-ACSV) (Donat and Bruland, 1990; van den Berg, 1985), which uses a competitive equilibrium between Zn-complexing ligands naturally present in the sample and a competing ligand (ammonium 1-pyrrolidinedithiocarbamate; APDC). Briefly, a ligand titration consisting of 9 Teflon vials (Savillex) was set up. In each vial, 10 mL of the seawater sample, 4 mM of borate buffer and a known concentration of Zn (0–9 nM) were added. The first two vials were not spiked with Zn and were used as replicates for the starting point of the titration. After 2 h, 25  $\mu$ M of APDC was added to each vial. At this APDC concentration, the detection window of the method is approximately from  $K'_{ZnL, Zn^{2+}} = 10^7$  to  $10^{12}$  for a  $C_L$  of 1 nM, where  $K'_{ZnL, Zn^{2+}}$  is the conditional stability constant of the Zn complex with respect to  $Zn^{2+}$  in seawater and  $C_L$  is the total ligand concentration, respectively. The APDC was allowed to equilibrate for 12 h. The samples were then transferred to a Teflon cell for the measurement.

## 3. Clean seawater sampling

### 3-1. Sample collection and storage

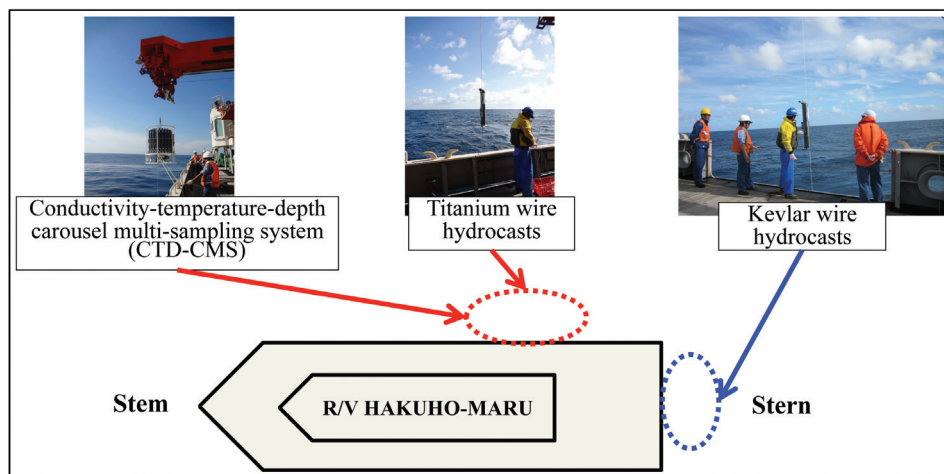
Seawater samples were collected in the subtropical North and South Pacific during the KH-11-10 cruise of R/V Hakuho-maru (from December 2011 to January 2012, Stn. 2 and Stn.15) and in the subarctic North Pacific during the KH-12-4 cruise of R/V Hakuho-maru (from August to October 2012, stations BD-7, BD-11 and BD-17). The stations are shown in Fig. 2. Seawater samples were collected using Teflon-



**Fig. 2.** Location of sampling stations. Seawater samples for subtropical North and South Pacific (Stn.2 and Stn.15, circle) 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, diamond), samples were collected as part of GEOTRACES GP 02 cruise (KH-12-4 R/V Hakuho-maru cruise, Aug-Oct 2012).

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 M 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,



**Fig. 3.** Sampling positions where sampling method were examined.

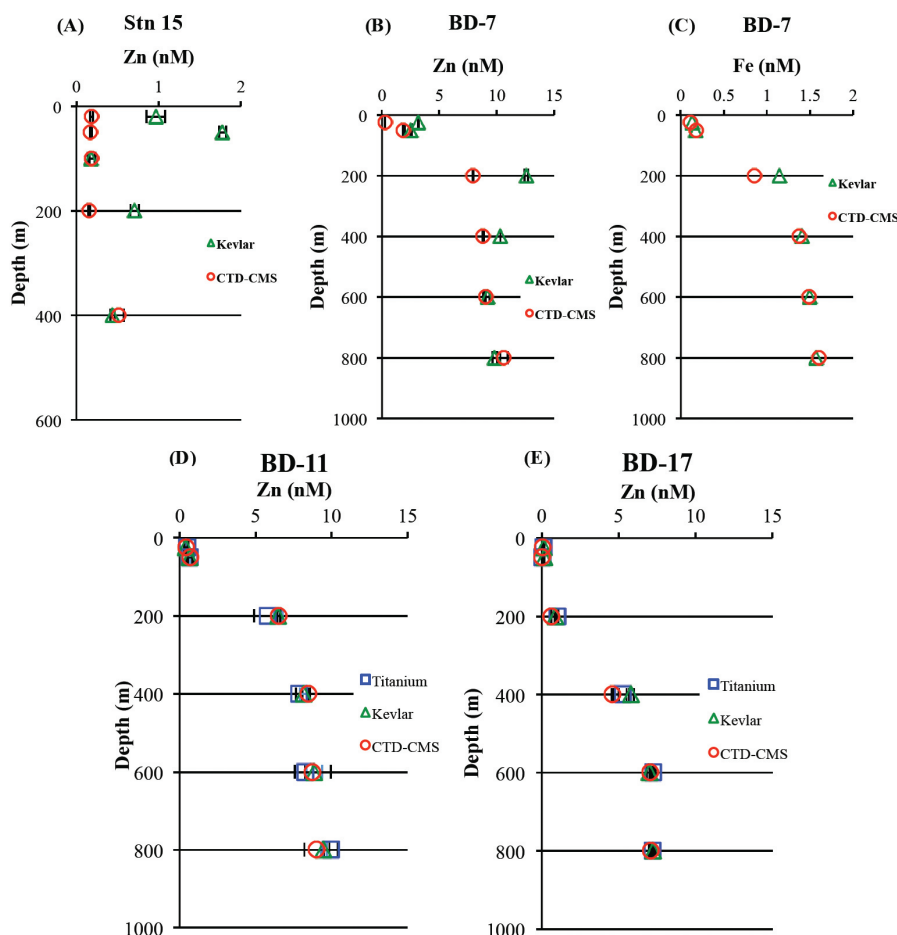
Hikari-kogyo); and (3) attached bottle by bottle to a Ti wire equipped in R/V Hakuho-maru. The acid-cleaned Niskin-X samplers attached to both wires were closed with Teflon messengers. In this study, seawater sampling using CTD-CMS and Ti wire hydrocasts were performed on the starboard side, while sampling using Kevlar wire hydrocasts took place off the stern of the research vessel (Fig. 3).

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. Seawater

samples were filtered using an acid-cleaned 0.2  $\mu\text{m}$  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 (Tama-pure AA-100), and were then stored.

### 3-2. 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



**Fig. 4.** (A, B, C) Comparison of dissolved Zn (Stn. 15 and BD-7) and Fe (BD-7) concentrations in seawater collected using Niskin-X samplers deployed onto Kevlar wire hydrocasts and CTD-CMS. (D, E) Comparison of dissolved Zn (BD-11 and BD-17) concentrations in seawater collected with three different sampling methods, Niskin-X samplers deployed onto titanium wire hydrocasts, Kevlar wire hydrocasts and CTD-CMS. Error bars on the Zn profiles represent the standard deviations between triplicate measurements.

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. 4-D and -E). Such consistency indicates that by performing water sampling on the starboard side of the R/V Hakuho-maru (Fig. 3), 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 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. 4-A and -B) 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 400 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. 4-D and -E). Our examination showed that a severe contamination problem did not occur for Fe (Fig. 4-C). 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.

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

##### 4-1. Sample collection and storage

Seawater samples were collected in the western subarctic North Pacific, Sea of Okhotsk

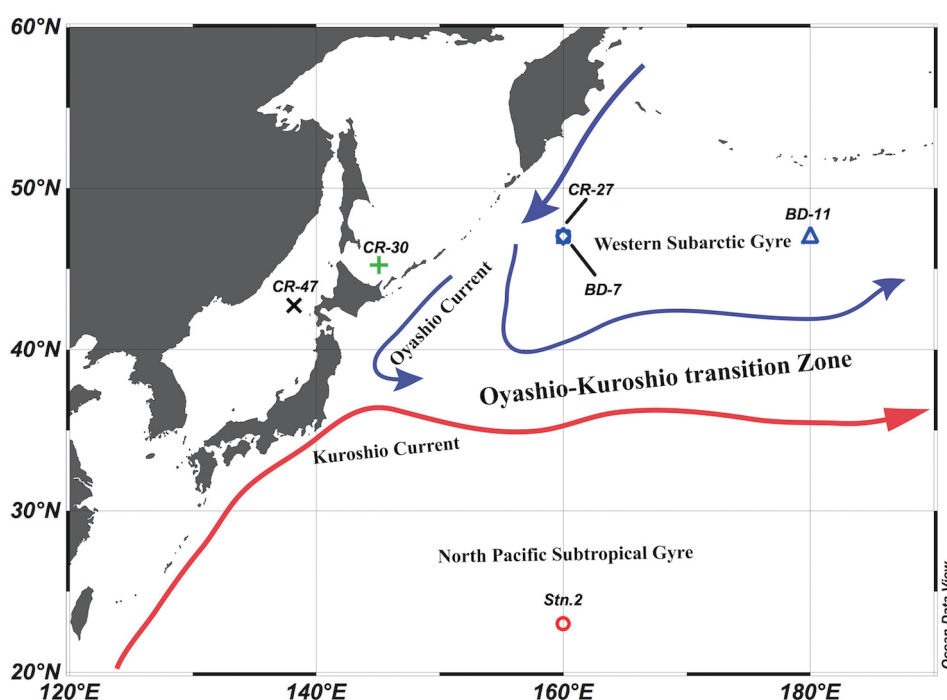


Fig. 5. Location of sampling stations in the subtropical North Pacific (Stn. 2, red), subarctic North Pacific (CR-27, BD-7 and BD-11, blue), Sea of Okhotsk (CR-30, green) and Sea of Japan (CR-47, black). Schematic illustration of the near-surface current is described based on Yasuda (2003).

and Sea of Japan (East Sea) during R/V Hakuho-mar research cruises KH-10-2 (in June and July 2010, at stations CR-27, CR-30 and CR-47) and R/V Hakuho-mar 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-mar research cruise KH-11-10. Locations of the sampling stations are indicated in Fig. 5. Seawater samples for total dissolved Zn are described above. The filtered samples for Zn speciation analysis were frozen immediately after collection and kept frozen until just before analysis.

#### 4-2. Total dissolved Zn in the western North Pacific and its adjacent seas

Total dissolved Zn profiles are nutrient type at all stations in this study (Fig. 6), 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). 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 nM.

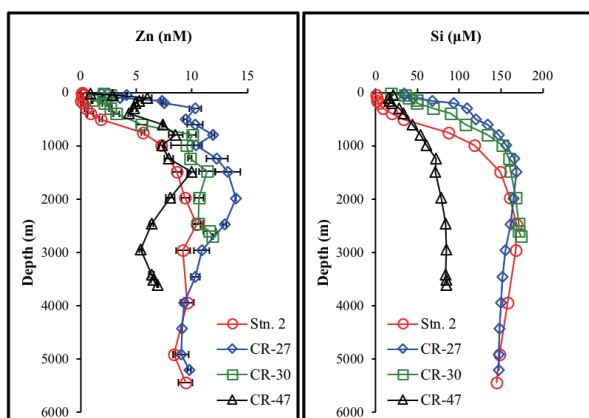


Fig. 6. Vertical distributions of total dissolved Zn and Si obtained in the subtropical North Pacific (Stn. 2, red), subarctic North Pacific (CR-27, blue), Sea of Okhotsk (CR-30, green) and Sea of Japan (CR-47, black).

These values are clearly lower than those in the subarctic North Pacific (CR-27, 1.9–7.5 nM), Sea of Okhotsk (CR-30, 1.0–2.7 nM) and Sea of Japan (CR-47, 0.9–6.0 nM). In the Sea of Okhotsk (CR-30), the total dissolved Zn concentration in the surface exceeded 2 nM, higher than those in the North Pacific. This result may indicate that there are additional sources of Zn in the Sea of Okhotsk.

#### 4-3. Probable sources of Zn complexing ligands

The  $\log K'_{ZnL, Zn^{2+}}$  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. Total ligand concentrations in the North Pacific ranged from 0.2 to 1.2 nM, which is slightly lower than in previous studies in the open ocean. In the marginal seas, total ligand concentrations were found to increase in the Sea of Okhotsk (2.6 nM) and Sea of Japan (1.3 nM) for each 20 m depth. Although there are few data, total ligand concentrations near surface in the marginal seas might be higher than those in the open ocean.

Fig. 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. 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



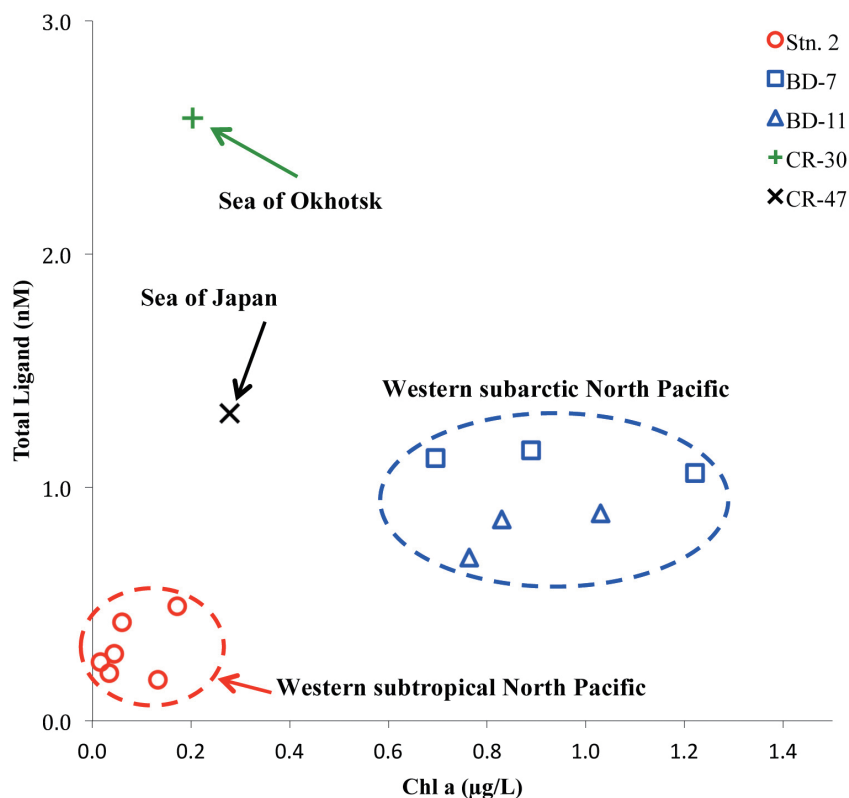


Fig. 7. Comparison of Chl *a* and total ligand concentrations.

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. 7). 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. 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.

## 5. Conclusions

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, I have investigated total dissolved Zn concentrations and Zn speciation in the western subtropical and subarctic North Pacific 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.

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