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TECHNICAL REPORT 3109
OCTOBER 2017

Water Quality Monitoring of Biofouling Removal from the ex-USS INDEPENDENCE (CV 62)

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Water Body Number
WA-15-0040 Sinclair Inlet

Approved for public release.

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M. K. Yokoyama, CAPT, USN
Commanding Officer

W. R. Bonwit
Executive Director

ADMINISTRATIVE INFORMATION

The work described in this report was performed for the Navy Sea Systems Command (NAVSEA), Naval Inactive Ships Program (SEA 21I) by the Environmental Sciences Branch (Code 71750) and the Energy and Environmental Sustainability Branch (Code 71760) of the Advanced Systems and Applied Sciences Division (Code 71700), of the Space and Naval Warfare Systems Center Pacific (SSC Pacific) San Diego, CA. Additional support was provided by the Naval Undersea Warfare Center (NUWC) Newport, RI.

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EXECUTIVE SUMMARY

BACKGROUND

On March 11, 2017, the ex-INDEPENDENCE (CV 62) was towed from Bremerton, WA arriving on June 1, 2017 in Brownsville, TX, for dismantling. In preparation for towing and based on an informal consultation with the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS), the Navy prepared and implemented a plan to remove biofouling from the ship's hull prior to towing to mitigate the transfer of invasive species to other regions. As part of this plan, the Space and Naval Warfare Systems Center Pacific (SSC Pacific), San Diego CA, and Naval Underwater Warfare Center (NUWC) Division, Newport RI, undertook a study to assess potential water quality impacts to Sinclair Inlet associated with biofouling removal from the ex-INDEPENDENCE.

STUDY DESIGN

Biofouling removal from the ex-INDEPENDENCE was conducted from January 6 to 27, 2017, at Mooring G at Naval Base Kitsap-Bremerton. The study was conducted to monitor and evaluate key water quality parameters at six sites located near the Ship (area of influence) and four Reference sites within western Sinclair Inlet. Four sampling events were conducted, which included before removal (Event 1, Baseline, November 9 to 10, 2016), during removal (Event 2, January 10, 2017), at the end of removal (Event 3, January 31, 2017), and 40 days after removal was completed (Event 4, March 7, 2017).

OBJECTIVES

Each sampling event consisted of sampling, measuring, and analyzing water quality parameters at 10 stations (six near the Ship and four at Reference locations) during four events over the evolution of the biofouling removal process. The objectives of the study were to evaluate potential water quality impacts associated with biofouling removal from the ex-INDEPENDENCE, including:

1. the release of copper (Cu) and zinc (Zn) associated with hull coatings;
2. depression of dissolved oxygen (DO) from the decay of organic matter removed from the hull to levels below the aquatic life DO criteria in marine water;
3. turbidity plumes that may exceed WQS;
4. the release of nutrients (nitrates [NO3], nitrites [NO2], and ammonia) which are precursors to reduction in DO that may contribute to degraded water quality; and
5. indicators of organic matter load (dissolved organic carbon [DOC] and biological oxygen demand [BOD]).

Where applicable, comparisons were made to WQS established by the state of Washington and recommended by the US Environmental Protection Agency (EPA) to be protective of aquatic life (Ecology, 2011, Ecology 2012; US EPA, 2003).

STATISTICAL ANALYSIS

Changes in water quality variables were assessed to determine whether any adverse impact could be attributed to biofouling removal from the ex-INDEPENDENCE. Potential adverse impacts were identified by determining if water quality parameters were statistically worse than baseline and reference conditions and assessing whether WQS were exceeded. Statistical tests were conducted using hypotheses for a Before - After - Control - Impact (BACI) statistical design:

$H1_O$: There are no differences between variables measured within the area of influence (Ship) and the same variables measured outside the area of influence (Reference sites) in western Sinclair Inlet.

$H2_O$: There are no differences between variables measured at the Ship before biofouling removal (Event 1) and the same variables measured at the Ship during subsequent events (Events 2, 3, 4) that may be affected by biofouling removal.

QUALITY ASSURANCE/QUALITY CONTROL

Concentrations of copper and zinc measured throughout this study were at or near the instrument and method detection limits making these values difficult to precisely and accurately quantify. The trace metal data were obtained with strict adherence to Quality Assurance/Quality Control (QA/QC) requirements which established how everything was accomplished from the type of sampling equipment that was utilized all the way through sampling procedures, laboratory processing, and data quality analysis. Satisfying the QA/QC procedures provides a high level of confidence in the results presented.

RESULTS

Study results showed that the concentrations of both dissolved copper and zinc were well below WQS throughout the study at all stations. The WQS for acute (1-hour average) and chronic (4-day average) exposures are 4.8 µg/L and 3.1 µg/L for dissolved copper and 90.0 µg/L and 81.0 µg/L for dissolved Zn, respectively. Dissolved copper concentrations measured at stations near the ship were significantly higher than reference stations during biofouling removal (Event 2 and Event 3) ranging from Non Detect (ND) < 0.10 µg/L to 1.58 µg/L compared to reference stations (< 0.10 µg/L to 0.82 µg/L). However, the dissolved copper concentrations decreased to baseline and reference levels within six weeks (Event 4) after biofouling removal was completed. Dissolved Zn concentrations measured at the Ship and Reference stations (ranging from < 0.2 µg/L to a maximum of 2.02 µg/L) were far below WQS.

For total copper, there were significant differences between Ship and Reference stations for Event 2 and Event 3. On average, total copper levels at Ship stations increased to about 2 to 3 times above Reference stations (Event 2 Ship: mean 3.65 ± 2.75, Reference: mean 0.93 ± 0.26, p=0.0021; Event 3 Ship: mean 2.69 ± 1.58, Reference: mean 0.78 ± 0.31, p=0.0003) which occurred during biofouling removal. However, the increase in total copper was not persistent as by Event 4 (6 weeks after hull cleaning completed), total copper measured at Ship stations (mean 0.77 ± 0.12) had returned to nearly the same level as Reference stations (mean 0.63 ± 0.04, statistically different at p = 0.0004). Particulate-bound copper (Total – Dissolved copper) increased from about 17% for Event 1 to 79% for Event 2 and 83% for Event 3 and returned to 22% by Event 4 indicating that the increase in total copper was likely non-labile particulates that were not toxic within the water column and not persistent as water column concentrations returned to baseline and reference levels by Event 4.

Overall, levels of turbidity, DO, and nutrients did not exceed water quality standards during the study and only small differences between the Ship and Reference sites were detected for turbidity and nutrients.

In addition to contributions from biofouling removal, runoff from storm events, discharges from municipal waste water treatment plants, freshwater runoff, and other sources also contributed to water quality conditions in Sinclair Inlet. More than 42 inches (in) of rain fell over the course of this study, including a major storm event (more than 3 in of rainfall within 24 hr) that occurred during biofouling removal. Despite these simultaneous contributions, dissolved copper and zinc, and nutrient concentrations were within the range of concentrations reported from previous and ongoing monitoring programs in Sinclair Inlet and no measurements exceeded WQS set by the State of Washington or water quality criteria recommended by US EPA.

Statistical tests indicated elevated levels of total copper, dissolved copper, and nutrient concentrations that were small in magnitude and temporary. This indicates that the study design was sensitive enough to discern potential changes in the environment associated with biofouling removal but does not indicate untoward environmental impacts.

CONCLUSIONS

A decision matrix was used to formalize conclusions about potential impacts to water quality resulting from the biofouling removal from the ex-INDEPENDENCE. The assessment was based on statistical significance of changes to water quality parameters during and after the biofouling removal operation, the magnitude of any effects and the risk of exceeding water quality standards. Conclusions were:

- Negative impacts from total and dissolved zinc, dissolved oxygen, nitrite, ammonia, DOC, and BOD were not found
- Statistically significant increases of total and dissolved copper, turbidity, and nitrate were measured
- Dissolved copper and zinc, dissolved oxygen, and turbidity did not exceed Water Quality Standards and were $\leq$ 25% of the threshold range
- All parameters returned to baseline levels and were similar to reference conditions within 40 days after biofouling removal was completed

In summary, there was no evidence of any parameter exceeding regulatory thresholds and no evidence of a persistent water quality impacts from the ex-INDEPENDENCE biofouling removal operation, as water quality indicators returned to ambient conditions within 40 days after biofouling removal was completed.

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1. INTRODUCTION

The ex-INDEPENDENCE (CV 62), which had been moored in Bremerton, WA since decommissioning in September 1998 (Seaforces.org, 2017), was towed on March 11, 2017 to Brownsville, TX, where it arrived on June 1, 2017 for dismantling (Navytimes.com, 2017). Based on a consultation with the National Marine Fisheries Service (NOAA NMFS, 2016), the Navy was required to clean the ship's hull prior to towing in order to mitigate the transfer of invasive species to other regions. While removing biofouling organisms prior to towing reduces the probability of spreading invasive species, there was concern by many within the Bremerton area that biofouling removal could have a detrimental impact on water quality in Sinclair Inlet. The Environmental Sciences and Energy & Environmental Sustainability Branches at Space and Naval Warfare (SPAWAR) Systems Center Pacific (SSC Pacific), San Diego CA and Naval Undersea Warfare Center (NUWC) Division, Newport RI, undertook a series of studies to assess any potential water quality changes to Sinclair Inlet associated with the removal of biofouling from the ex-INDEPENDENCE.

Prior to biofouling removal, comprehensive biological surveys of taxonomy and biomass present on the hull of CV 62 were conducted by SSC Pacific and NUWC at randomly selected stations along transect belts on the hull, as well as other isolated areas of the hull where fouling was known to occur (Earley et al. 2018a). Water quality monitoring was conducted to evaluate key water quality parameters at six sites located near the Ship (area of influence) and four Reference sites within western Sinclair Inlet during four sampling events conducted before removal (November 9 to 10, 2016), during removal (January 10, 2017), at the end of removal (January 31, 2017), and 40 days after removal was completed (March 7, 2017). Sediment monitoring (Johnston et al. 2018) was conducted before removal (Pre-Removal on December 13, 2017) and after the ship was towed from Sinclair Inlet (Post-Removal on March 30, 2017).

This report summarizes the results of water quality monitoring in Sinclair Inlet from November 2016 to March 2017 and subsequent water chemistry and statistical analysis. The objectives of this study were to monitor water quality changes over the evolution of biofouling removal from the ex-INDEPENDENCE, by analyzing water quality parameters including:

- Metals (dissolved and total Copper [Cu] and Zinc [Zn])
- Dissolved Oxygen (DO)
- Turbidity
- Nutrients (Nitrate, Nitrate and Ammonia)
- Dissolved organic carbon (DOC) and Biological oxygen demand (BOD)

This report presents field observations and laboratory data analysis of these water quality parameters compared to Washington State Department of Ecology (Ecology) and the US Environmental Protection Agency (EPA) water quality standards (WQS), in order to assess any potential environmental impacts associated with this biofouling removal event.

The data quality objectives (DQOs) and Quality Assurance and Quality Control (QA/QC) procedures were identified in the Project Work Plan (PWP) and Sampling and Analysis Plan (SAP) prepared for the study (SSC Pacific and NUWC 2016).

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2. BACKGROUND

2.1 EX-INDEPENDENCE AND INACTIVE SHIPS

Naval Sea Systems Command, Inactive Ships (NAVSEA 21I) manages U.S. Navy ships that have been taken out of commission or out of service. NAVSEA 21I is responsible for planning, programming, budgeting, and execution of the Navy's inactivation and disposal of conventionally powered surface ships and other smaller vessels. Inactive ship disposition typically results in ship donation, ship dismantling, or use in fleet training (e.g., sinking exercises). The Forrestal-Class aircraft carrier, ex-INDEPENDENCE, was commissioned on January 10, 1959. The vessel was decommissioned in 1998 after 39 years of active service and transferred to the NAVSEA Inactive Ships Maintenance Office (NISMO). The ship was moored at Mooring G at Puget Sound Naval Shipyard (PSNS) on December 21, 1999, where it remained until it was towed on March 11, 2017, arriving in Brownsville, TX on June 1, 2017 for dismantling.

2.2 EX-INDEPENDENCE PAINTING HISTORY

The most recent painting report for ex-INDEPENDENCE, dated December 17, 1986, reported that the body of the ship was blasted 100 percent to near white metal and her underwater body was coated with both anti-corrosive paints and anti-fouling paints from the keel to the lower limit load line. Based on ship records, the two anti-fouling paints used on the ex-INDEPENDENCE in 1985-1986 contained cuprous oxide and zinc oxide as the active anti-fouling ingredients (Painting Report, USS INDEPENDENCE [CV 62], 17 December 1986). In the 30 years since then, the antifouling compounds in the paint have been depleted from the original concentrations and are no longer preventing biofouling growth on the hull (NUWC, 2016).

2.3 EX-INDEPENDENCE HULL BIOFOULING

Hull biofouling inspections were conducted by the US Navy's hull cleaning experts, NAVSEA 00C prior to in-water removal to characterize the species and mass of biological material on the hull and what specific equipment and procedures would be employed to reduce the potential transport of invasive species. On November 5, 2016, Seaward Marine Services, Inc. under contract by NAVSEA assessed and documented the amount of biofouling on the hull of the ex-INDEPENDENCE. This inspection determined the average biofouling growth to be approximately two inches (in) in thickness, however, inspection was limited to the bow of the vessel and 300 feet (ft) aft due to barges and other equipment tied up to the ex-INDEPENDENCE. In December 2016, prior to biofouling removal, SSC Pacific in collaboration with NUWC conducted in-water hull surveys and documented dense biofouling on all parts of the vessel (SSC Pacific and NUWC, 2016). In these surveys, biomass thickness was observed to vary across the hull, with areas of a few large organisms (i.e., tubeworms) interspersed with areas of densely concentrated smaller organisms (i.e., barnacles). Figure 1 shows an example of biofouling observed on the hull. Biomass wet weight ranged from <0.1 kg/m^2 to 2.0 kg/m^2 in stratified random samples. Biomass across the entire wetted hull surface was estimated to be 54,010 kg (geometric mean of total wet weight) comprised of water (76%) calcareous matter, i.e. shells (18%) and organic matter (6%) or 3,146 kg (SSC Pacific and NUWC, 2016).

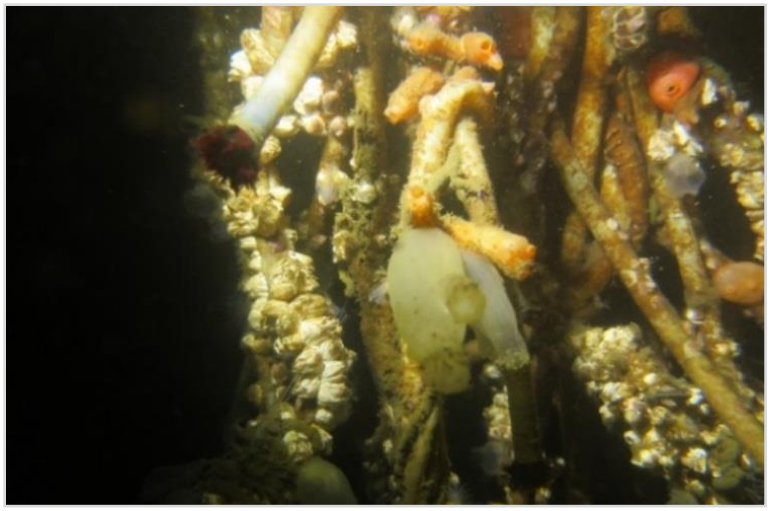

Figure 1. Example of biofouling density and diversity on the hull of the ex-INDEPENDENCE observed during pre-removal inspections.

2.4 BIOFOULING REMOVAL PROCESS

Biofouling removal was conducted by Seaward Marine Inc., under contract to the Navy, from 6 to 27 January 2017. Biofouling removal was conducted using the self-propelled, diver driven SCAMP® cleaning machine in accordance with the Naval Ships' Technical Manual (NSTM) Chapter 081 (Naval Sea Systems Command, 2006). In addition, NSTM Chapter 081 provides a description of the various tools used to clean ship hulls such as diver-operated machines with rotating brushes. This equipment uses either multi-brushes or single-brushes fitted with different brush types depending on the type of machine and fouling conditions present. The multi-brush machines utilize an impeller to hold the vehicle against the hull, while wheels move the large unit along the easily accessible areas of the hull. Single-brush units are held in place by both the diver and the suction force generated from the rotating brush, and are used to clean appendages and hull areas that the large multi-brush unit cannot access. For areas that are more difficult to reach, divers employ high-pressure water jets. Post-removal dive surveys showed 99% of the hull surface was free of biofouling organisms (SSC Pacific and NUWC, 2016) such as those shown in Figure 2 of the port bilge keel.

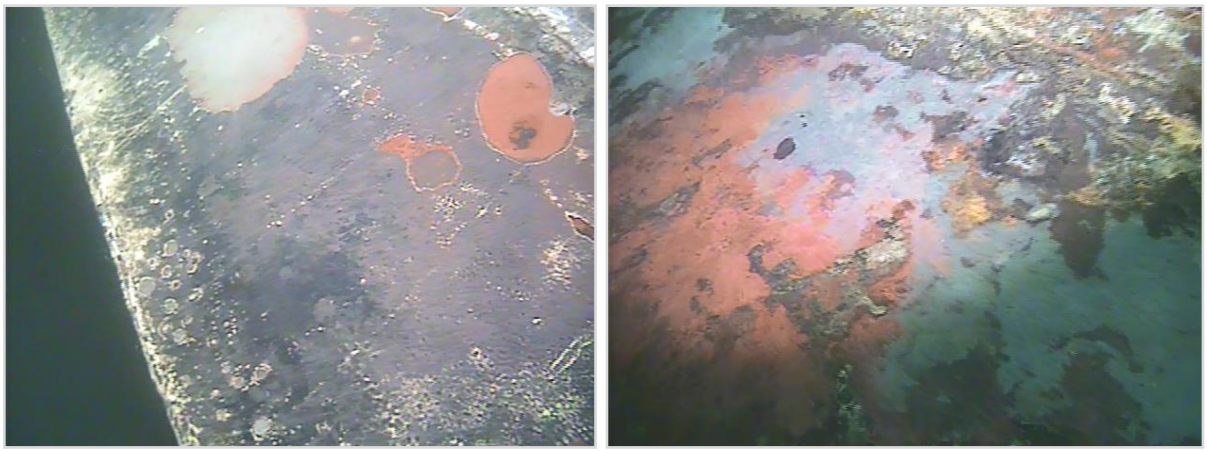

Figure 2. Top edge of port bilge keel showing paint (left) and top of port bilge keel showing paint and areas of bare hull (right).

2.5 COPPER AND ZINC IN SHIP HULL PAINT: LEACH RATES AND RELEASE DURING BIOFOULING REMOVAL

Copper has been used since the 18th century or perhaps much earlier to control marine growth on ships by acting as a toxicant that inhibits the settling and growth of marine organisms (Woods Hole Oceanographic Institution, 1952). Several decades of studies have shown that biocide release rates from antifouling ship hull coatings are influenced by a number of factors, including paint formulation and paint age (Johnson, Grovhoug, and Valkirs 1999, Valkirs, Seligman, Hasbeck, and Caso, 2003), physical factors such as hydrodynamics, temperature, pH and salinity, as well as biological factors such as the presence of biofilms (communities of bacteria and algae) at the paint surface (Woods Hole Oceanographic Institution, 1952; Mihm and Loeb, 1988). Generally, passive metal leach rates are higher at freshly painted surfaces and decline substantially within several months (Valkirs et al., 2003; Earley et al., 2014), in response to biofouling growth. As fouling increases due to reduced coating efficacy, the demand for surface refreshment (biofouling removal and re-painting) increases (Earley et al., 2014). Biofouling removal includes active removal of fouling organisms and varying amounts of antifouling paint, which can temporarily increase localized environmental loading of copper and zinc (Yebra, Kiil, and Dam-Johansen, 2004). However, passive leaching from ship hull paint is the primary contributor to ambient toxicity. For example, Valkirs et al. (1994) found that 99% of the dissolved copper loading in San Diego Bay was contributed by antifouling paints on pleasure craft (65%) and active Naval vessels (34%) in contrast to <1% contributed from biofouling removal.

Water toxicity associated with copper loading is mediated by the biological, physicochemical and hydrographic conditions of the surrounding environment. Once released from a coating, dissolved copper may take a number of chemical forms in natural seawater environments, including the hydrated free copper ion (Cu^{2+}), dissolved organic copper (labile and inert), inorganic copper complexes, and colloidal and particulate copper (Morel, 1983). In general, only the free copper ion represents the bioavailable fraction of copper in the marine environment (Morel, 1983). Ligands, dissolved organic carbon (DOC), and other compounds associated with biofilms may be present in sufficient quantity in the surrounding water to bind with the released copper, decreasing its bioavailability and toxicity. For example, biofilms such as organic copper-binding ligands in coastal estuaries have been shown to effectively buffer copper toxicity even at relatively high copper loadings (Buck and Bruland, 2005; Rivera-Duarte et al., 2005). Copper concentrations that exceed the binding capacity of natural ligands can lead to potentially toxic copper conditions (Brand, Sunda, and Guillard, 1986; Rivera-Duarte et al., 2005), which can be an issue in harbors and marinas, where there are large concentrations of active vessels and where water circulation may be limited (Schiff, Diehl, and Valkirs, 2004). Similar physiochemical behavior is associated with dissolved zinc released into the marine environment (Hirose 2006; Bryne, Kump, and Cantrell, 1988; Millero and Hawke 1992; Stanley and Byrne 1990).

Hydrographic conditions, such as currents, wind turbulence, tidal exchange, and naturally occurring organic matter, have a large role in diminishing toxicity from antifouling paint passive leaching in bays or bodies of water with more open geography. In Sinclair Inlet, open geography, currents, wind, tides and DOC produce conditions that naturally buffer copper toxicity. Using procedures recommended by the US EPA for calculating Water-Effect Ratios (WER, US EPA, 1994) Rosen, Rivera-Duarte, Johnston, and Podegracz (2009) reported reduced copper toxicity that supports the adjustment of the national Water Quality Criteria (WQC) in Sinclair Inlet by a factor of 1.41 for the dissolved copper criteria. This indicates that an upward adjustment of WQC from 4.8 µg/L to 6.8 µg/L for acute and 3.1 µg/L to 4.4 µg/L for chronic would provide the same level of protection to aquatic life intended by US EPA (US EPA 1994).

2.6 ORGANIC MATTER IMPAIRMENT TO WATER BODIES

Water bodies can be adversely impacted by elevated concentrations of organic matter, the decay of which could result in increases of nutrients, primary productivity (e.g. plankton bloom), and associated decreases in dissolved oxygen (DO). The active removal of fouling organisms from a vessel releases organic matter to the immediate aquatic environment. A portion of the released material is composed of living organisms that continue living on the sediments or strata where they are deposited, and another portion is made up of organic matter (i.e. dead organisms) that undergo biochemical decomposition and could potentially drive increases in nutrients and decreases in DO (Valkirs et al., 1994). A portion of the released organic material is also consumed by other organisms, buffering the effect of organic material release. Also, similar to metal loadings, the effect from organic matter loading is diminished by hydrographic conditions. Currents, tidal effects, and wind-induced water turbulence add oxygen to the water, buffering the impacts from organic matter loading.

A simplified diagram illustrating the complex processes and interactions influencing the fate of organic matter release in a coastal marine embayment is provided in Figure 3. In addition to biofouling removal from the ship hull, there are other continuous and intermittent sources (i.e., stormwater runoff) and sinks (i.e., hydrography) of organic material that influence any effects of nutrient loading. Other sources of organic matter, hydrodynamics of the water body, meteorological affects and decay rates of organic matter are additional factors contributing to the complexity of the system and the challenges associated with understanding the effects of organic matter release.

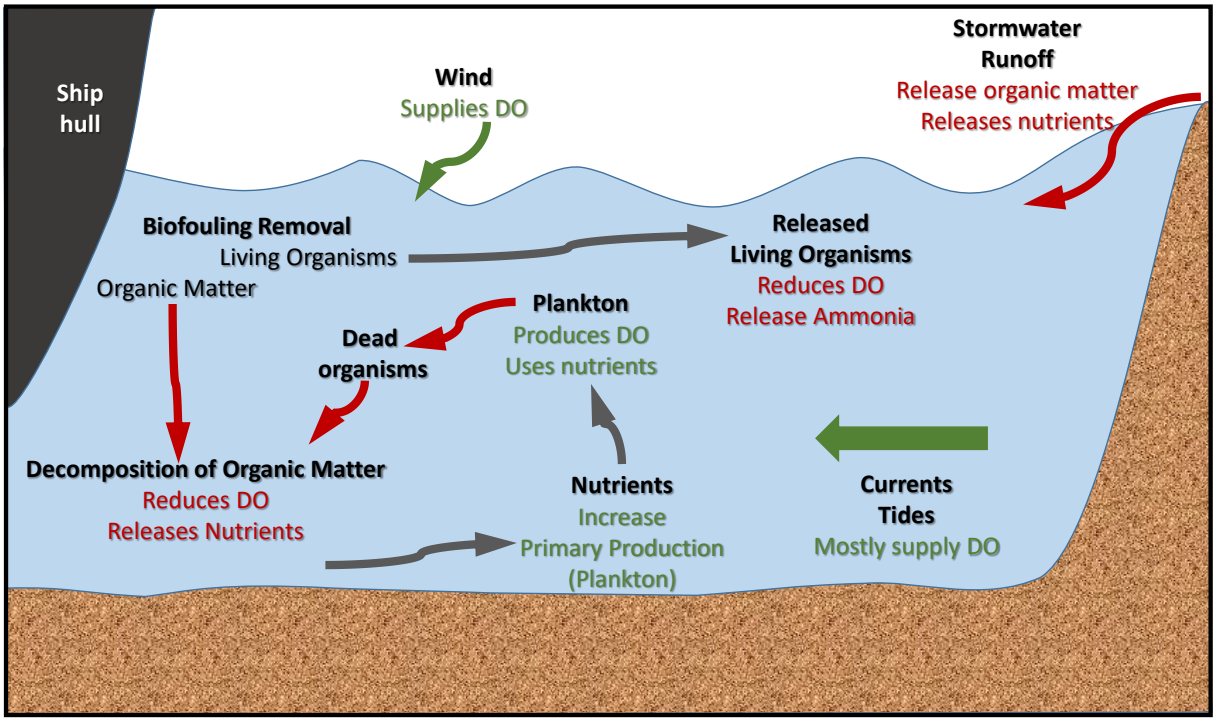

Figure 3. Simplified diagram of the processes affecting the release of organic matter in a coastal marine embayment.

The most significant deleterious effect associated with excessive organic matter loading is the development of hypoxic or anoxic conditions. Hypoxia is a condition where the amount of DO in the water is not adequate for aerobic organisms, while anoxia is a complete lack of DO. Hypoxic (i.e.,

Dead Zones) and anoxic conditions are associated with excessive anthropogenic nutrient pollution (e.g., waste water treatment plant effluent, stormwater runoff, fouling removal, etc.) coupled with other factors (i.e., water stagnation, persistent thermocline) that combine to deplete the oxygen levels required to support most marine life in bottom and near-bottom water (NOAA 2017). The conditions associated with the generation of hypoxic conditions are part of a complex ecological system, described as an imbalance between inputs of nutrients and sources of DO. Generation of hypoxic and anoxic conditions are diminished by active sources of DO, mostly hydrographic conditions (i.e., currents, tides, wind, and stream inputs of DO), exchange with the atmosphere, and primary production (i.e., photosynthesis; Figure 3).

2.7 WATER QUALITY EVALUATION AND STANDARDS

Increases in dissolved copper (Cu) and zinc (Zn) concentrations in Sinclair Inlet associated with biofouling removal from the ex-INDEPENDENCE were evaluated by comparing measured data, including reference sites, to established water quality standards. These standards are developed to ensure the maintenance of water quality conditions appropriate to the category assigned to the water body. The aquatic life category for Sinclair Inlet is Class A, Excellent quality (Department of Ecology, Specific Use Designations for Marine Water, Washington Administrative Code (WAC) 173-201A-130). This designates Sinclair Inlet as having excellent quality for aquatic life uses, including salmonid and other fish migration, rearing, and spawning; clam, oyster, and mussel rearing and spawning; crustaceans and other shellfish (e.g. crabs, shrimp, crayfish, scallops, etc.) rearing and spawning (Department of Ecology, WAC 173-201A-610).

Water quality standards adopted by the Department of Ecology for aquatic life in the state of Washington that are relevant to this study are presented below for copper and zinc (Table 1), turbidity (Table 2), and DO (Table 3). There are no numerical standards for nutrients, where the end point of concern is low DO. For the case of ammonia which is measured as total ammonia, the water quality standard is based on un-ionized ammonia (Ecology, 2011, 2012) which is a function of physicochemical conditions of the receiving water (salinity, pH and temperature) and can be calculated for total ammonia (US EPA, 1989). Based on ambient data collected in Sinclair Inlet for this effort, the average (± 1 standard deviation) salinity was 30.00 ± 0.51 PSU, pH 7.91 ± 0.15, and temperature 9.35 ± 1.97°C. Rounding up to salinity 30 PSU, pH 8.0, and temperature 10°C, the US EPA recommended Water Quality Criteria for total ammonia is 15 mg/L and 2.1 mg/L, for acute and chronic exposure, respectively (US EPA, 1989).

Table 1. Water quality criteria for copper and zinc in marine waters (Ecology, 2011, 2012).

Metal	Acute[1] (µg/L)	Chronic[2] (µg/L)
Dissolved copper	4.8	3.1
Dissolved zinc	90.0	81.0

Notes:

[1] 1-hour average concentration not to be exceeded more than once every three years on the average.

[2] 4-day average concentration not to be exceeded more than once every three years on the average.

Table 2. One-day maximum turbidity for the aquatic life use category of Sinclair Inlet (Ecology,

2012).

Category	Nephelometric Turbidity Units (NTUs)
Excellent quality[1]	Turbidity must not exceed: • 5 NTU over background when the background is 50 NTU or less; or • 10 percent increase in turbidity when the background turbidity is more than 50 NTU

Notes:

[1] Turbidity allowed as a result of human actions for "Excellent Quality" aquatic life use category.

Table 3. Aquatic life dissolved oxygen (DO) criteria (Ecology, 2012).

DO criteria in Marine Water for aquatic life use categories[123]	
Category	Lowest 1-Day Minimum
Extraordinary quality	7.0 mg/L
Excellent quality[4]	6.0 mg/L
Good quality	5.0 mg/L
Fair quality	4.0 mg/L

Notes:

[1] When a water body's DO is ≤0.2 mg/L of the criteria and that condition is due to natural conditions, then human actions considered cumulatively may not cause the DO of that water body to decrease more than 0.2 mg/L.

[2] Concentrations of DO are not to fall below the criteria at a probability frequency of more than once every ten years on average.

[3] DO measurements should be taken to represent the dominant aquatic habitat of the monitoring site. This typically means samples should not be taken from shallow stagnant backwater areas, within isolated thermal refuges, at the surface, or at the water's edge.

[4] The aquatic life use category for Sinclair Inlet is "Excellent quality"

3. OBJECTIVES AND ASSESSMENT CRITERIA

The objectives of this study were to monitor water quality changes over the evolution of biofouling removal from the ex-INDEPENDENCE, by analyzing water quality parameters including:

- Metals (total and dissolved Cu and Zn)
- Dissolved Oxygen
- Turbidity
- Nutrients (nitrates, nitrites and ammonia)
- DOC and BOD

In order to address these objectives, water quality sampling was conducted before, during and after biofouling removal to measure: metals (total and dissolved Cu and Zn), nutrients (nitrate, nitrite, and ammonia), indicators of organic matter (BOD and DOC) and hydrological conditions (turbidity, DO, pH, temperature, and salinity). In addition, currents at different depths were measured in the area during the study. The data collected were used to assess water quality changes from biofouling removal by comparing the change in parameters between ex-INDEPENDENCE study sites (Ship or CV 62) and locations in western Sinclair Inlet outside of the area of influence (Reference sites) before, during and after conclusion of the biofouling removal activity. The results were compared to historical studies, ongoing local and regional monitoring within Sinclair Inlet and Puget Sound ecosystem, Washington State water quality standards (WAC 173-201A) and US EPA Water quality standards. A sampling and analysis plan (SAP) for water quality monitoring was developed to guide this effort and establish quality control procedures (SSC Pacific and NUWC, 2016).

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4. METHODS

4.1 STUDY LOCATION

The study was conducted in the western portion of Sinclair Inlet, Bremerton WA (Figure 4). Sampling locations were distributed in the vicinity of the ex-INDEPENDENCE (CV 62) Figure 5, which was berthed at Mooring G of Naval Base Kitsap, Bremerton.

Figure 4. Berthing Location of the ex-INDEPENDENCE (CV 62) along Mooring G at Naval Base Kitsap, Bremerton in Sinclair Inlet, Puget Sound, WA.

Figure 5. Ex-INDEPENDENCE docked at Mooring G of the Navy Inactive Ships Maintenance Office in Naval Base Kitsap, Bremerton, WA.

4.2 STUDY AREA AND SAMPLING DESIGN

4.2.1 Area of Influence

The spatial boundaries of the study are defined as the area of influence near the ex-INDEPENDENCE (Figure 6) and reference locations outside the area of influence but within the western portion of Sinclair Inlet. The area of influence is defined as the area most likely to be impacted by material released during biofouling removal, and was determined using the particle-tracking model, General NOAA Operational Modeling Environment (GNOME; NOAA, 2014). GNOME was linked to output from the model curvilinear hydrodynamics in 3 dimensions (CH3D) developed for Sinclair and Dyes Inlets (Johnston et al., 2009). For details on the GNOME/CH3D modeling process, parameters and simulation results, see Appendix A of the Sampling and Analysis Plan (SAP) for water quality monitoring in the PWP (SSC Pacific and NUWC, 2016).

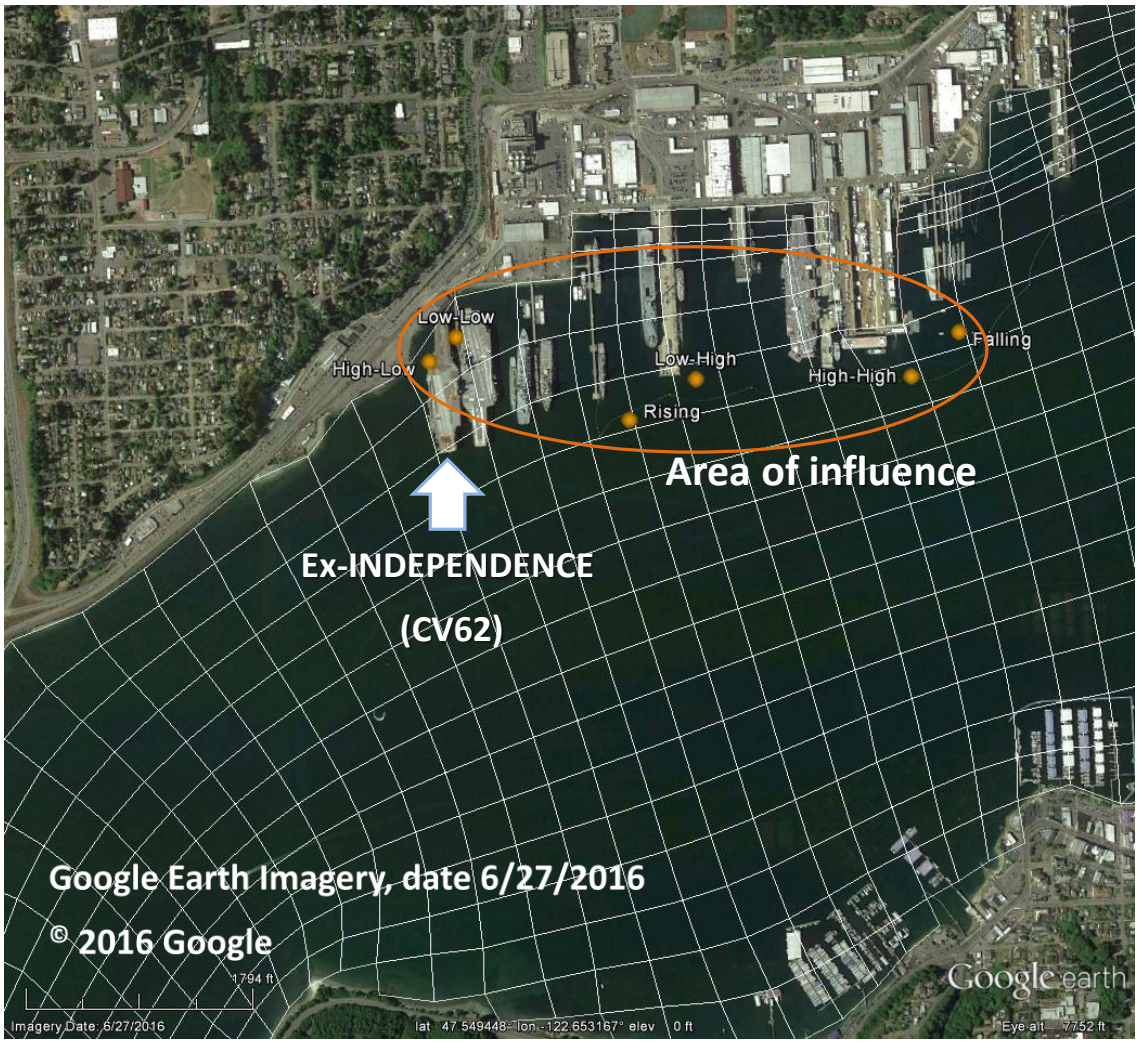

Figure 6. Location of the ex-INDEPENDENCE and area of influence (orange oval) defined by GNOME/CH3D simulations for the varying tide states.

4.2.2 Water Column Monitoring Stations

Water column monitoring stations (Table 4) were established to include stations directly adjacent to the CV 62 at Mooring G (Figure 7) and reference locations outside of the area of influence (Figure 8). Current data was collected with an upward facing acoustic Doppler current profiler (ADCP) moored on the bottom approximately 200 m from the ship (Figure 7).

Table 4. Water quality sampling locations adjacent to CV 62 and at reference locations.

Station ID	Type	Description	Latitude	Longitude
CV62-1	Ship	Bow of ship at Mooring G	47.55400	-122.65698
CV62-2	Ship	Midship forward starboard side at Mooring G	47.55334	-122.65666
CV62-3	Ship	Midship aft starboard side at Mooring G	47.55204	-122.65662
CV62-4	Ship	Stern of ship at Mooring G	47.55108	-122.65695
CV62-5	Ship	Midship aft port side at Mooring G	47.55206	-122.65753
CV62-6	Ship	Midship forward port side at Mooring G	47.55330	-122.65731
R500-1	Reference	500 m 245° (West) of CV 62	47.54913	-122.66321
R500-2	Reference	500 m 168° (South) of CV 62	47.54316	-122.65063
R1000-1	Reference	1000 m 250° (West) of CV 62	47.54359	-122.66584
R1000-2	Reference	1000 m 140° (South East) of CV 62	47.54650	-122.65684

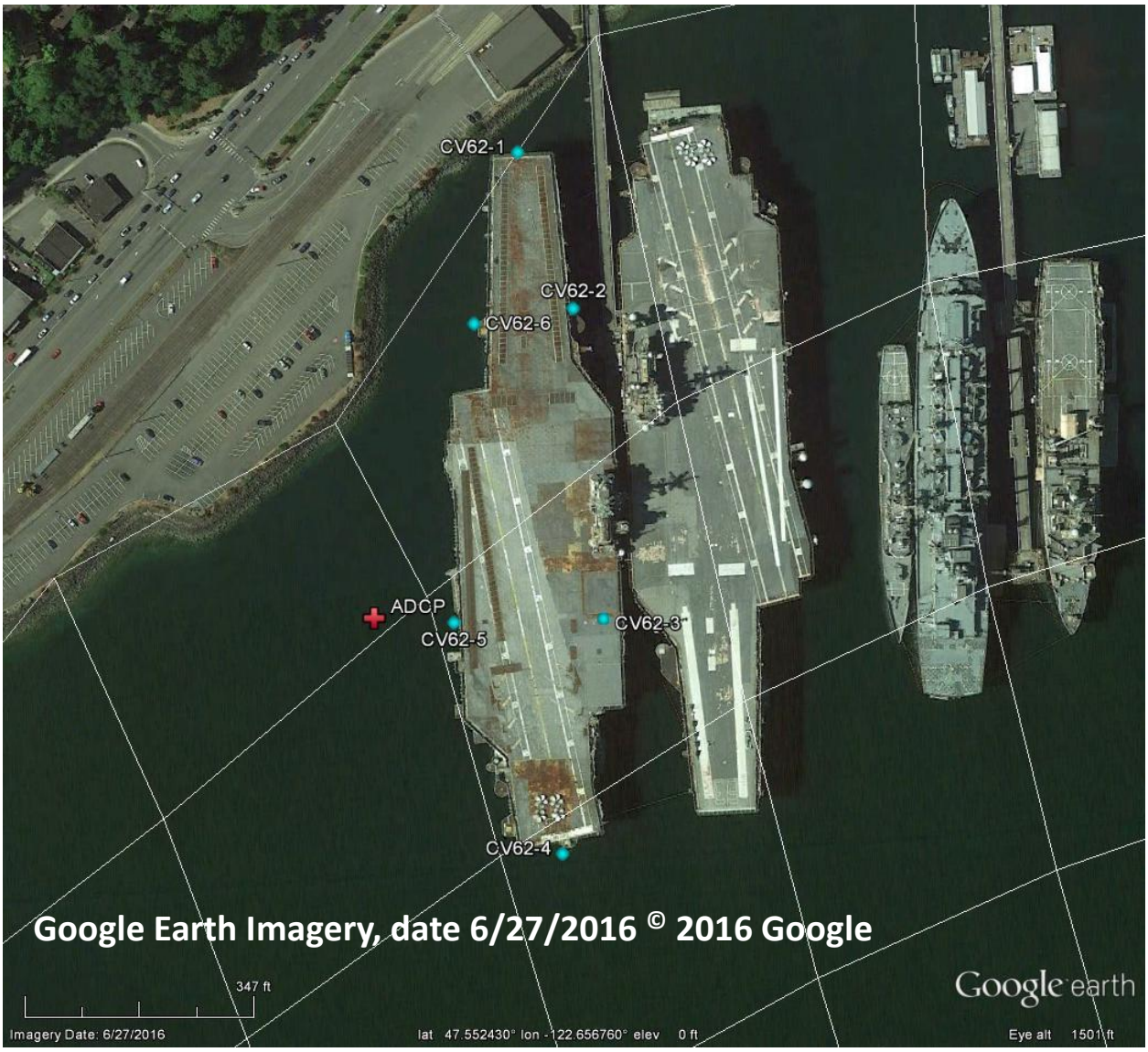

Figure 7. Location of water quality monitoring stations directly adjacent to CV 62 (blue circles), and location of ADCP (red cross). Note that CV62-3 is located at the water under the flight deck of the ex-INDEPENDENCE.

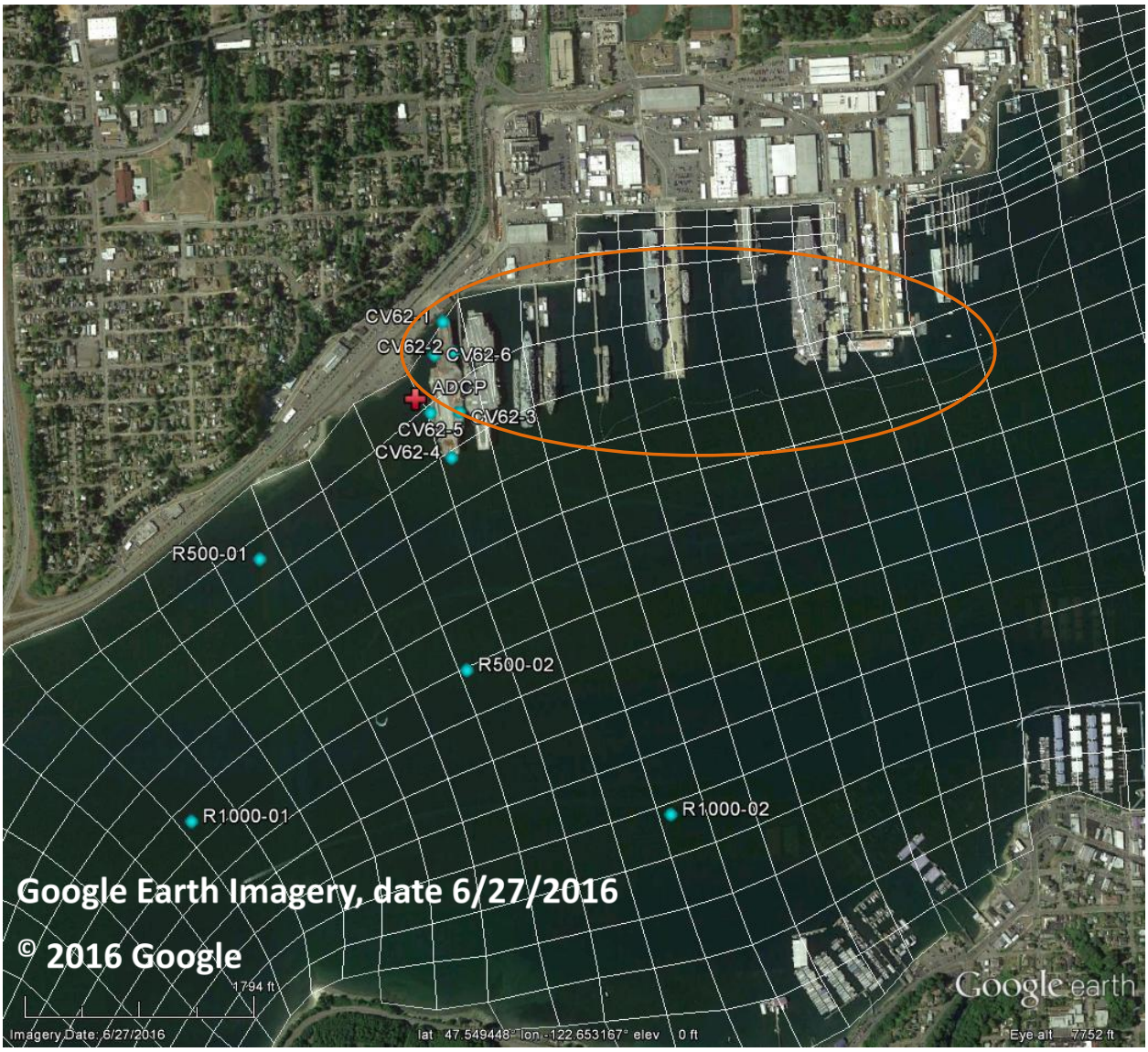

Figure 8. Location of water quality monitoring stations directly adjacent to the ex-INDEPENDENCE (CV62-1 to CV62-6), reference stations (R500-01, R500-02, R1000-01, and R1000-02) outside of the area of influence (orange oval), location of ADCP (red cross), and overlay of CH3D model grid (white rectangles).

4.2.3 Water Quality Sampling Events and Parameters Measured

Sampling was conducted two months prior to biofouling removal to represent baseline conditions, and then over course of the removal evolution at three separate events Table 5.

Table 5. Water quality sampling events during the biofouling removal evolution.

Event Number	Event Name	Description	Dates(s)
Event 1	Baseline	Two months (55 days) prior to removal	November 9-10, 2016
Event 2	During-removal	One week (4 days) after beginning of biofouling removal	January 10, 2017
Event 3	Week-post-removal	One week (4 days) after biofouling removal completed	January 31, 2017
Event 4	Month-post-removal	Six weeks (38 days) after biofouling removal completed	March 7, 2017

Water quality conditions were assessed by measuring the concentrations of copper and zinc, DO, turbidity, nutrients, DOC and BOD. For each sampling event, water samples were collected at each station in three strata: surface (S) (within top 1 meter), mid-depth (M) (at or near the thermocline if present), and near bottom (B) (approximately 1 m above the bottom) (Table 6). Unfiltered (i.e., total) samples were analyzed for nitrate (NO_3), nitrite (NO_2), total ammonia, Cu and Zn. Samples filtered (i.e., dissolved) through 0.45 µm pore size were analyzed for DOC, Cu and Zn. A subset of unfiltered samples collected from the near bottom strata were also analyzed and used to evaluate whether organic matter released by the biofouling removal would affect ambient conditions. Copper (Cu) and Zinc (Zn) were quantified (µg/L) in seawater as predictors of the metal loading associated with the biofouling removal from the ex-INDEPENDENCE. These metals are the active ingredients of the antifouling system used on the ship hulls by the Department of the Navy (Seligman et al., 2001; Valkirs et al., 2003). Water column profiles were taken at each station to collect in situ data for temperature, pH, salinity, and DO using oceanographic sensors capable of logging data continuously throughout the water column.

Table 6. The number of planned sampling events (A), parameters (B), and water column profiles (C).

A. Sampling events and discrete water chemistry samples

Events						Discrete Water Chemistry			BOD
Group	Stations	Event 1 (Baseline)	Event 2 (During Removal)	Event 3 (Week Post Removal)	Event 4 (Month Post Removal)	Surface	Mid-Depth	Near-Bottom	Near-Bottom
CV 62	6	1	1	1	1	24	24	24	24
R500m	2	1	1	1	1	8	8	8	8
R1000m	2	1	1	1	1	8	8	8	8
Total	10	3	3	3	3	40	40	40	40

Table 6. The number of planned sampling events (A), parameters (B), and water column profiles (C). (Continued)

B. Parameters analyzed for discrete water chemistry samples

Parameter	Samples (n)	Field Duplicates	MS/MSD	CRM	Total Analyzed
Cu & Zn Total	120	12	12	12	156
Cu & Zn Dissolved	120	12	12	12	156
DOC	120	12			132
Nutrients	120	12			132
BOD	40	4			44

MS= matrix spike; MSD= Matrix Spike Duplicate; CRM= Certified Reference Material

C. Water column profiles collected for in situ temperature, pH, salinity, DO, and turbidity

Group	Profiles
CV 62	24
R500m	8
R1000m	8
Total	40

4.3 WATER SAMPLING PROCEDURES

Trace metal sampling procedures followed ultra-clean sampling techniques (US EPA, 1995, 1996). Ultra-clean water column sampling and analysis was conducted following the methods and procedures described in Bosse et al. (2014). Ultra-clean sampling involves implementing trace-metal clean techniques (US EPA, 1996) throughout preparation of sampling equipment, during field sampling, as well as during laboratory manipulation and analysis of the samples. Ultra-clean procedures include acid-soaking of all sampling material and equipment, collecting water samples using clean hands-dirty hands techniques, using quartz-still grade nitric acid (Q-HNO_3) for sample acidification, and performing laboratory processing of the samples within a high-efficiency particulate air (HEPA) class-100 all polypropylene working area (US EPA, 1996).

Water samples were collected using a peristaltic pump with Teflon® pump-head and weighted Teflon® tubing, and the option for in-line filtration with acid-cleaned high-volume polypropylene 0.45 µm Calix filter cartridges (US EPA, 1996; Figure 9). With the pump engaged and water pumping continuously through the tubing, the weighted Teflon® tubing was lowered to the desired depth (surface, mid-depth, and near-bottom) and the desired sample volume was collected in pre-cleaned sample bottles after rinsing the bottle three times with the flowing sample water. Dissolved samples were collected in a similar manner by toggling the flow through the in-line filtration cartridge (US EPA, 1996). Samples for BOD, nutrients and total metals were collected using the unfiltered option in the system. Samples for DOC and dissolved metals were collected using the filtered option in the system. All samples were labeled and a strict chain-of-custody and data log were kept for all field sampling activities.

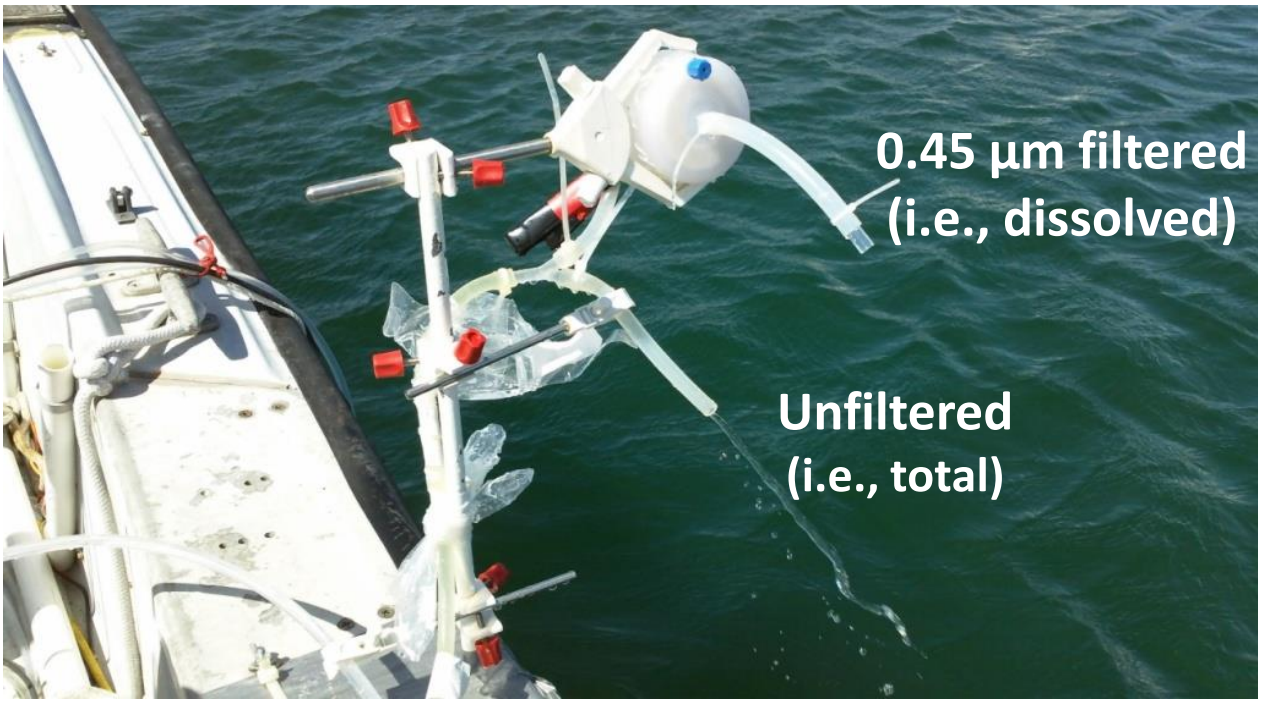

Figure 9. Peristaltic pump set up for collection of unfiltered and filtered water chemistry samples.

4.4 WATER COLUMN PHYSIOCHEMICAL MONITORING

Water column physiochemical properties were monitored with an In-situ Troll 9500 (In-situ, Inc., Fort Collins, CO) water quality parameter sensor calibrated and programmed to collect data continuously every 10 sec during the water quality monitoring surveys. The water quality parameters monitored with the Troll 9500 sensor included temperature (°C), salinity (calculated from conductivity and temperature, PSU), DO (mg/L), percent dissolved oxygen saturation (%), pH, turbidity (NTU), and pressure, which was then used to deduce the exact depth at which the sample was taken (ft). The Troll 9500 was attached to a weighted line 5 ft above a Secchi disk marked at 5 ft increments. The pump intake line of Teflon® tubing was also attached in a similar manner to avoid possible contamination from any disturbances of the seafloor. Continuous water quality data were logged using the Troll 9500, and discrete samples (single events) were collected at each station using the peristaltic system. Samples were taken from three strata: near-bottom, mid-depth and near-surface. These water samples were measured onboard the vessel. A YSI300A Ecosense water quality probe (Yellow Springs Instruments, Yellow Springs, OH) was used to measure temperature (°C), conductivity (mS/cm) and specific conductivity (mS/cm), total dissolved solids (g/L), and salinity (ppt). A desktop turbidity meter (MicroTurbidimeter, HF Scientific, Inc.) was used to measure discrete turbidity (NTU). Measurements obtained from the discrete samples collected at depth were logged for informational purposes only, as the accuracy of these measurements is affected by adiabatic processes occurring in samples collected at depth and measured on the surface. The YSI300A water quality probe was also used to measure surface water conditions directly. The presence/absence of surface sheens and fresh water plumes from rainfall runoff were noted in the field log.

At each sampling location, an onboard fishfinder fathometer (Lowrance 000-12636-001 Hook-3X DSI) was used to estimate the bottom depth. The instrument package was deployed to acquire Secchi

disk depth and then lowered to approximately 3-10 ft. from the bottom to begin water quality sampling.

4.5 ADCP DATA COLLECTION

An upward looking acoustic Doppler current profiler (ADCP, Workhorse Sentinel, Teledyne RD Instruments, San Diego, CA) was moored adjacent to the ex-INDEPENDENCE on November 9, 2016 (Figure 7) and recovered on March 7, 2017. The ACDP was programed to record current speeds and directions through the water column at 5 min intervals for 3 months. Unfortunately, the ADCP was towed out of position on 2 January 2017, as it was attached to a barge that was moved to facilitate biofouling removal. Fortunately, the ADCP was able to provide continues current data for 54 days of the spring-neap tidal cycle, which repeats about every 14 days in Sinclair Inlet.

4.6 DATA ANALYSIS

4.6.1 Analytical Chemistry

Prior to analysis all samples were acidified to pH ≤ 2 with Q-HNO_3 in a HEPA class-100 all polypropylene working area. Both total and dissolved copper and zinc concentrations in the samples were measured with a Perkin-Elmer SCIEX ELAN DRC II inductively coupled plasma mass spectrometer (ICP-MS; US EPA, 1997), following inline concentration and salt matrix removal using flow injection for atomic spectroscopy (FIAS).

Metal concentrations in seawater samples were quantified by flow injection analysis (Beck, Franks, and Bruland, 2002; Bose, Biller and Bruland, 2012) using procedures documented in (Bosse et al., 2014). An on-line Perkin-Elmer FIAS 400 was used for pre-concentration and salt matrix removal using TOYOPEARL AF-Chelate- 650M from Tosoh Corp (Shunan-shi, Yamaguchi, Japan). The FIAS 400 is coupled with an Autosampler 100 and set to inject the treated sample directly into the ICP-MS. Analytical standards were made with Perkin-Elmer multi-element standard solution, PEMES-3, diluted in 0.45 µm filtered and acidified (pH ≤ 2 with Q-HNO_3) seawater collected outside San Diego Bay in September 1999 to match the salinity of the test samples. Standards were analyzed at the beginning and end of the run, with acceptable calibration curves where R ≥0.999. Seawater blanks were analyzed every five samples, and had an average ± standard deviation of 0.73 ± 0.16 µg/L for copper and of 0.43 ± 0.22 µg/L for zinc. The blanks resulted in an average method detection limit (MDL= 3 blank standard deviations [SD]) for the four different ICP-MS runs accepted for this report of 0.47 ± 0.20 µg/L for copper and 0.66 ± 0.28 µg/L for zinc, and a method reporting limit (MRL = 10 SD) of 1.55 ± 0.68 µg/L for copper and 2.20 ± 0.93 µg/L for zinc. The analysis also included measurement of sample duplicates and the certified reference material (CRM) CASS-6, Nearshore Seawater Certified Reference Material for Trace Metals and other Constituents originating from the National Research Council of Canada. Duplicate sample recoveries averaged 101 ± 16 % for copper and 105 ± 47 % for zinc. CASS-6 is certified to 0.530 ± 0.032 µg/L for copper, and 1.27 ± 0.18 µg/L zinc. A coefficient of variation (CV) of ≤ 15% for replicate measurements, as well as a recovery within 15% of CASS-6 were required for acceptance of the quantifications. The actual recovery for CASS-6 was 89 ± 14 % for copper and 92 ± 17 % for zinc. **Note that the metal data presented in this report was not corrected for blanks or CRMs. All quantified values are reported and used in the calculations, whether or not these values are below the MDL or MRL.** Table 7 summarizes all water chemistry analysis parameters, holding times, and accuracy or detection limits for water samples.

Table 7. Water chemistry analysis parameters, holding times, and accuracy/detection limits.

Analytical Parameter	Total Sample Volume (L)	Min volume needed from each replicate (L)	Lab Preservation	Holding Time	Detection Limit	
					Accuracy / Limit of Detection	Units
Measured in situ (Troll 9500)						
Pressure (to derive depth)					± 0.1%	ft
pH					± 0.1	pH
Conductivity					± 0.5%	µS/cm
Salinity					0.1	PSU
Temperature					± 0.1	°C
Turbidity					0.1	NTU
Dissolved Oxygen (DO)					± 0.1	mg/L
Nutrients, BOD and DOC						
Dissolved Organic Carbon (DOC)	1	0.25	Cool, 4° C, H_2SO_4 to pH<2.0	28 days	0.1	mg/L
Nitrate + Nitrite Nitrogen				28 days	0.13	mg/L
Ammonia Nitrogen				28 days	0.008	mg/L
Biological Oxygen Demand (BOD)	0.5	0.3	Cool, 4° C, dark amber bottle	48 hr	0.5	mg/L
Total Metal						
Copper	0.060	0.030	Q-HNO_3 to pH<2.0	6 months	0.47	µg/L
Zinc				6 months	0.66	µg/L
Dissolved Metal (0.45 µm filter)						
Copper	0.060	0.030	Filtered within 24 hours, Q-HNO_3 to pH<2.0	6 months	0.47	µg/L
Zinc				6 months	0.66	µg/L

4.6.2 Statistical Analysis

The raw data were validated based on the pre-defined performance based QA/QC procedures identified in the PWP (SSC Pacific and NUWC, 2016), and all useable data were combined into a flat file (MS Excel) for statistical analysis. The raw data from the Troll 9500 casts were downloaded, imported into Excel, verified for QA/QC, and assigned to station location and depth for measurements that corresponded to the discrete water samples. Pressure data were converted into sample depth (ft) after adjusting for surface displacement. In-situ temperature (°C), salinity (psu), pH, DO (mg/L), and percent dissolved oxygen saturation (%) were used as recorded, and the raw turbidity readings were converted into NTU using a two-point calibration curve generated during instrument calibration. Any spurious data records from bubbling, non-equilibration, or hitting bottom with the instrumentation were eliminated during the QA/QC review. Raw data from the analytical chemistry analysis by SSC Pacific and contract laboratories (ALS Inc., Kelso, WA, and EnviroMatrix Inc., San Diego, CA) were subjected to an independent unbiased QA/QC review to validate data quality. The analytical chemistry raw data that passed QA/QC review were merged with the in-situ and other discrete data. Non-detected (ND) values were replaced with the sample-specific MDL/2, and any sample results failing QA/QC review were omitted from the statistical analysis.

The flat file was imported into R-Studio (v98.1091) running R (v3.01.1, R Foundation for Statistical Computing) for statistical analysis. Descriptive statistics were compiled for the data by Event (1 Baseline, 2 During-Removal, 3 Week-Post-Removal, and 4 Month-Post-Removal), Type (Ship and Reference), and Strata (Surface, Middle, and Bottom) for the in-situ and discrete data. Any missing values were ignored. To visualize the data, a series of x-y plots were generated for the salient variables, where x=days since November 8, 2016 (start of the study) and y = variable of interest (APPENDIX C).

The aim of this study was to assess whether changes in water quality variables could be attributed to biofouling removal from the ex-INDEPENDENCE. To do this, hypotheses were developed following the widely used Before - After - Control - Impact (BACI) design.

The first hypothesis was:

$H1_o$: There are no differences between variables measured within the area of influence (Ship) and the same variables measured outside the area of influence (Reference sites) in western Sinclair Inlet.

The second hypothesis was:

$H2_o$: There are no differences between variables measured at the Ship before biofouling removal (Event 1) and the same variables measured at the Ship during subsequent events (Events 2, 3, 4) that may be affected by biofouling removal.

Prior to conducting statistical tests, histograms of the data were plotted to determine whether the data distribution conformed to a normal distribution and were suitable for parametric analysis of variance (ANOVA) or would be better evaluated using non-parametric statistical tests that do not require assumptions of normality. For practicality, both parametric and non-parametric tests were calculated for both hypotheses.

For H1 the following tests were used

Parametric ANOVA: $F = aov(Y \sim Type, data = EVENTn)$ Equation 1

Non-Parametric: $KW = kruskal.test(Y \sim Type, data = EVENTn)$ Equation 2

For H2 the following tests were used:

Parametric T-test: $T = t.test(Y_{base}, Y_{EVENTn})$ Equation 3

Non-Parametric: $W = wilcox.test(Y_{base}, Y_{EVENTn})$; $n>1$ Equation 4

Where

Y = variable of interest
Type = type of sample (Ship or Reference)
EVENTn = Subset of data from Event n
Y_{base} = variable of interest from Event 1
$Y_{ENVENTn}$ = variable of interest from subsequent event
n = number of sampling events

And

F, KW, T, and W = statistical result
p(F), p(KW), p(T), and p(w) = probability of random result
$p \leq 0.05$ denotes statistical significance

Box and whisker plots by Event (1, 2, 3, 4), Type (Ship or Reference), and Strata (Surface, Mid-Depth, Bottom) were constructed to visualize statistical comparisons and evaluate the magnitude of the differences detected.

4.6.3 Decision Framework

A decision matrix Table 8) was used to evaluate whether biofouling removal caused any impacts to water quality in Sinclair Inlet. The decision takes into account whether there were statistical differences between the ship and reference sites, the magnitude of the difference, and the potential of exceeding a regulatory benchmark or threshold, if applicable. Accordingly, if there is no difference between the ship and reference sites for a water quality parameter, or the data from the ship site indicates better water quality at the ship sites compared to reference sites, then the conclusion would be no water quality impact from biofouling removal. If there are statistically significant differences showing water quality conditions at the ship worsened relative to the reference sites, the conclusion about water quality impact would depend on the magnitude of the difference and potential of exceeding a benchmark or standard (Table 8). The decision matrix allows the degree of water quality impact to be evaluated in a quantitative manner and is similar to approaches commonly used in environmental risk and assessment studies (Johnston et al., 2002; Thom et al, 2005; Labisoa et al., 2014; Diefenderfer et al., 2016). Table 8. Decision matrix used to assess the impact of biofouling removal from the hull of the ex INDEPENDENCE on water quality in Sinclair Inlet, WA.

			Potential of Exceeding Benchmark or Standard					
			% of Threshold					
			≤25%	>25% and <50%	>50% and <75%	>75% and <100%	>100% and <150%	>150%
	Magnitude of Difference		Very Low	Low	Medium	High	Very High	Adverse
Statistical Difference from Reference	No Difference or Better than Reference	None	No Impact	No Impact	No Impact	No Impact	No Impact	No Impact
	≤2x Reference	Slightly Different	Negligible Impact	Low Potential for Impact	Low Potential for Impact	Medium Potential for Impact	High Potential for Impact	Adverse Impact Likely
	>2x and <5x Reference	Different	Negligible Impact	Low Potential for Impact	Medium Potential for Impact	Medium Potential for Impact	High Potential for Impact	Adverse Impact Likely
	≥5x and <10x Reference	Highly Different	Negligible Impact	Low Potential for Impact	Medium Potential for Impact	High Potential for Impact	High Potential for Impact	Adverse Impact Likely
	>10x Reference	Very Highly Different	Negligible Impact	Medium Potential for Impact	High Potential for Impact	High Potential for Impact	Adverse Impact Likely	Adverse Impact Likely

Note: The conclusion (shown in Table cells) is based on the magnitude of statistical differences between the ship and reference sites (Table rows) combined with the potential of exceeding a regulatory benchmark or standard (Table columns). The colors within each conclusion box inform the severity of impacts ranging across the following values: negligible (Yellow), low (lite pink), medium (dark pink), high(bright pink), and adverse (Red).

4.7 QUALITY CONTROL

A Project Work Plan (PWP) was prepared to document the sampling and analysis procedures (SSC Pacific and NUWC, 2016). The quality assurance (QA) and quality control (QC) procedures identified in the PWP were used to assure transparency, consistency, comparability, completeness, and confidence in meeting the data quality objectives defined for the study.

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5. RESULTS

5.1 FIELD SAMPLING CONDITIONS

During the field sampling events, there was a 10-12°C drop in air temperature between the Event 1 survey and subsequent events. Weather varied across the events from clear and warm for Event 1 to wet and cold for Event 2, clear and cold for Event 3, and very wet and freezing cold for Event 4 (Table 8). The weather created some logistical problems resulting in the discrete turbidity and Secchi disk depths not being measured during Event 2, and field duplicates were not collected in several cases.

Table 8. Field conditions during each of the sampling events (A) and antecedent rainfall and weather (B) associated with sampling.

A. Weather Conductions During Sampling Events

		Stations Sampled		Tide		Air Temp (°C)	
Event	Date	Ship	Ref	Condition	Avg (ft)	High	Low
1-Baseline	11/9/2016	5		High	11.2	14.8	10.5
	11/10/2016	1	4	Rising	6.8	16.7	12.2
2-During Removal	1/10/2017	6	4	High	11.1	2.2	1.1
3-End of Removal	1/31/2017	6	4	High-Low	6.6	8.1	1.0
4-After Removal	3/7/2017	6	4	Falling	7.9	3.3	0.0

B. Antecedent Rainfall and Weather

	Antecedent Rainfall Cumulative Rainfall (in) Prior to Sampling					
Event	06 hrs	12 hrs	24 hrs	48 hrs	72 hrs	Weather .
1-Baseline (11/9)	0.08	0.13	0.13	0.25	0.38	Overcast, cool and calm with rain
1-Baseline (11/10)	0.01	0.02	0.15	0.15	0.27	Partly cloudy, calm
2-During Removal	0.16	0.21	0.21	0.86	0.86	Overcast, cold, showers and wind 5-7mph
3-End of Removal	0.00	0.00	0.00	0.00	0.00	Clear, cold and windy 2-7mph
4-After Removal	0.42	0.42	0.74	1.53	1.80	Cold pouring rain, sleet at times

The cumulative rainfall over the study period from October 1, 2016 to April 14, 2017 is shown in Figure 10. About 15 inches of rainfall occurred prior to sampling Event 1, 11.56 inches of rain fell between Event 1 and Event 2, 3.51 inches of rain fell between Event 2 and Event 3, and another 9.45 inches of rain fell between Event 3 and Event 4. A total of 4.33 inches of rain fell during biofouling removal including a storm event of over 3 inches of rain that occurred between January 18 and 19, 2017. The relatively high amount of rainfall that occurred during the study period indicates that stormwater and freshwater runoff into Sinclair Inlet were important contributing factors during the study.

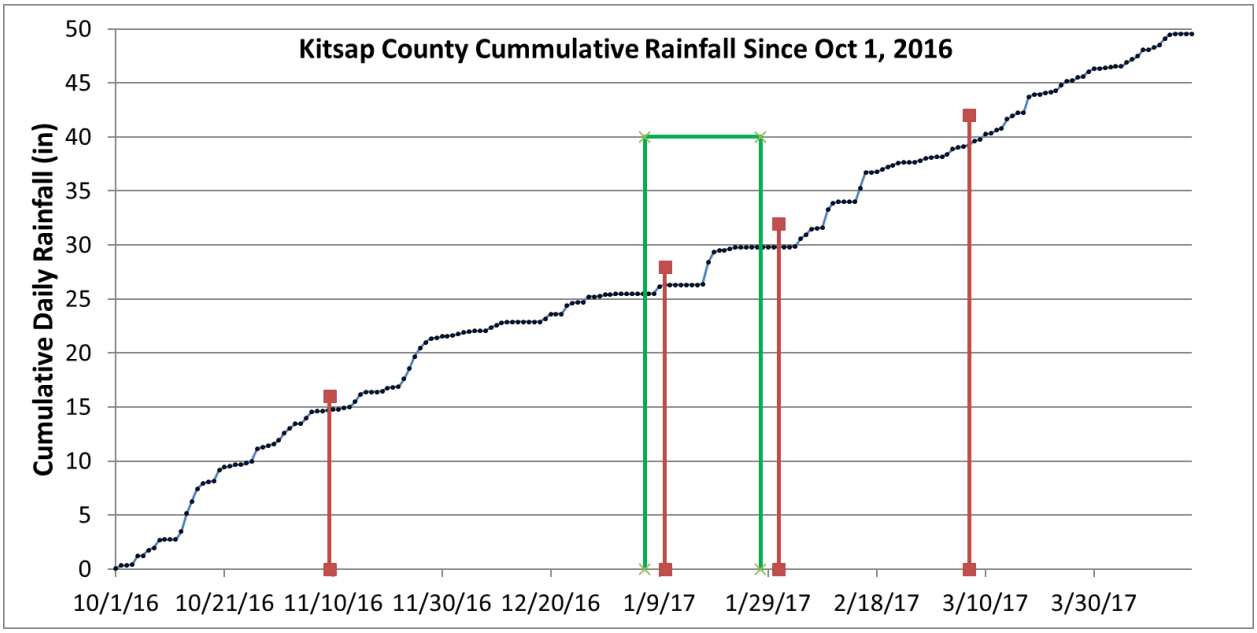

Figure 10. The average cumulative rainfall reported for rain gauges in Kitsap County from October 1, 2016 to April 17, 2017 (www.cocorahs.org/ViewData/RainyDaysReport.aspx). The water quality monitoring events (red bars) and biofouling removal period (green box) are also shown.

5.2 CURRENT METER DEPLOYMENT

The ADCP current meter recorded current velocities and directions throughout the water column at 5 min intervals from November 9, 2016 until January 2, 2017 at the location near CV 62 (see Figure 7**).** The data record from the ADCP showed that the current speed averaged throughout the water column ranged from 0 – 5 cm/sec for most of the deployment period (Figure 11. A), with higher current speeds of up to 55 cm/sec near the surface (Figure 11. B) and lower speeds near the bottom (Figure 11. C). The prevailing direction of the surface currents toward the Northeast was consistent with predictions from the GNOME/CH3D simulations used to identify the area of influence (see Appendix A of Water Quality SAP, SSC Pacific and NUWC 2016).

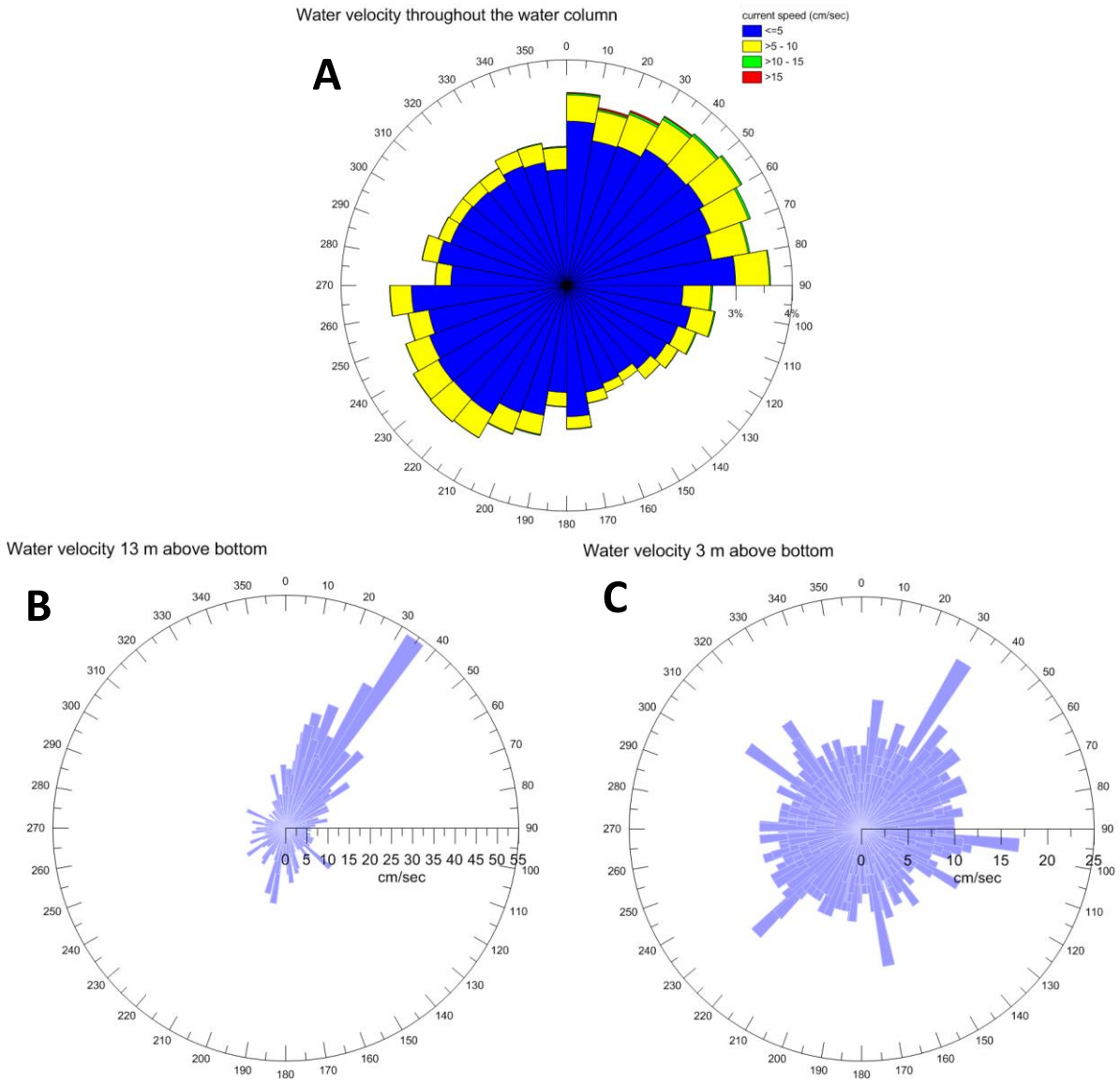

Figure 11. Current speed and direction recorded by ACDP moored near CV 62 showing the average velocities throughout the water column (A), near the surface (B, about 40 ft water depth), and near the bottom (C, about 9ft from bottom).

5.3 FIELD DATA, STATISTICAL DATA AND QA/QC

The raw data from field sampling is provided in Appendix A.2, A.5 and A.7. The data flat file used for statistical analysis is provided in Appendix A.6. The independent, non-biased QA/QC narrative and raw data tables are provided in Appendix B.

5.4 EXPLORATORY DATA ANALYSIS, DESCRIPTIVE STATISTICS AND HYPOTHESIS TESTING

Descriptive statistics were compiled for the data by *Event* (1 Baseline, 2 During-Removal, 3 Week-Post-Removal, 4 Month-Post-Removal), *Type* (Ship and Reference), and *Strata* (Surface, Mid-Depth, and Bottom) for in-situ and discrete data (Appendix C: Table C-1). To visualize the data, a series of x-y plots were generated for the salient variables, where x=days since November 8, 2016 (start of the study) and y = variable of interest (APPENDIX C: Figure C-1 to C-7). Results of statistical tests are shown in Appendix C: Table C-2. Box and whiskers plots for measured parameters are displayed in APPENDIX C, Figure C-8 to C-176).

5.5 TEMPERATURE, SALINITY, PH

There was a sharp decrease in water temperature between Event 1 and subsequent events from the onset of winter (Appendix C: Table C-1, Figure C-1, and Figure C-7). The significant reduction in water temperature has effects on the results of the study. With the onset of winter, the decrease in temperature and reduction in sunlight cause plankton growth to be diminished; microbial growth and metabolism are essentially shutdown; and the decrease in water temperatures increases the solubility of dissolved oxygen. Winter storm-related wind and rain increase the already substantial tidal mixing, and the water column is more likely to be stratified by fresh water plumes from storm runoff and increased stream flow from the watershed than from temperature which occurs in the warmer months.

The salinity measured during the study varied between events (Appendix C: Figure C-1, Table C-1). Lower salinities at the Ship compared to Reference stations for Event 2 and Event 3, can be attributed to the proximity of a major storm drain from the City of Bremerton that drains directly in front of Mooring G, < 200 ft from the bow of ex-INDEPENDENCE.

The water column pH remained relatively constant during the study (Appendix C: Figure C-1, Table C-1), ranging between pH 7.7 and 8.1. A slight upward drift in pH during the course of the study was probably related to an aging pH probe.

5.6 DISSOLVED AND TOTAL METALS

A summary of dissolved Cu, total Cu, dissolved Zn, and total Zn measured at Ship and Reference sites for each sampling event is provided in Table 9. The raw data are provided in Appendix B2, statistical summaries are provided in Appendix C Table C1, and the statistical hypothesis testing results are provided in Appendix C Table C2. For the statistical analysis, data that did not meet data validation criteria were omitted and non-detected (ND) values were substituted for half of the detection limit (DL/2). The results and magnitude of differences calculated for statistical tests for hypothesis $H1_O$ (no difference between Ship and Reference) and $H2_O$ (no difference at the Ship during Event 1 and subsequent events) are also summarized in Table 9.

Table 9. Summary of dissolved Cu (A), total Cu (B), dissolved Zn (C), and total Zn (D) measured at Ship and Reference sites and results of statistical tests for hypothesis $H1_O$ (no difference between Ship and Reference) and $H2_O$ (no difference at the Ship during Event 1 and subsequent events). Note that for the statistical analysis ND values were substituted for the half of the detection limit (DL/2).

A.	**SHIP**					**REFERENCE**						
	Dissolved Cu (µg/L)					**Dissolved Cu (µg/L)**					**Statistical Significance**	
	n	mean	stdev	min	max	n	mean	stdev	min	max	**$H1_O$**	**$H2_O$**
Event 1	18	0.72	0.19	0.48	1.14	12	0.49	0.06	0.38	0.61	+	
Event 2	18	0.77	0.35	0.38	1.58	12	0.47	0.26	0.05	0.82	+	NS
Event 3	18	0.46	0.44	0.05	1.54	12	0.14	0.09	0.05	0.36	↑	NS
Event 4	18	0.60	0.08	0.50	0.74	12	0.56	0.03	0.51	0.60	NS	-
B.	**SHIP**					**REFERENCE**						
	Total Cu (µg/L)					**Total Cu (µg/L)**					**Statistical Significance**	
	n	mean	stdev	min	max	n	mean	stdev	min	max	**H1o**	**H2o**
Event 1	18	0.87	0.26	0.49	1.69	12	0.71	0.14	0.51	0.94	NS	
Event 2	18	3.65	2.75	0.99	10.21	12	0.93	0.26	0.45	1.36	↑↑	↑
Event 3	18	2.69	1.58	1.04	7.16	12	0.78	0.31	0.38	1.44	↑↑	↑
Event 4	18	0.77	0.12	0.60	0.97	12	0.63	0.04	0.57	0.70	+	NS
C.	**SHIP**					**REFERENCE**						
	Dissolved Zn (µg/L)					**Dissolved Zn (µg/L)**					**Statistical Significance**	
	n	mean	stdev	min	max	n	mean	stdev	min	max	**H1o**	**H2o**
Event 1	8	1.25	0.33	0.83	1.92	9	1.47	0.42	0.85	1.89	NS	
Event 2	18	0.39	0.31	0.10	1.03	12	0.91	0.35	0.10	1.38	-	↓
Event 3	18	1.84	0.39	1.30	2.56	12	1.75	0.19	1.41	2.01	NS	+
Event 4	18	1.10	0.07	1.00	1.23	12	1.06	0.02	1.01	1.10	+	NS
D.	**SHIP**					**REFERENCE**						
	Total Zn (µg/L)					**Total Zn (µg/L)**					**Statistical Significance**	
	n	mean	stdev	min	max	n	mean	stdev	min	max	**H1o**	**H2o**
Event 1	18	1.99	1.02	0.94	5.40	12	1.59	0.84	0.10	2.93	NS	
Event 2	18	1.50	0.73	0.10	3.82	12	1.61	0.31	1.02	2.02	NS	NS
Event 3	18	1.14	0.24	0.84	1.61	12	1.39	0.34	1.03	2.15	↓	–
Event 4	18	1.27	0.11	1.10	1.45	12	1.13	0.04	1.07	1.19	+	-

NS Not Significant
\+ Ship sites slightly higher than reference
\- Ship sites slightly lower than reference
↑ Ship sites higher than reference
↓ Ship sites lower than reference
↑↑ Ship sites much higher than reference

5.6.1 Dissolved Copper

Throughout this study, all copper concentrations measured at Ship stations as well as Reference stations were well below acute (4.8 µg/L) and chronic (3.1 µg/L) water quality criteria for copper in marine waters (Figure 12; Appendix C: Figure C-5; see Table 1 for WQS). Dissolved copper concentrations ranged from non-detect (ND) (< 0.47 ± 0.20 µg/L) to 1.58 µg/L with the highest concentrations measured during Event 2 and Event 3 at station CV62-6S. Narrow data ranges and low concentrations were characteristic of all events and stations. For Event 1, data ranges were 0.48 to 1.14 µg/L and 0.38 to 0.61 µg/L for Ship and Reference stations respectively. For Event 2, data ranges were 0.38 to 1.58 and 0.0 (ND) to 0.82 µg/L respectively. For Event 3, data ranges were ND to 1.54 and ND to 0.36 µg/L respectively. For Event 4, data ranges were 0.50 to 0.74 and 0.51 to 0.60 µg/L respectively. (Appendix C: Table C-1; Figure C 12).

Examination of spatial differences (Ship vs. Reference sites; Hypothesis 1) show that dissolved copper measured at Ship stations was statistically higher than Reference stations for Event 1 (p= 0.0001), Event 2 (p= 0.0156) and Event 3 (p= 0.0195), but differences were very small (less than a factor of 2.0) and dissolved copper concentrations were less than 50% of the chronic threshold of 3.1 µg/L. There was no statistical difference in dissolved copper concentrations between Ship and Reference sites at Event 4 after the end of biofouling removal activities (Appendix C: Table C-1, and Table C-2).

Examination of temporal variation (Event 1 vs. Events 2, 3, 4; Hypothesis 2) shows that there was no statistical difference in dissolved copper concentrations at the Ship stations between Event 1 and Event 2 or between Event 1 and Event 3. Event 4 showed a small but significant (p= 0.0270) reduction in dissolved copper concentrations compared to Event 1 (Appendix C: Table C-1, and Table C-2).

In summary, the key findings for dissolved copper are: 1) all measurements across all stations and sampling events were well below the WQS; 2) ship stations showed slightly elevated concentrations of dissolved copper compared to reference stations at Events 1, 2 and 3 but no difference between ship and reference stations at Event 4 after biofouling removal; and 3) among ship stations there was no statistical difference in dissolved copper concentrations between Event 1 and Events 2 and 3, with a slight decrease in concentration to below Baseline levels at Event 4.

5.6.2 Total Copper

The spatial (i.e., between stations) and temporal (i.e., between Events) trends were more pronounced for total copper concentrations (Figure 13; Appendix C: Figure C-5, and Figure C-14). At reference stations, the range in total copper concentration was fairly narrow (0.38 to 1.44 µg/L) across the four sampling events. In contrast, the range in total copper concentration at ship stations was more broad (0.49 to 10.21 µg/L) with the largest ranges and highest maximums measured during Event 2 (0.99 to 10.21 µg/L) and Event 3 (1.04 to 7.16 µg/L).

Statistical tests for Hypothesis 1 showed no difference in total copper measurements between Ship and Reference stations at Event 1. However, there were significant differences between Ship and Reference stations for Events 2 and 3. On average, total copper levels at Ship stations increased to about 2 to 3 times above Reference stations (Event 2 Ship: mean 3.65 ± 2.75, Reference: mean 0.93 ± 0.26, p=0.0021; Event 3 Ship: mean 2.69 ± 1.58, Reference: mean 0.78 ± 0.31, p=0.0003) which occurred during biofouling removal. However, the increase in total copper was not persistent as by Event 4 (6 weeks after hull cleaning completed), total copper measured at Ship stations (mean 0.77 ± 0.12) had returned to nearly the same level as Reference stations (mean 0.63 ± 0.04, statistically

different at p=0.0004) (Figure C-5). Particulate-bound Cu (Total – Dissolved Cu) increased from about 17% for Event 1 to 79% for Event 2 and 83% for Event 3 and returned to 22% by Event 4 (Table 9)) indicating that the increase in copper was likely non-labile particulates that were not toxic within the water column and not persistent as water column concentrations returned to baseline and reference levels by Event 4 (Table 10). The raw data are provided in Appendix B2, Appendix C: Table C-1, and Table C-2 contain the statistical summaries.

Statistical tests for Hypothesis 2 (difference between Events at Ship stations) showed significant differences in total copper levels between Event 1 and Event 2 (p=0.0005) and between Event 1 and Event 3 (p=0.0001), but no difference between Event 1 and Event 4 where total copper levels dropped below baseline levels (Appendix C: Table C-1, and Table C-2).

In summary, total copper measurements showed: 1) low levels and narrow ranges of total copper at Reference stations 2) elevated total copper concentration (2-3 times ambient levels) at Ship stations during and soon after biofouling removal 3) no difference or very slight difference in total copper between Ship and Reference stations before and one month after biofouling removal.

5.6.3 Dissolved Zinc

Zinc is a problematic constituent to accurately measure at low-level concentrations, as it is ubiquitous throughout the environment and easily confounds and contaminates samples creating inaccurate results. There was evidence of contamination effects in samples for dissolved zinc in Event 1, where outliers showed concentrations 2-4 orders of magnitude higher than mean levels (Figure 14). Note that the scale range shown on the graph in Figure 14 was chosen to show fine-scale patterns in dissolved zinc concentrations, therefore measurements above this range (n=5) are displayed as measured values with arrows pointing up. These outlier values for dissolved zinc were several times larger than their correspondent total zinc samples (maximum total zinc measured was 5.40 µg/L for station CV62-2S in Event 1). All samples were filtered on-line while sampling, and most samples were quantified as having dissolved zinc concentrations below those for total zinc, as expected. Samples of dissolved zinc with much higher concentrations than the corresponding total zinc samples were considered to be contaminated samples and these data were omitted from the statistical analysis (n=13). These included the following stations during Event 1: CV62-1B (17.48 µg/L), CV62-1M (2.16 µg/L), CV62-1S (3,287 µg/L), CV62-2S (63.68 µg/L), CV62-3B (2.85 µg/L), CV62-3M (1.79 µg/L), CV62-4S (14.52 µg/L), CV62-5B (41.89 µg/L), CV62-5M (5.32 µg/L); CV62-6B (12.41 µg/), CV62-R500-1B (8.10 µg/L), CV62-R1000 2B (1.49), and CV62-R500-1S (3.10 µg/L) (See Appendix B2). All other samples, including those with dissolved zinc concentrations that are larger than the corresponding total zinc concentrations by a range similar to the MDL were considered adequate for the assessment.

The sources of contamination was unknown but corrective actions of thoroughly cleaning the filtering apparatus prevented similar problems from occurring during the subsequent sampling events.

All zinc concentrations measured at the ship and reference stations (ranging from Non Detect (ND) to a maximum of 2.02 µg/L) were well below the acute (90.0 µg/L) and chronic (81.0 µg/L) water quality criteria for zinc in marine waters throughout this study (Figure 14; Appendix C: Figure C-16, see Table 1 for WQS). There were no clear spatial or temporal patterns for dissolved zinc concentrations; the data were generally homogeneous across events and have low concentrations throughout the sampling area.

Statistical tests for Hypothesis 1 show no difference between ship and reference stations for Events 1 and 3, with a very small but significant (p=0.0001) difference for Event 2 (zinc slightly lower at

Ship stations) and Event 3 (p=0.0499, zinc slightly higher at Ship stations). Statistical tests for Hypothesis 2 show small differences among Ship stations with lower levels of dissolved zinc measured at the Ship during Events 2 and 3. (Appendix C: Table C-1, Table C-2).

Overall these data indicate: (1) dissolved zinc levels did not exceed WQS thresholds 2) very low levels of dissolved zinc overall (3) little to no difference in dissolved zinc between Ship and Reference stations and (4) little to no difference between Events at Ship stations.

5.6.4 Total Zinc

Total zinc concentrations did not have definitive spatial or temporal patterns, although a wider range of measurements were recorded for total zinc (0.01 µg/L – 5.40 µg/L) compared to dissolved zinc (0.10 µg/L – 2.02 µg/L) as expected (Figure 15); Appendix C: Figure C-16). Event 1 showed the broadest range and highest maximum (0.72 to 5.40 µg/L) compared to Event 2 (ND to 3.82 µg/L) and Event 3 (0.84 to 2.15 µg/L) and Event 4 (1.07 to 1.45 µg/L), which had the narrowest range and lowest maximum across the study.

Statistical tests for Hypothesis 1 show no difference in total zinc between Ship and Reference stations during Events 1 and 2, with slightly lower concentrations measured at the Ship in Event 3 (Ship: Mean 0.29 ± 0.38, Reference: Mean 1.03 ± 0.86) and slightly higher at the Ship in Event 4 (Ship: Mean 1.27 ± 0.11, Reference: Mean 1.13 ± 0.04). Statistical tests for Hypothesis 2 show no difference in total zinc for Ship stations between Events 1 and 2, with small but statistically lower measurements at Events 3 and 4. (Appendix C: Table C-1, Table C-2).

Overall these data indicate: 1) very low levels of total zinc overall 2) little to no difference in total zinc between Ship and Reference stations and 3) little to no difference between Events at Ship stations.

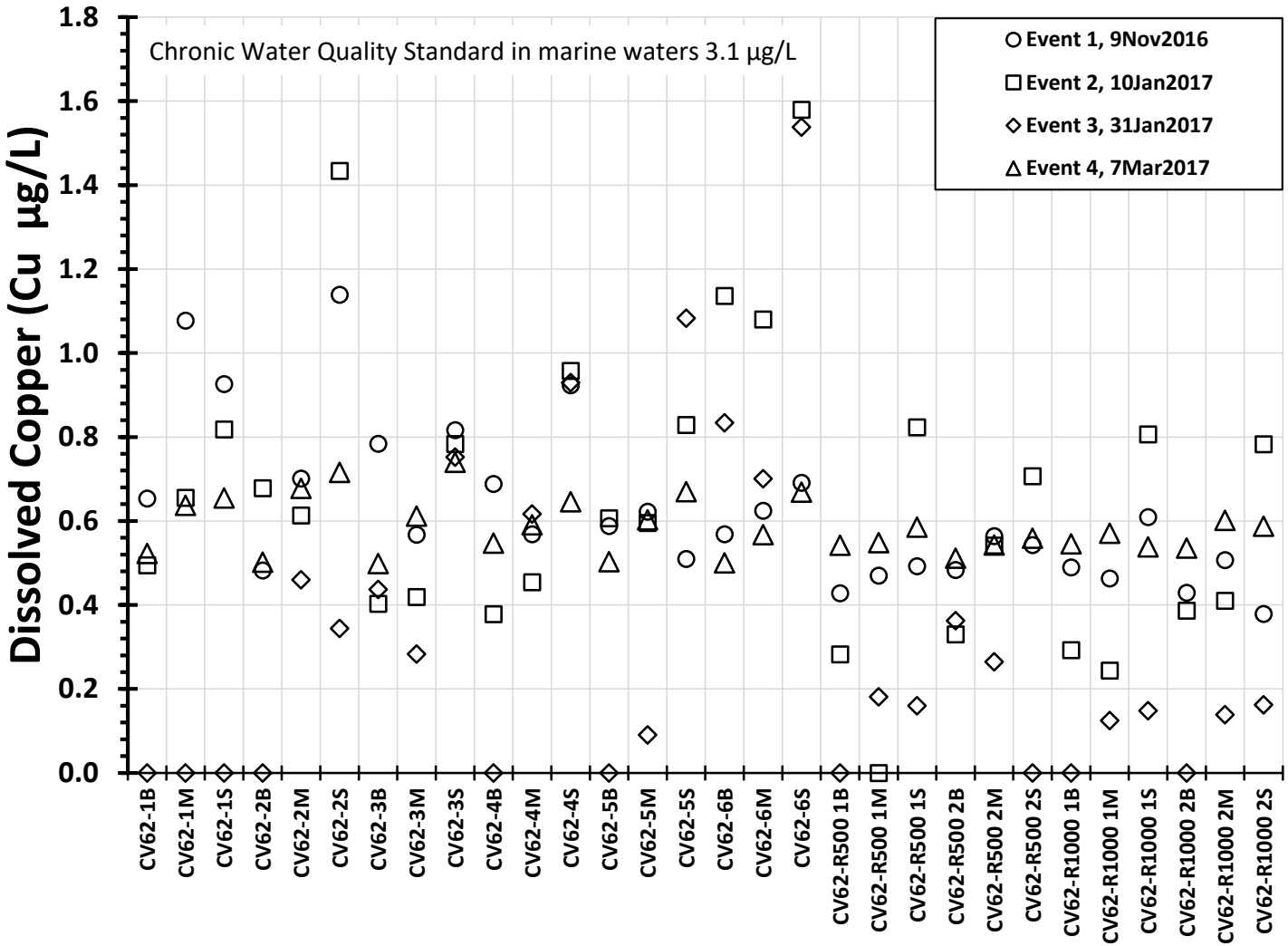

Figure 12. Dissolved copper concentration (Cu µg/L) distributions measured during the four sampling events to assess metal loading generated by biofouling-removal from the hull of the ex-INDEPENDENCE (CV 62). Non-detects (ND) are plotted as zero concentration (MDL 0.47 ± 0.20 µg/L).

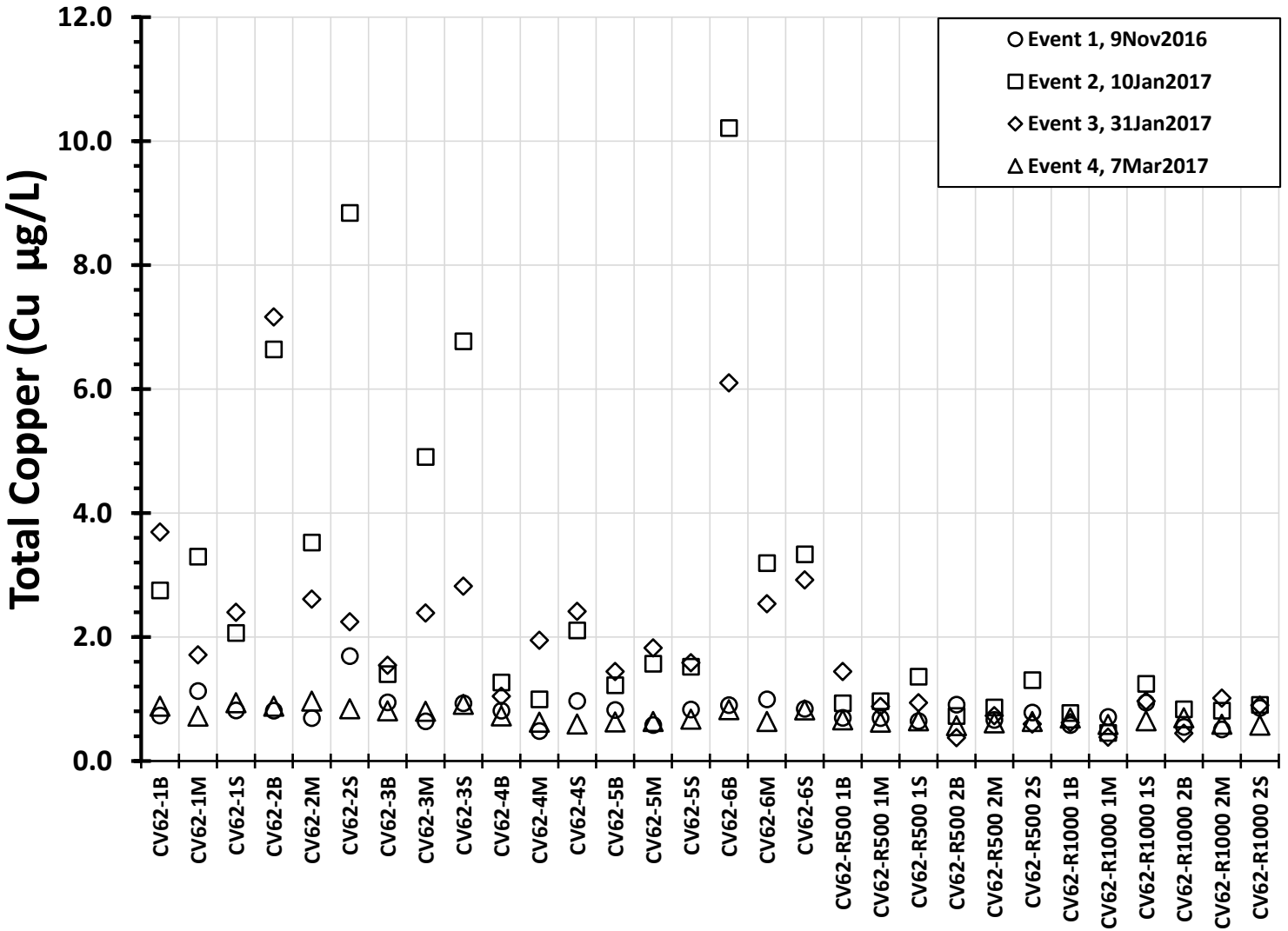

Figure 13. Total copper concentration (Cu µg/L) distributions measured during the four sampling events to assess metal loading generated by biofouling-removal from the hull of the ex-INDEPENDENCE (CV 62).

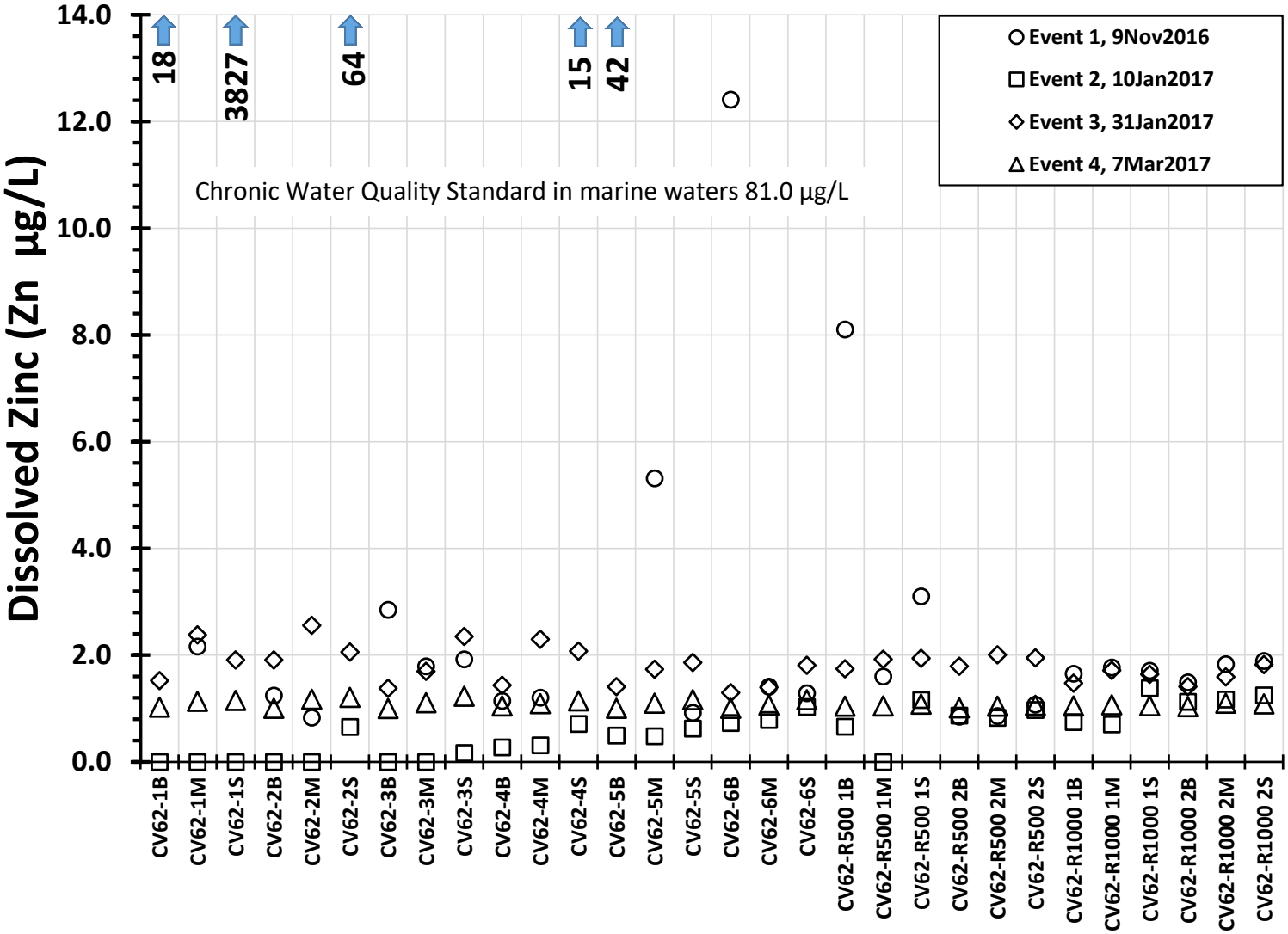

Figure 14. Dissolved zinc concentration (Zn µg/L) distributions measured during the four sampling events to assess metal loading generated by biofouling-removal from the hull of the ex-INDEPENDENCE (CV 62). Numerical data and arrows are shown for Event 1 where values are above 14.0 µg/L. Non-detects (ND) are shown as zero concentration (MDL 0.66± 0.28 µg/L).

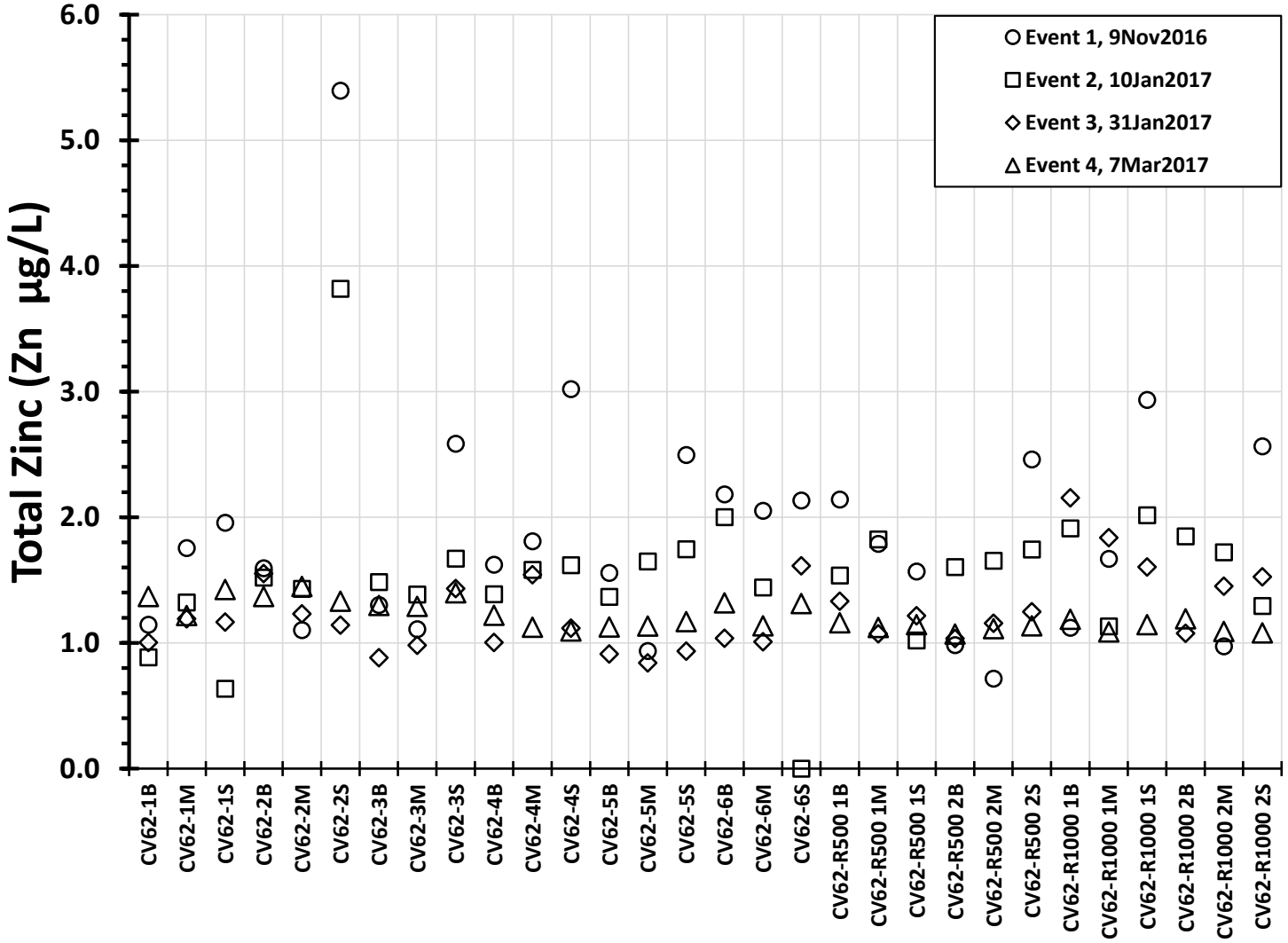

Figure 15. Total zinc concentration (Zn µg/L) distributions measured during the four sampling events to assess metal loading generated by biofouling-removal from the hull of the ex-INDEPENDENCE (CV 62).

5.7 DISSOLVED OXYGEN (DO)

There was high variation in dissolved oxygen (DO) during the Event 1 survey (%Saturation range 52.5% – 128.30%) owing to the naturally-occurring draw down of DO during the end of the fall season, especially in bottom water. However, once temperatures decreased, DO remained at or near saturation levels for all water column strata for the remainder of the study (Appendix C: Table C-1, Figure C-17). DO levels did not decrease below the WQS for any measurements during and after biofouling removal.

Statistical tests for Hypothesis 1 show no difference in DO between Ship and Reference stations for Events 1, 2 and 4, with slightly reduced DO at Ship stations during Event 3 (Ship: mean 95.99 ± 3.14, Reference: mean 99.49 ± 3.86, p=0.0108) (Appendix C: Table C-1, Table C-2).

Statistical tests for Hypothesis 2 show improved of DO levels at the Ship stations throughout the study compared to Baseline: Event 1 mean 87.86 ± 19.49; Event 2 mean 100.60 ± 1.71; Event 3 mean 95.99 ± 3.14; Event 4 mean 105.03 ± 3.23. (Appendix C: Table C-1, Table C-2).

5.8 TURBIDITY

Very slight and short term differences in turbidity were measured during the study by both the in-situ (Appendix C: Table C-1, Figure C-3, Figure C-10) and the discrete turbidity sensors (Appendix C: Table C-1, Figure C-4, Figure C-11). Turbidity levels did not exceed WQS for any measurements during the study. Secchi disk depth observations also corroborate the low turbidity measurements obtained by field sensors (Appendix C: Figure C-4).

Statistical tests for Hypothesis 1 show no difference in turbidity between Ship and Reference stations for Events 1, 2 and 4. There was a slight increase in turbidity at Ship stations during Event 3 based on in-situ turbidity measurements (Ship: mean 0.11 ± 0.08 NTU, Reference: mean 0.04 ± 0.02 NTU, p = 0.0117), but there was no significant difference in discrete turbidity. (Appendix C: Table C-1, Table C-2).

Statistical tests for Hypothesis 2 show a small but significant difference in in-situ turbidity at Ship stations between Events 2, 3, and 4 compared to Baseline: Event 1 mean 0.05 ± 0.04 NTU; Event 2 mean 0.07 ± 0.04 NTU; Event 3 mean 0.11 ± 0.08 NTU; Event 4 mean 0.07 ± 0.04 NTU. There was no significant difference in discrete turbidity. (Appendix C: Table C-1, Table C-2).

5.9 NUTRIENTS

5.9.1 Nitrates

Nitrate (NO_3) concentrations measured during the study were in the range of 1.42-2.61 mg/L (detection limit 0.05 mg/L) throughout the study period, with nitrate levels for both Ship and Reference stations trending up slightly from Event 1 (range 1.42-2.30) to Event 2 (range 1.81-2.21 mg/L), reaching maximum levels during Event 3 (range 1.95 to 2.61 mg/L), and trending down slightly during Event 4 (range 1.95 to 2.52 mg/L) (Figure 16, Appendix C: Table C-1, Figure C-7, Figure C-17). Note that the highest concentration measured during Event 4 was at a reference station (CV62-R1000 2B, 2.52 mg/L; Figure 16). There were no differences in nitrate concentrations as a function of depth, however, slightly lower nitrate levels (range 1.42 to 1.51 mg/L) were measured in the surface samples from the reference stations during Event 1. Event 1 was the singular event exhibiting lower nitrate concentrations on the surface (S), in comparison to the bottom (B) and mid-depth (M). The data for the other three sampling events tend to be more homogeneous, with some

stations having slightly higher concentrations on the surface, but no definitive stratification in nitrate measurements (Figure 16).

Statistical tests for Hypothesis 1 show no difference in nitrate between Ship and Reference stations for Events 1, 2 and 4, with slightly higher nitrate concentrations measured at the Ship during Event 3 (Ship: Mean 0.55 mg/L ± 0.02, Reference Mean 0.52 ± 0.04, p=0.0032) (Appendix C: Table C-1, Table C-2, Figure C-7, Figure C-16).

Statistical tests for Hypothesis 2 show slightly higher values of nitrate at the Ship stations for all events compared to the baseline. Despite the statistical significance, these differences are very small: Event 1 mean 0.41 ± 0.04; Event 2 mean 0.45 ± 0.03; Event 3 mean 0.55 ± 0.02; Event 4 mean 0.49 ± 0.03. This small magnitude of effect is discussed further in Section 6.5.

Overall these data indicate: 1) a slight upward trend in nitrate levels at both Ship and Reference Stations during the study 2) slightly higher nitrate levels between the Ship and Reference stations at Events 3 but no difference at the other three Events 3) very small but statistically higher levels of nitrate at Ship stations compared to Reference stations during all Events.

5.9.2 Nitrites

Most of the nitrite (NO_2) concentrations were not detected (ND) at a MDL of 0.023 mg/L, and a MRL of 0.16 mg/L. The only quantifiable concentrations were measured one week after initiation of the biofouling removal (Event 2). These NO_2 concentrations were measured at the CV62-6S station and the reference stations (Figure 17), and comprise a range of 0.023 to 0.030 mg/L.

5.9.3 Ammonia

Similar to nitrite (NO_2), total ammonia was not detected (ND) in many samples by the analytical capabilities at an MDL of 0.062 mg/L, and an MRL of 0.13 mg/L. The most complete set of ammonia data is for Event 1. Patterns in these data show a narrower range of ammonia concentrations in the first five Ship stations, and a slightly wider range of concentrations in reference stations (Figure 18). A similar distribution in ammonia concentrations is depicted by the data from Event 4. Most notably is the small overall range in the data (0.05 – 0.14 mg/L) very close to detection limits. There were no discernable patterns in the data in terms of stratification. And all detectable levels of ammonia were well below the chronic level of 2.2 mg/L based on the physiochemical conditions of Sinclair Inlet.

Statistical tests for Hypothesis 1 show no difference in ammonia between Ship and Reference stations for any Event. Statistical tests for Hypothesis 2 show small but significantly lower levels of ammonia at Ship stations during Events 2, 3, and 4 compared to Baseline: Event 1 mean 0.08 ± 0.02; Event 2 mean 0.05 ± 0.00; Event 3 mean 0.05 ± 0.00; Event 4 mean 0.06 ± 0.02. (Appendix C: Table C-1, Table C-2).

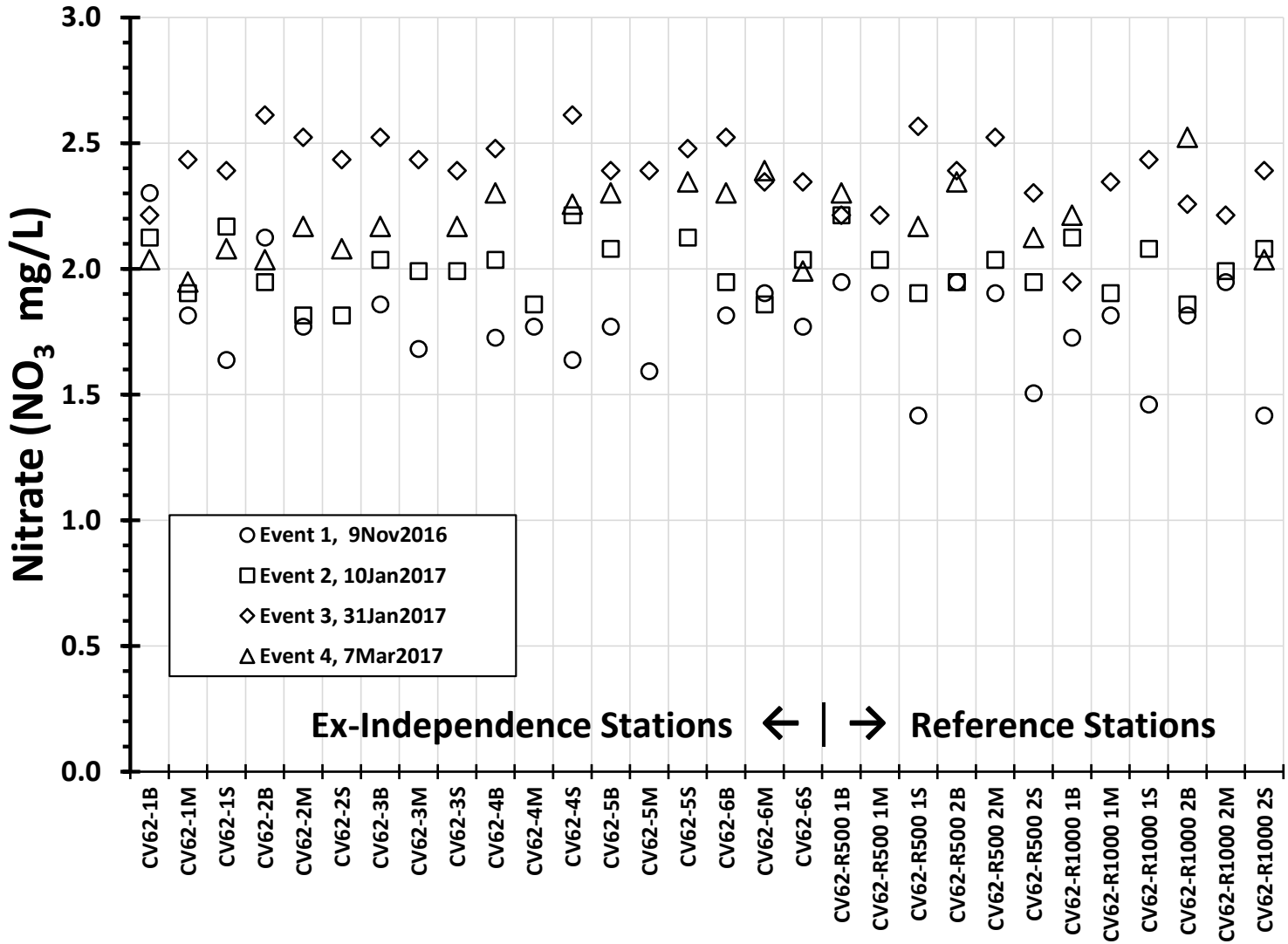

Figure 16. Nitrate (NO_3) concentration (mg/L) distributions measured during the four sampling events associated with biofouling removal from the ex-INDEPENDENCE (CV 62).

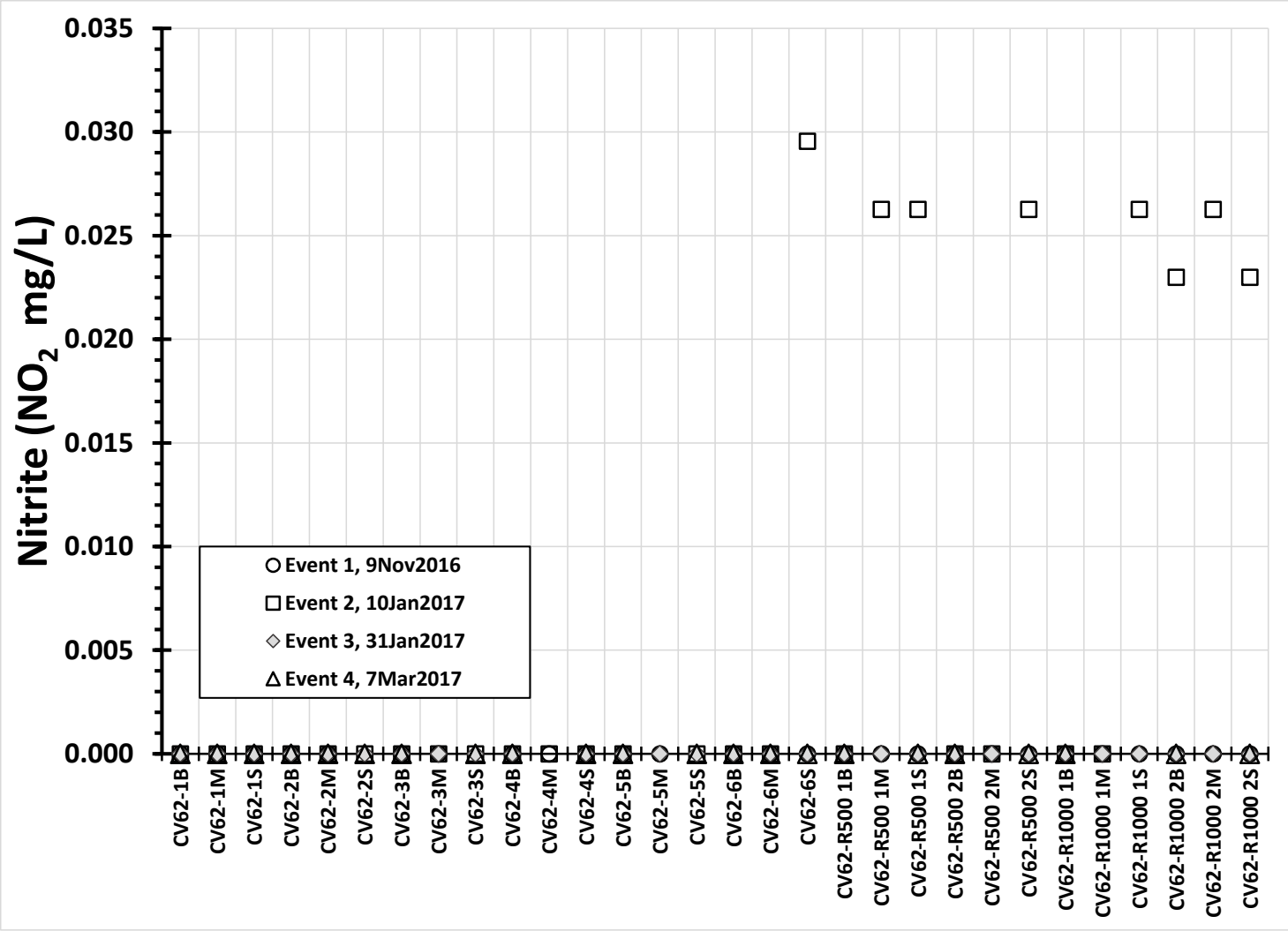

Figure 17. Nitrite (NO_2) concentration (mg/L) distributions measured during the four sampling events associated with biofouling removal from the ex-INDEPENDENCE (CV 62). Non-Detect samples are plotted as zero concentration.

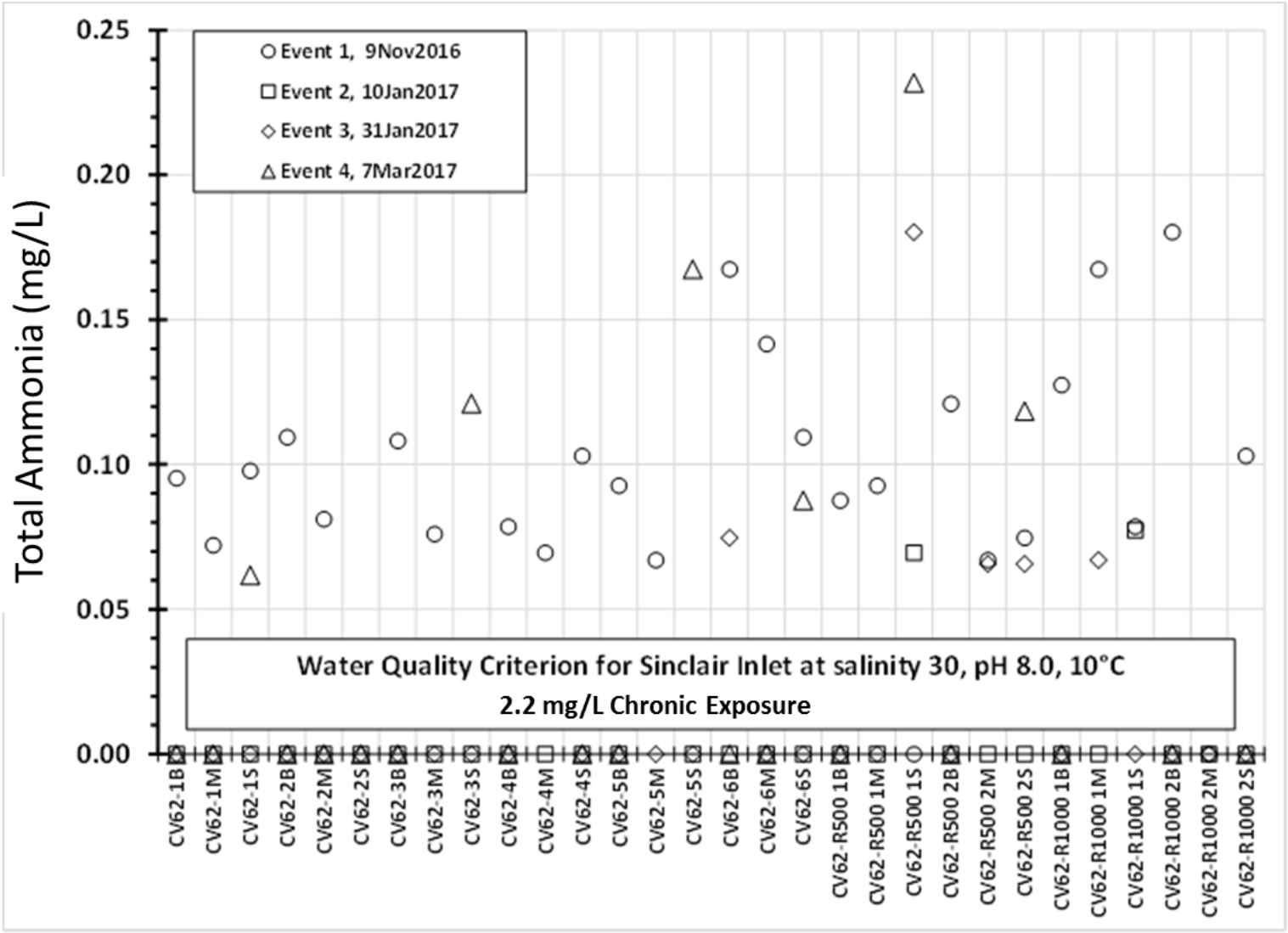

Figure 18. Total ammonia concentration (mg/L) distributions measured during the four sampling events associated with biofouling removal from the ex-INDEPENDENCE (CV 62). Non-Detect samples are plotted as zero concentration.

5.10 ORGANIC MATTER

5.10.1 Dissolved Organic Carbon (DOC)

Most of the DOC samples were below the MDL of 0.2 mg/L, with a MRL of 1.0 mg/L (Figure 19). There were two measurable concentrations in Event 2 of 0.20 mg/L for the ship station CV62-2M, and 0.41 mg/L for reference station CV62-R500 1B. The four measured concentrations for Event 3 are 0.30 mg/L at CV62-1S, 0.80 mg/L at R1000 1B and 0.40 mg/L at both CV62-R1000 1M and CV62-R1000 2M.

5.10.2 Biological oxygen Demand (BOD)

All the BOD concentrations were below the detection level throughout the study, with an MDL of 2.0 mg/L and a MRL of 4.0 mg/L (APPENDIX B).

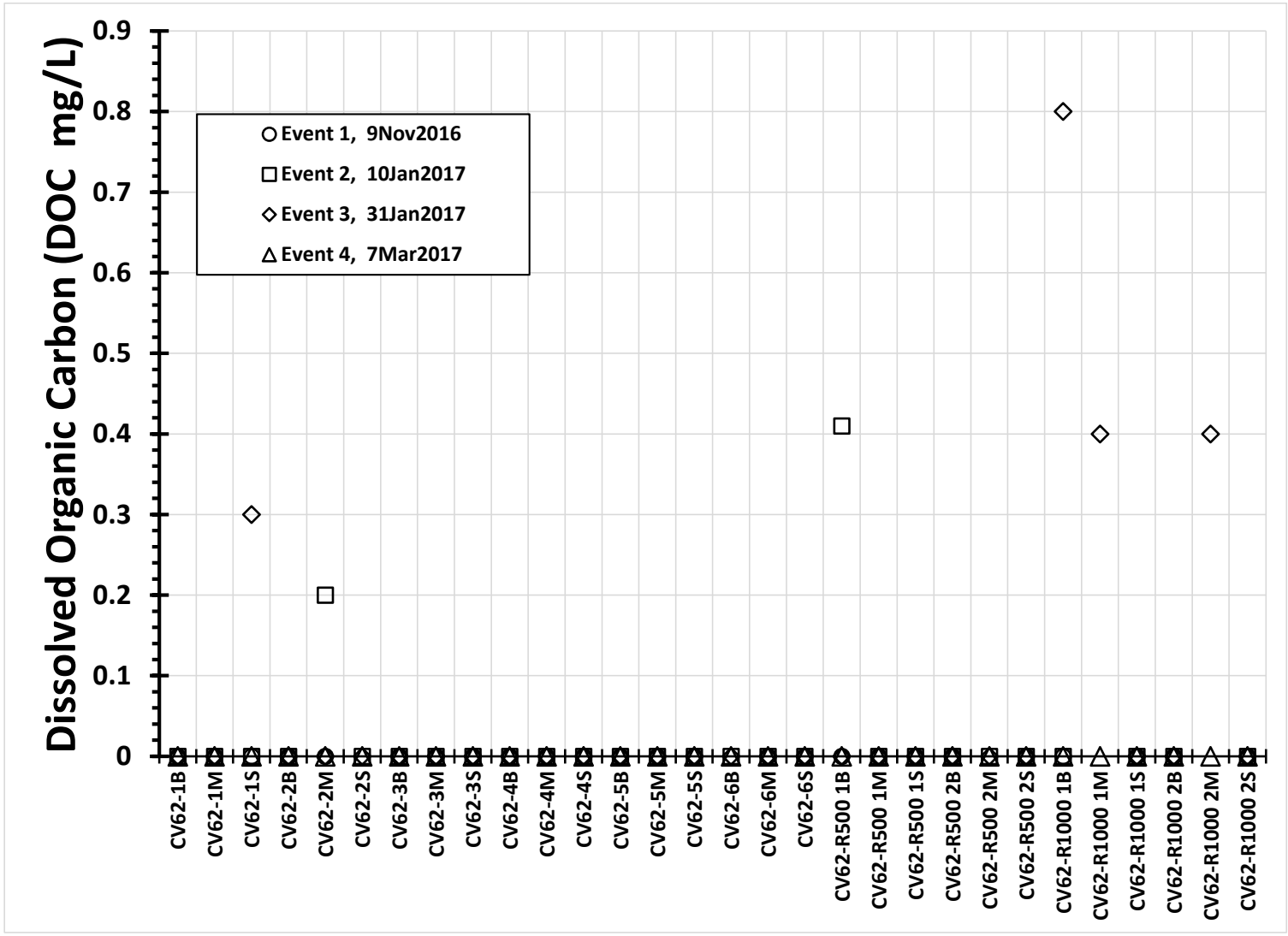

Figure 19. Dissolved organic carbon (DOC) concentration (mg/L) distributions measured during the four sampling events associated with biofouling removal from the ex-INDEPENDENCE (CV 62). Non-Detect samples are plotted as zero concentration.

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6. DISCUSSION

6.1 COPPER AND ZINC

The US EPA updated the aquatic life copper criteria for seawater in 2003 (www.epa.gov/wqc/fact-sheet-2003-draft-updated-aquatic-life-copper-criteria, accessed I June 2017) to a Criterion Continuous Concentration (CCC) of 3.1 µg/L and Criterion Maximum Concentration (CMC) of 4.8 µg/L dissolved copper, which is also the water quality standard (WQS) adopted by the State of Washington (Ecology, 2012). The overall range of dissolved copper measured in this study was ND to 1.58 µg/L. Therefore, even the largest dissolved copper concentration measured in the study was almost two times below the water quality threshold deemed to be protective of aquatic life. It should be noted that the CCC only applies to dissolved copper and not to total copper concentrations.

The WQS are based on protecting the most sensitive lifecycle of the most sensitive species, which includes invertebrate larvae known to be very sensitive to copper exposures during their early life stage. Recent work conducted by the NOAA National Marine Fisheries Service, Northwest Fisheries Science Center in Seattle, WA has shown that the seawater WQS are also protective of sublethal effects of copper on juvenile salmon olfaction, because juvenile salmon are much less sensitive to copper exposure in seawater than in freshwater (Baldwin, 2015; Sommers, Mudrock, Labenia, and Baldwin, 2016). Furthermore, out-migrating juvenile salmon are primarily present in late spring early summer (Fresh et al., 2006) which is well after the biofouling removal from ex-INDEPENDENCE was completed.

In order to assess water quality measurements from this study in the context of previous and ongoing monitoring efforts in Sinclair Inlet, study data are presented together in a series of “box and whiskers” plots. Data from this study are summarized for Ship and Reference stations by sampling event. For each set of data, the bottom box represents the second quartile of data, the top box represents the third quartile of data, with both boxes together representing 50% of the data. The border line between these boxes represents the median, and the whiskers are the minimum and maximum measured concentrations. Concentrations not detected at the MDL are included as zeros in the figures.

In this study, there were small differences in dissolved copper concentrations measured at the Ship stations compared to Reference stations for Events 1, 2, and 3 (Figure 20, Appendix C: Table C-2). Between sampling events, dissolved copper increases at Event 2, reaches a maximum at Event 3 then trends towards baseline at Event 4 (Figure 20, Appendix C: Figure C-5). These statistical changes are very small and these effects of biofouling removal from the ex-INDEPENDENCE were detected only after extensive analytical work on samples collected during Events 2 and 3, where the largest dissolved copper concentrations were measured (Figure 20). All measured values are well below the CCC, as discussed above, and are within the previously reported ranges of dissolved copper concentrations for Sinclair Inlet. The overall range in dissolved copper concentration (ND 0.47 µg/L to 1.58 µg/L,) is within the ranges reported by Katz et al. (2004; 0.44 to 2.21 µg/L) and those reported by Rosen et al. (2009). Rosen et al. (2009) included analysis of samples by two separate laboratories, the Environmental Sciences laboratory at SSC-Pacific in San Diego, CA, which reported a range of 0.60 to 1.80 µg/L, and the Battelle Marine Science Laboratory in Sequim, WA, that reported a range of 0.70 to 1.60 µg/L.

The data from the present study were also compared to more recent monitoring conducted by Puget Sound Naval Shipyard & Intermediate Maintenance Facility (PSNS&IMF) as part of the cooperative Project ENVironmental enVESTment (ENVVEST). Developed under a Final Project

Agreement among PSNS&IMF, US EPA, Ecology and Participating Stakeholders (US Navy, US EPA, Ecology 2000), Project ENVVEST is being conducted to address Total Maximum Daily Load (TMDL) requirements and help achieve clean water goals for Sinclair and Dyes Inlets (Johnston et al. 2009, Lawrence et al. 2011). As part of the ENVVEST monitoring program, seasonal ambient monitoring for trace metals and other water quality constituents has been conducted since August 2009 (Johnston et al. 2017b).

The ENVVEST stations closely located to either CV 62 or Reference sites that were sampled in December 2016 and March 2017 are shown in Figure 21, and the results obtained during the same time period as the ex-INDEPENDENCE water quality assessment are summarized Table 10 (Pacific Northwest National Laboratory, PNNL, 2017a, 2017b). These comparisons indicate that any increase in dissolved copper concentrations that could be attributed to the biofouling removal were only short term increases which did not exceed the ranges of copper reported in historical data, and did not persist after the biofouling removal was completed. Note that the dissolved copper data collected for this study, with measurements ranging from 0.05 to 1.58 µg/L, are similar to levels expected for nearshore areas around PSNS (Figure 20).

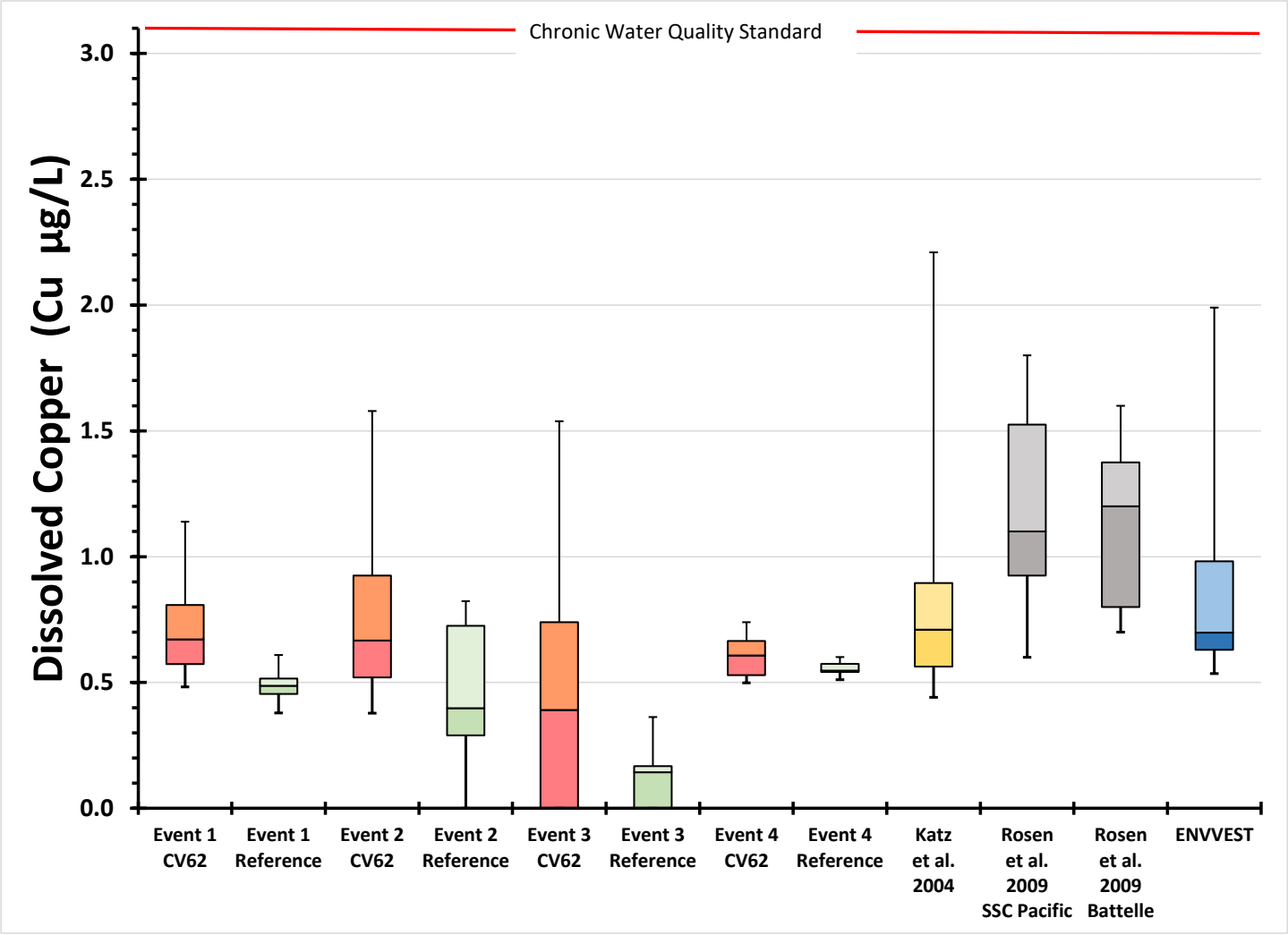


Figure 20. Comparison of the range in dissolved copper (Cu) measured (µg/L) during this study compared to Katz et al. (2004), Rosen et al. (2009), and data collected in December 2016 and March 2017 by the ENVVEST program.

Table 10. ENNVEST ambient monitoring results for samples collected December 6-7, 2016 and March 28-April 5, 2017 at stations located within the CV 62 study area.

Station ID	Type	Date	Salinity (psu)	DOC (mg/L)	TOC (mg/L)	Dissolved Cu (µg/L)	Total Cu (µg/L)	Dissolved Zn (µg/L)	Total Zn (µg/L)
PS01	Ship	12/6/2016	29.2	1.01	0.98	1.27	1.71	3.37	3.67
PS13	Ship	12/6/2016	29.4	0.99	0.98	0.63	0.87	1.50	1.74
M4	Reference	12/6/2016	29.2	1.00	1.01	0.75	1.04	3.49	3.89
SNO3	Reference	12/7/2016	28.6	1.07	1.06	0.54	0.88	1.68	2.06
PS01	Ship	3/28/2017	27.6	1.12	1.18	0.62	0.84	2.97	3.34
PS13	Ship	3/28/2017	27.4	1.06	1.06	1.99	2.75	6.22	6.93
M4	Reference	4/5/2017	25.4	1.28	1.60	0.64	0.82	1.59	2.29
SNO3	Reference	4/5/2017	18.3	1.62	1.66	0.89	1.26	3.77	4.56

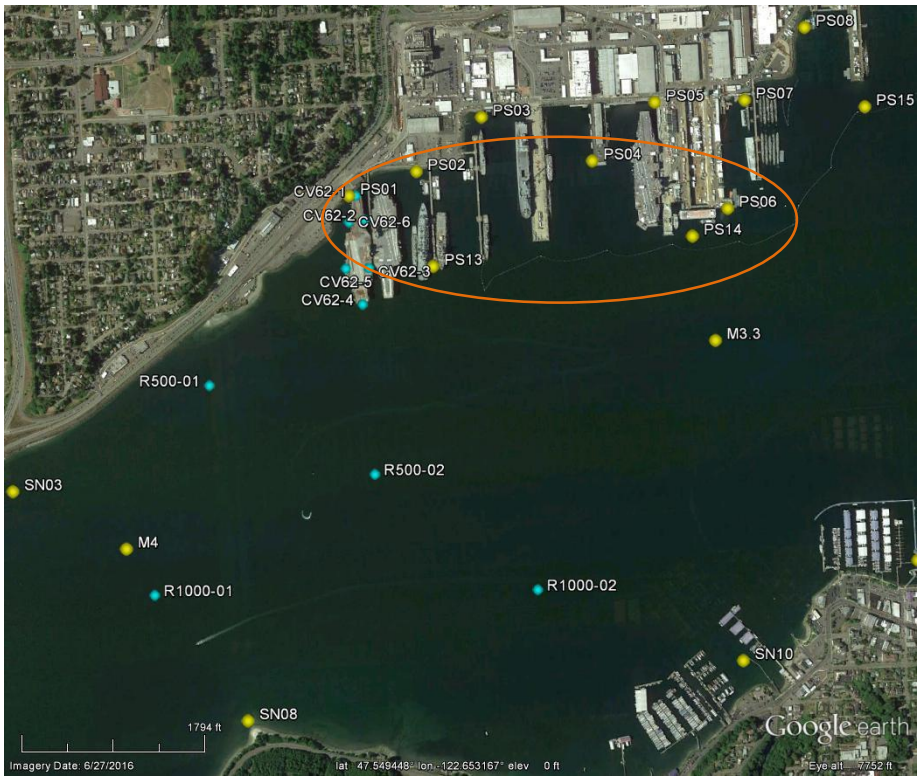


Figure 21. Location of CV 62 monitoring stations (blue circles) and the ENVVEST ambient monitoring stations (yellow circles) within Sinclair Inlet.

A summary of dissolved copper concentrations from ENVVEST monitoring from Aug 2009 to Sep 2015 is shown in Figure 22. The data show the trend of dissolved copper concentrations at selected locations within the inlets, including nearshore PSNS stations that are located directly adjacent to industrial outfalls, storm drains, dry docks, and ship berthing areas including Mooring G, where the ex-INDEPENDENCE was berthed; stations located along the PSNS security barrier; nearshore stations throughout Sinclair and Dyes Inlets; and marine stations located within the central channels of the inlets and passages that connect with Greater Puget Sound. The trend shows a gradient of dissolved copper concentrations of about 1.5 µg/L within nearshore areas of PSNS, about 1.0 µg/L near the PSNS security barrier and nearshore areas of Sinclair and Dyes Inlets, and decreasing to about 0.5 µg/L for the marine stations connecting to central Puget Sound. Generally, the data show that copper levels are below the acute water quality criteria standard for protection of aquatic life and that magnitude and frequency of levels that exceed aquatic life thresholds has been decreasing (Figure 22).

Dissolved copper data collected for this study (0.05 to 1.58 µg/L) were similar to expected for nearshore areas of PSNS. These areas are affected by industrial discharges, runoff during storm events, leaching from active and inactive vessels, leaking sanitary sewer systems, groundwater seepage, resuspension of contaminated sediment, and transport of contaminants from the other sources within the watershed. Furthermore, many areas within PSNS, such as the berthing area at Mooring G, are subjected to low mixing from restriction of currents due to the irregular shoreline, pier pilings, and the presence of ship hulls that block currents and restrict flushing. Consequently, if there were a major releases of copper from biofouling removal, observed copper concentrations near the area around Mooring G would likewise be expected to reflect these major increases in a respective manner.

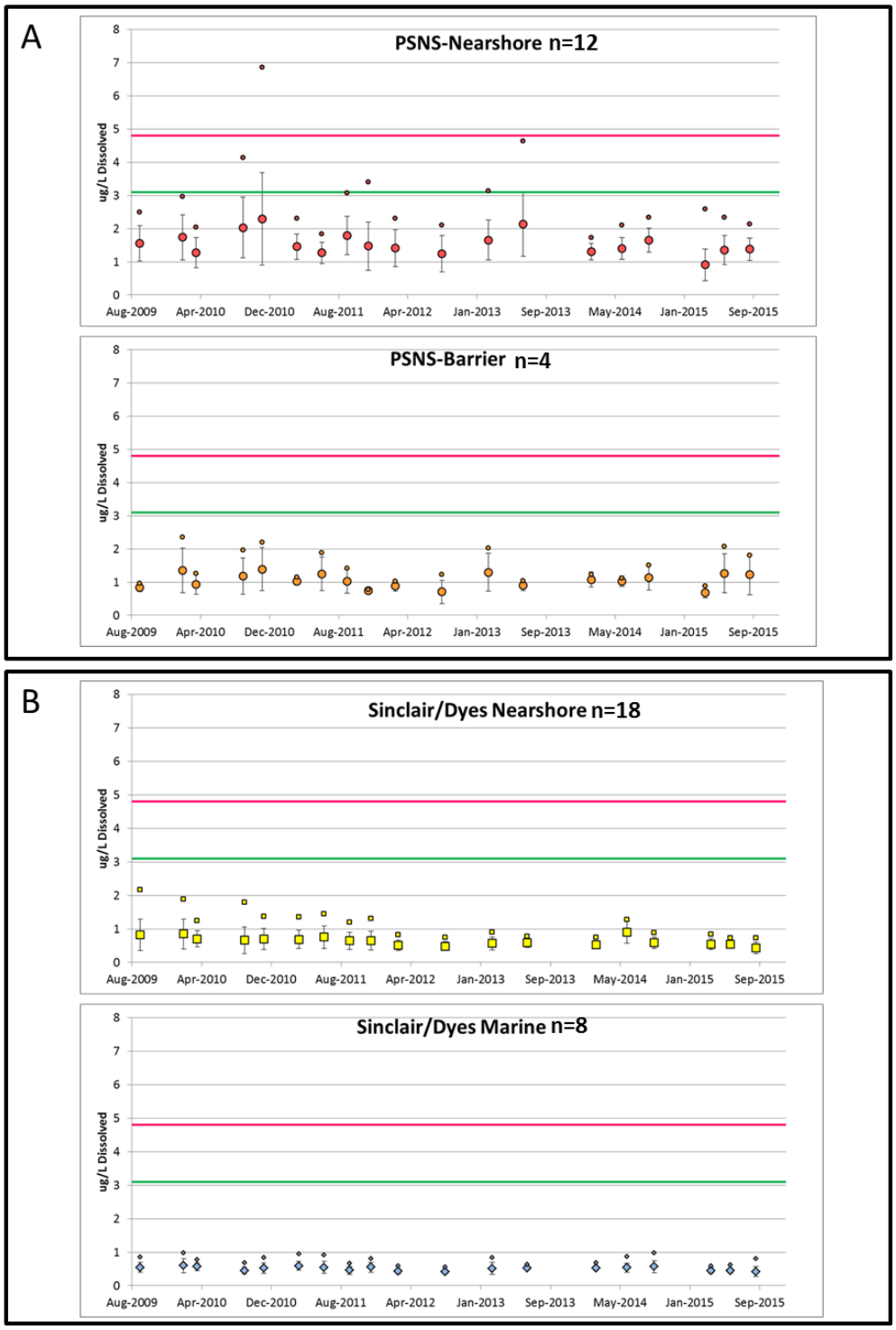


Figure 22. Dissolved Cu (µg/L) measured during ENVVEST seasonal monitoring events at locations within PSNS (A) and reference nearshore and marine stations in Sinclair and Dyes Inlets (B), from Johnston et al. (2017a). Large symbols are means with standard deviation for error bars, smaller points are maximum for sampling event, the green and red horizontal lines are the water quality CCC and CMC for Cu, respectively.

In contrast to dissolved copper, total copper (i.e., unfiltered) concentrations demonstrated elevated levels that may be attributed to the biofouling removal from the ex-INDEPENDENCE. As shown in Figure 23 total copper concentrations measured at Ship stations during Event 2 (0.99 to 10.21 µg/L) and Event 3 (1.04 to 7.16 µg/L) are seven to ten times larger than any sample data from reference stations (0.45 to 1.36 and 0.38 to 1.44 µg/L respectively), Ship station data from Event 1 (0.49 to 1.69 µg/L) and Event 4 (0.60 to 0.97 µg/L), as well as data previously reported by Katz et al. (2004), Rosen et al. (2009), and by the ENVVEST Program in December 2016 and March 2017 in nearby sites. Total copper, however, does not have a water quality criterion associated with it and the associated concentrations are not considered biologically available (US EPA 1993).

The temporal increase in total copper concentration associated with the biofouling removal from the ex-INDEPENDENCE had short-term effects on total copper concentrations in the water. Total copper data for Ship stations collected during Event 4 has a similar range (0.60 to 0.97 µg/L) to the Reference stations (0.52 to 0.70 µg/L), and is within the overall range for Reference stations in the other three events (0.38 to 1.44 µg/L). These data are also in the lowest range of the data reported by Katz et al. (2004), Rosen et al. (2009) and collected for the ENVVEST Program in December 2016 and March 2017 at nearby stations (Figure 23 and Figure 24). These comparisons support the conclusion that the concentration of total copper returned to background conditions within six weeks after the biofouling removal from the ex-INDEPENDENCE was completed.

Good coherence found within the data reinforces quality of analysis and increases confidence in the results. However, other sources of copper, such as stormwater runoff or some other unknown discharge may have contributed to the elevated levels observed. There is a prominent stormwater drain just near Mooring G (<200 ft from site CV62-1) and a former landfill (OUBA) is located along shoreline at Charleston Beach (about 330 ft from Mooring G). Moreover, the area around Mooring G is a “dead end” and “catchall” for contaminants transported along the northern shoreline of Sinclair Inlet, which can be prominent feature that is enhanced by strong winds blowing from the south or west.

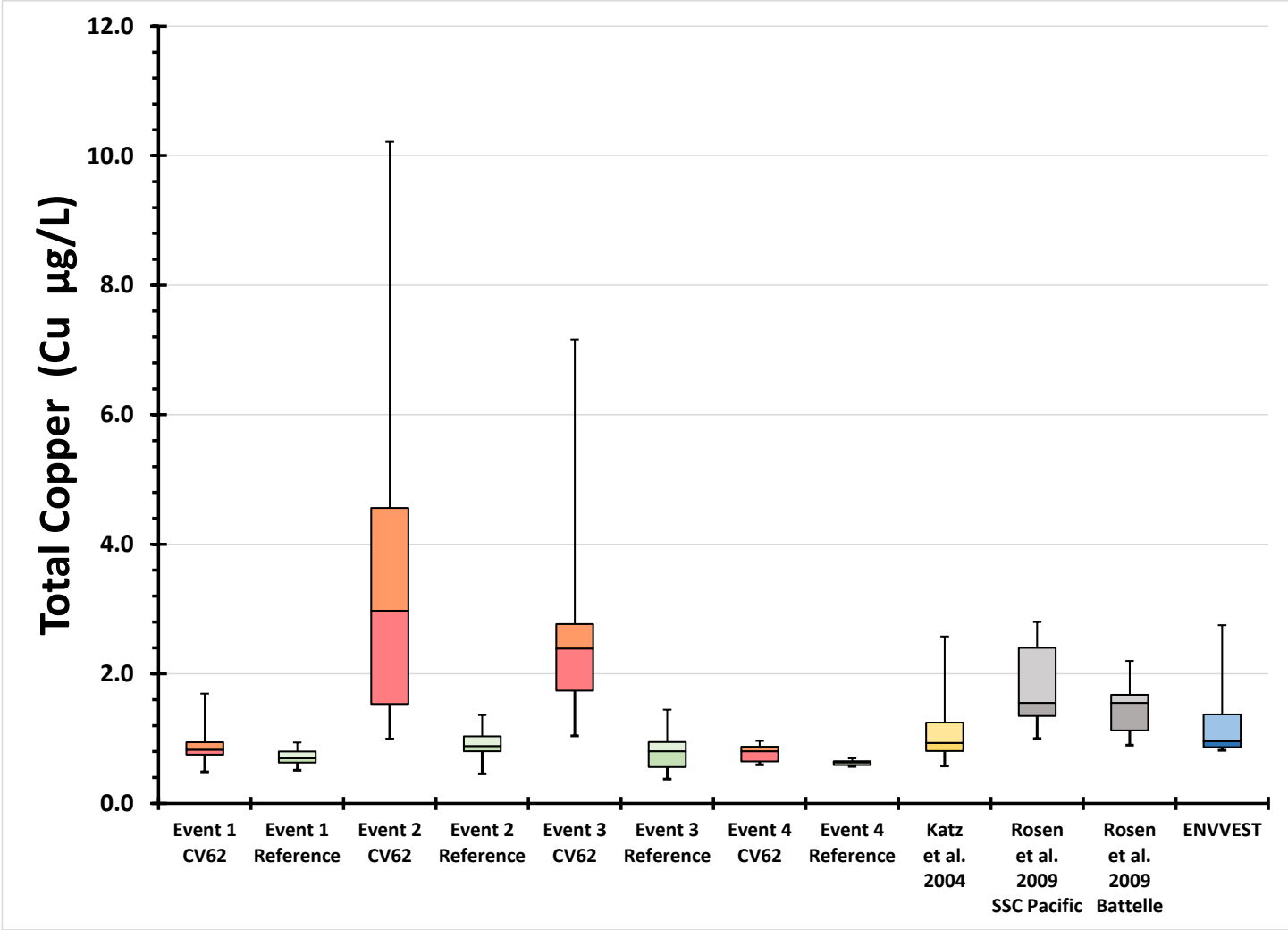


Figure 23. Comparison of the range of total copper (Cu) measured (µg/L) in this effort to those from Katz et al. (2004), the data for winter reported by Rosen et al. (2009), and data collected in December 2016 and March 2017 by the ENVVEST program.

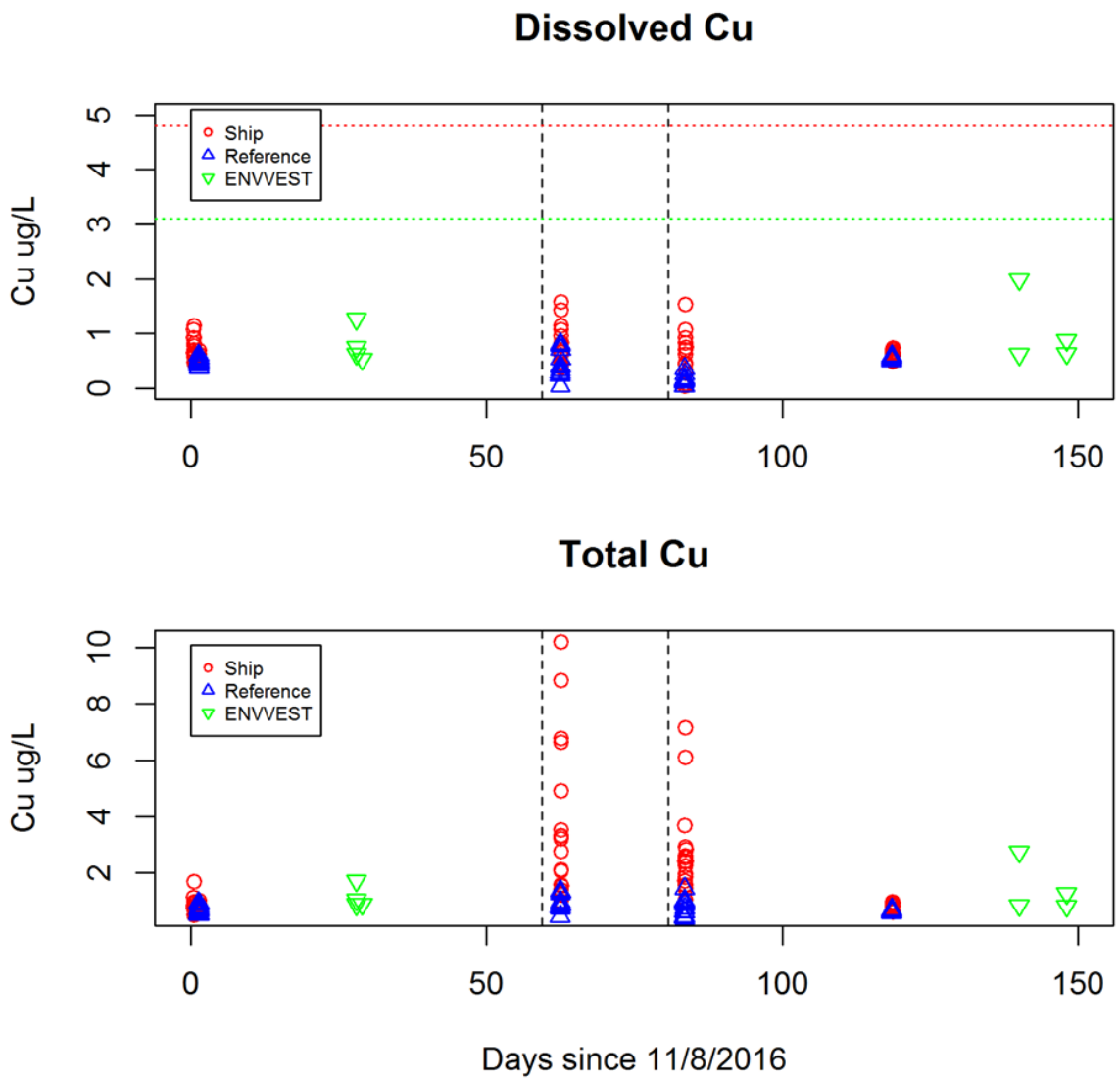


Figure 24. Results for dissolved and total copper (µg/L) measured at Ship (red circles), Reference (blue triangle) and ENVVEST (green triangles) sampling sites during the study. The upper panel shows chronic (green dotted line) and acute (red dotted line) water quality standards and the vertical dashed lines denote the beginning and end of biofouling removal. See Figure 21 for sampling locations.

The overall range of dissolved zinc measured during this study was ND to 2.56 µg/L. Therefore, the highest dissolved zinc concentrations were significantly below the chronic WQS of 81.0 µg/L (Ecology, 2011) that is protective of aquatic life. It should be noted that the WQS only applies to dissolved zinc and not to total zinc concentrations.

Contamination of dissolved zinc in Event 1 was confirmed by comparison to corresponding total zinc concentrations, previously measured values from Katz et al. (2004), and data from the ENVVEST Program (Figure 25). In theory, total metal must include dissolved metal and metal in particles larger than 0.45 µm in diameter; therefore, total metal concentration must be larger or equal

to dissolved metal. In practice, dissolved metal is sometimes slightly larger than total copper due to detection limits and noise in the analytical information. Dissolved zinc concentrations that substantially exceeded total zinc concentrations were omitted from the analysis (see Appendix B2). Aside from being greater than their associated total zinc concentrations, these measures are also well above reference station samples during Event 1, stations in other sampling events, and data previously reported by Katz et al. (2004) and PNNL (2017a, 2017b) (Figure 25). These comparisons provide further evidence that the dissolved zinc concentrations in these stations for Event 1 were contaminated (n=13) and do not represent the water quality characteristics in Sinclair Inlet. Therefore, the contaminated zinc results were not used for statistical analysis and interpretation.

For the usable dissolved zinc data, the range in concentration was very similar for all four sampling events (Figure 25, Appendix C: Figure C-6). Dissolved zinc measured at Ship stations was significantly lower than Reference stations for Event 2 and slightly higher than Reference for Event 4 (Figure 25). All of the data is within the lower end of the ranges reported by Katz et al. (2004) and PNNL (2017a, 2017b) (Figure 25). The lack of any clear trend, and the low range of measured dissolved Zn precludes attributing dissolved Zn levels to the biofouling removal from ex-INDEPENDENCE.

Concentrations of total zinc are mostly on the lower range of the data reported by Katz et al. (2004) and collected for the ENVVEST Program in December 2016 and March 2017 (Figure 26). There was no clear pattern from the comparison of total zinc between ship and reference stations. Total Zn concentrations at Ship sites were significantly lower than reference sites for Event 3 and slightly higher than Reference for Event 4, for example. However, in general, the measured range in total zinc is within the range reported by Katz et al. (2004) and collected for the ENVVEST Program (Figure 26). These findings support the conclusion for dissolved zinc, that total zinc concentrations were not attributed to the biofouling removal from the ex-INDEPENDENCE.

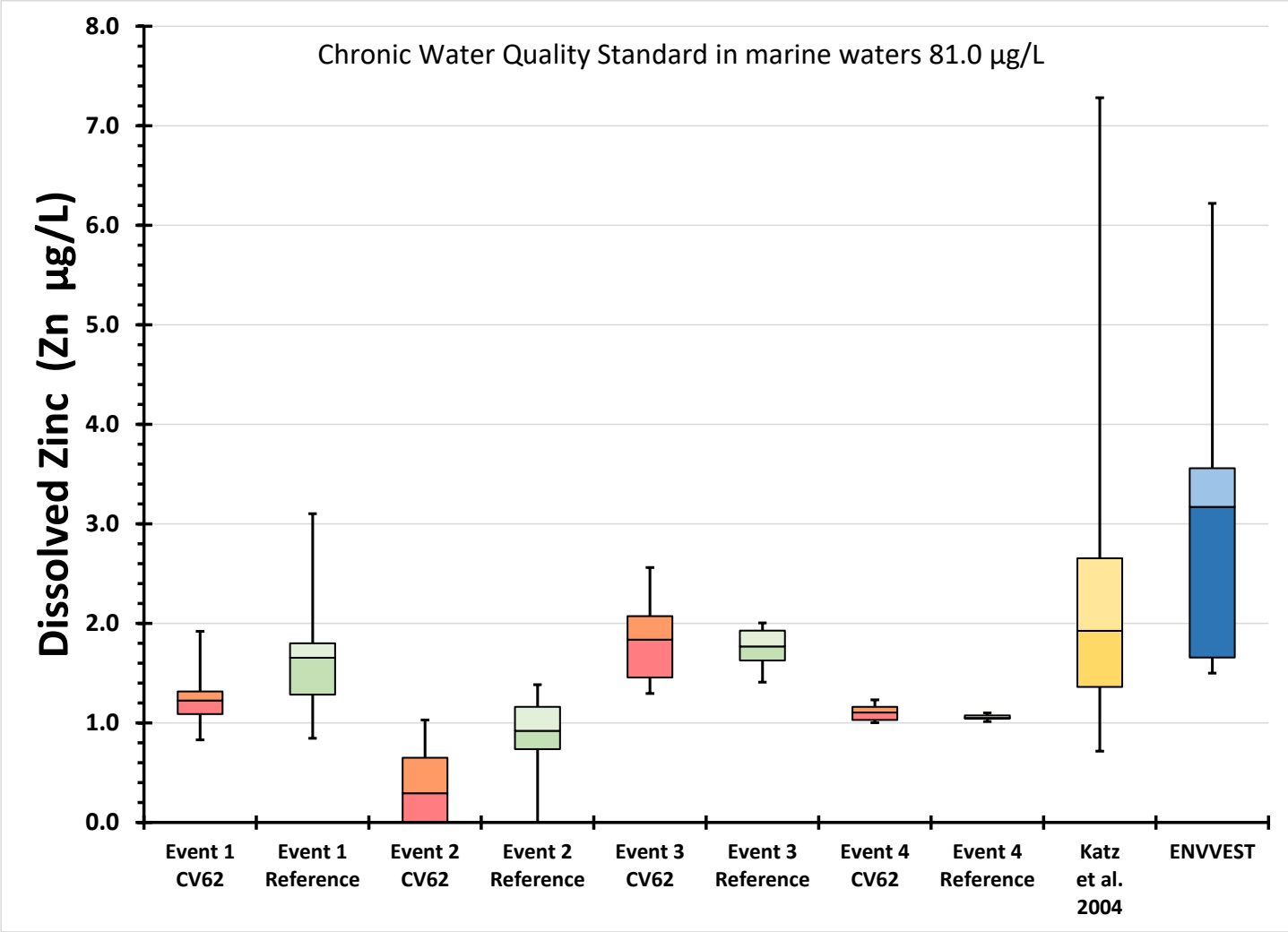


Figure 25. Comparison of the range of dissolved zinc (Zn) measured (µg/L) in this effort to those from Katz et al. (2004), and data collected in December 2016 and March 2017 by the ENVVEST program. Dissolved Zn data considered contaminated from Event 1 was omitted for this figure.

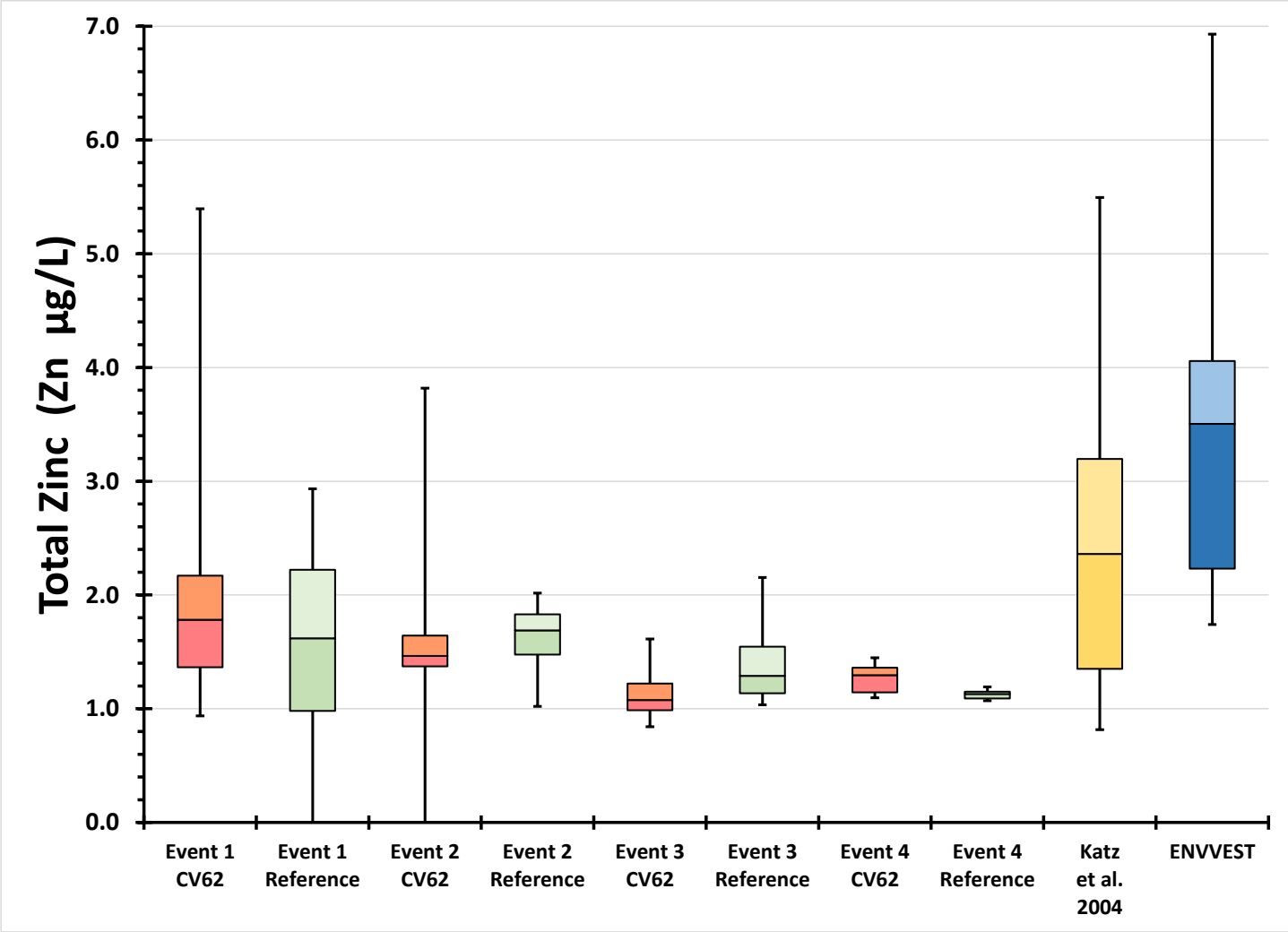
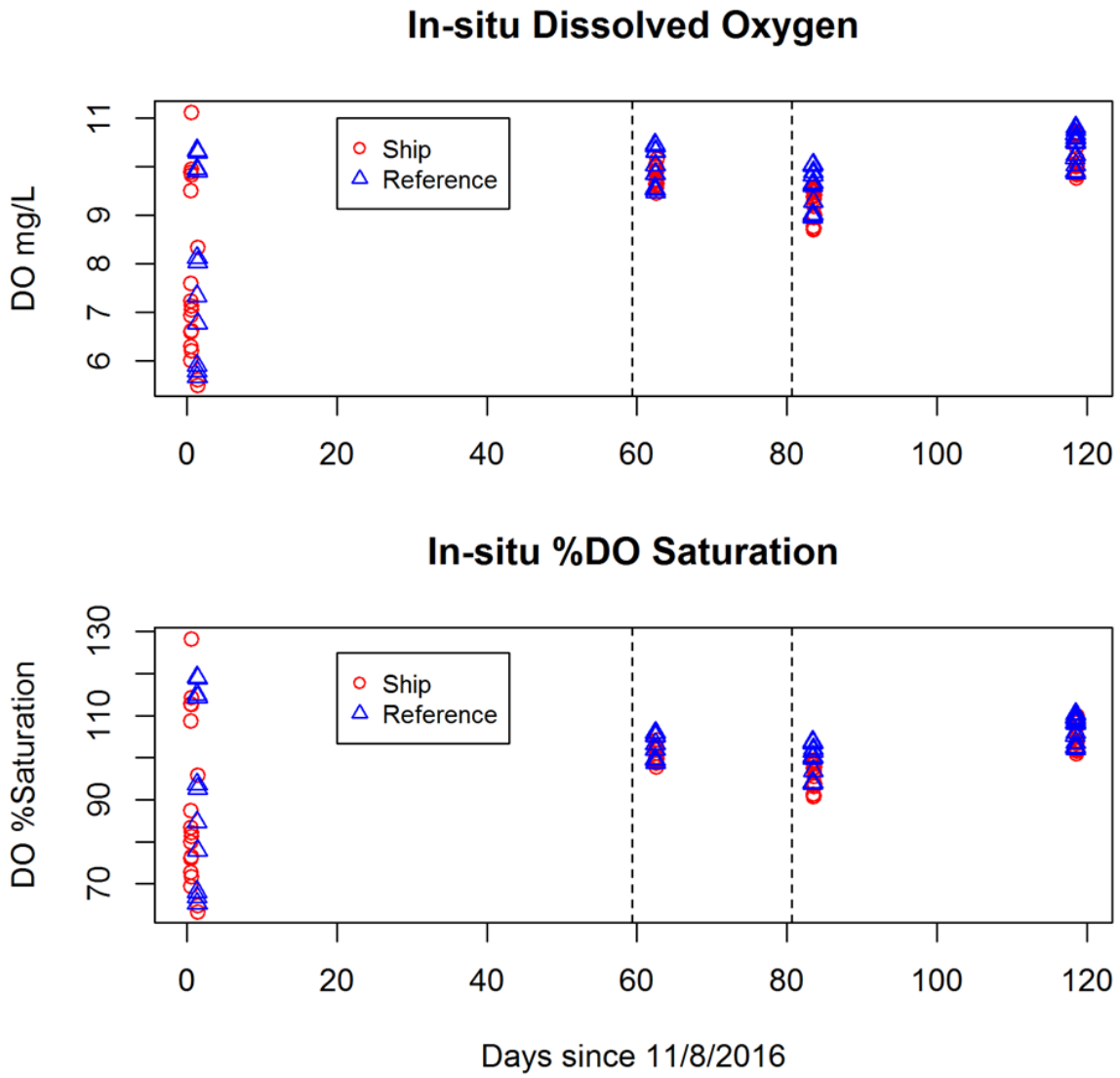


Figure 26. Comparison of the range of total zinc (Zn) measured (µg/L) in this effort to those from Katz et al. (2004), and data collected in December 2016 and March 2017 by the ENVVEST program.

6.2 DISSOLVED OXYGEN (DO)

Except for the baseline sampling event DO remained at or near saturation levels for all surface and bottom stations throughout the study, and no impacts to DO could be attributed to biofouling removal during the study period. In South and Central Puget Sound, the lowest DO levels are usually measured in the bottom waters during September and October (Amed et al., 2014). The relatively high, near-saturation DO levels measured during this study were not unexpected, however, potential impacts from decreased DO may not be manifested for months or years into the future. Any future studies of DO should recognize the difficulty of separating out potential effects of biofouling removal from other potential sources of oxygen depletion such as naturally occurring algal blooms and other sources of nutrient loading from the watershed. Currently, there are already 303(d) listings for impaired DO for waterbody segments within Sinclair and Dyes Inlets (Ecology 2017), so careful monitoring would be necessary to determine if the trend of DO worsens in the waters of Sinclair Inlet.



6.3 TURBIDITY

Very slight and short term differences in turbidity were measured during the study by both the in-situ and discrete turbidity sensors. Turbidity levels did not exceed the WQS for any measurements during the study. Secchi disk depth observations also corroborated the low turbidity measurements obtained by field sensors. Sampling events were scheduled independent of the biofouling removal operations, so only Event 2 sampling occurred during when cleaning operations were underway. On the cleaning support vessel, topside personnel on vessel did not observe plumes of red paint particles in the water during cleaning operations (McCue, 2017).

6.4 NUTRIENTS

6.4.1 Nitrates

The minimal difference in the range of nitrate (NO_3) concentrations measured within each sampling event attest to the similarity in nutrient concentration across the whole sampled area. The marginal temporal variation also attests to the absence of nutrient loading effects as result of the biofouling removal from the ex-INDEPENDENCE. Measured ranges in concentrations are at the higher end of previously measured nitrate concentrations in Sinclair Inlet (Figure 27). Katz et al. (2004) conducted seasonal monitoring within Sinclair Inlet, including the Shipyard, by collecting discrete samples for nutrient and trace metal (Cu and Zn) analysis from several stations during Spring (March 1998), Summer (July 1998) and Fall (September 1999) events. In addition to receiving waters, Katz et al. (2004) also sampled creeks and waste water treatment plant effluents to identify sources of contaminants in discharges and runoff. Data from these creeks and waste water treatment plant effluents are not included in Figure 27. The Ecology's Environmental Assessment Program (EAP) Marine Waters environmental monitoring program includes station SIN001_0, located at 47.5500 latitude north and -122.6417 longitude west in Sinclair Inlet, at 18 meters (~55 feet) depth (Figure 21, near Station M3.3). Water quality data from that station is reported monthly from October 1991 to July 2016 in fortress.wa.gov/ecy/eap/marinewq/mwdataset.asp?staID=129 (accessed on 15 May 2017), and appropriate data from that station is used in this report for comparison purposes (Ecology, 2017a).

The range of nitrate concentrations measured in this effort are at the higher range of the concentrations previously measured by Katz et al. (2004), and within the range reported in the Ecology's Environmental Information Management website (Ecology, 2017; Figure 27). The overall range in concentration for the CV 62 samples was 1.59 to 2.61 mg/L, and 1.42 to 2.57 mg/L for the reference stations. These ranges are within the range of the data reported by Katz et al. (2004; 0 to 1.67 mg/L) and Ecology (2017; 0.005 to 2.67 mg/L; Figure 27). The minimal temporal variation in concentration ranges measured, indicate that biofouling removal from the ex-INDEPENDENCE had negligible effects on increasing the nitrate concentrations in Sinclair Inlet during the study period.

The differences in nutrient concentration ranges measured when comparing between the different sampling events attest to overall changes in the oceanographic and hydrologic conditions in Sinclair Inlet. There was a slight increase in nitrate concentration near the CV 62 on Event 3; however, the differences were not statistical significant after Event 4, suggesting that any input associated with hull cleaning was short-term and within the range of normally occurring nitrate concentrations in Sinclair Inlet (Figure 27).

While the biofouling removal of the ex-INDEPENDENCE may be partially responsible for the slight increases in nitrate concentration, these changes can also be attributed to changes in precipitation and effects from stormwater runoff. Meteorological conditions varied during the sampling events. Weather conditions in Event 1 and Event 2 were dry (no rain), clear days. In contrast, during the two post removal events, there was cold rain on Event 3, and wind, snow and cold rain during Event 4. Therefore, stormwater runoff may have contributed to the increased nutrient loading observed during these sampling events. While the variations in meteorological conditions may have had an effect in the nutrient levels observed in this effort, the total change in nitrate concentrations was minimal and within the range of nitrate concentrations previously measured in Sinclair Inlet (Figure 27, Katz et al., 2004; Ecology, 2017).

The effects of meteorological and hydrographic conditions could also support the homogeneity of the nitrate data at each station (Figure 16). As presented in the results section above, some stratification of the water column could be derived from the data from Event 1. However, the other three sampling events show more homogeneity in the depth profiles for nitrate concentration, indicating that the water column was well-mixed and fairly homogeneous. This is an indicator that dissolved oxygen concentrations were replenished to bottom waters more efficiently under these conditions, reducing any potential effect that organic loading associated with biofouling removal from ex-INDEPENDENCE could have in the area, and increasing the potential for distribution of this organic matter load to a larger volume of water or sediment surface area.

6.4.2 Nitrites

Lack of detectable nitrite data hinders the use of this parameter in assessing any effect of nitrite release from biofouling removal from the ex-INDEPENDENCE. Available data originated from Event 2, which had detectable nitrite levels. If attributable to biofouling removal, the levels were within the range of previously reported data (Figure 28). These data, though sparse, corroborate the findings for nitrates, that biofouling removal had a negligible impact on nutrient levels in Sinclair Inlet. Secondarily, the data may also be indicative of water column oxygenation, which could have increase oxidation of nitrite to nitrate.

6.4.3 Ammonia

Total ammonia concentrations (Figure 29) were not statistically different from ship and reference stations during any of the sampling events, and concentrations measured throughout this study were an order of magnitude lower than the calculated US EPA WQC of 2.1 mg/L (average salinity 30, pH 8.0 and 21°C). Measured ammonia concentrations were in the upper range of previously reported values from independent studies, however, the range (ND to 0.23 mg/L) was consistent with the timeframe of this study.

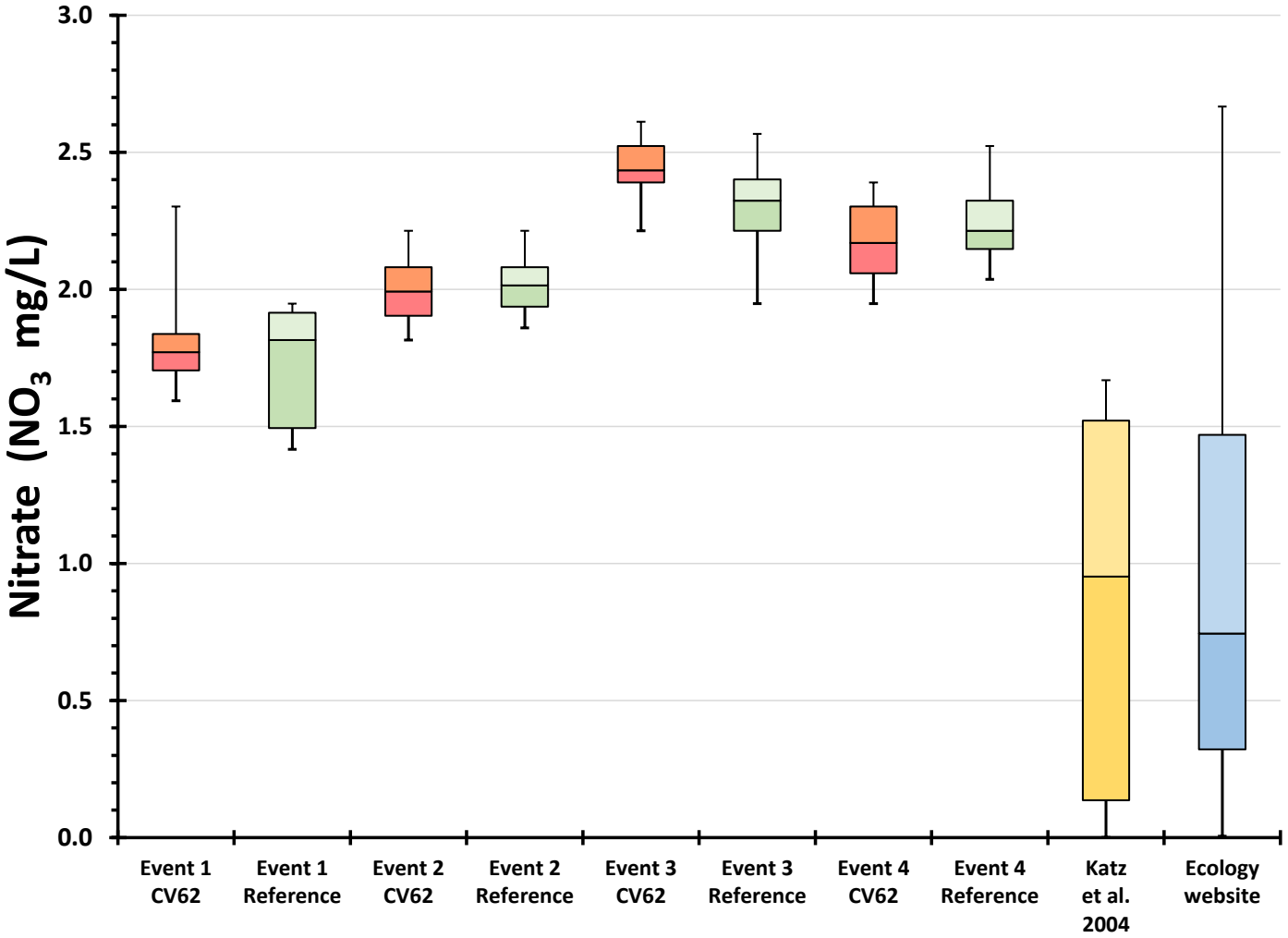


Figure 27. Comparison of the range in nitrate (NO_3) measured (mg/L) in this effort to those from Katz et al. (2004) and data reported for station SIN001_0 in Sinclair Inlet monitored by the State of Washington Department of Ecology (fortress.wa.gov/ecy/eap/marinewq/mwdataset.asp?staID=129, accessed 15 May 2017). (Ecology 2017a)

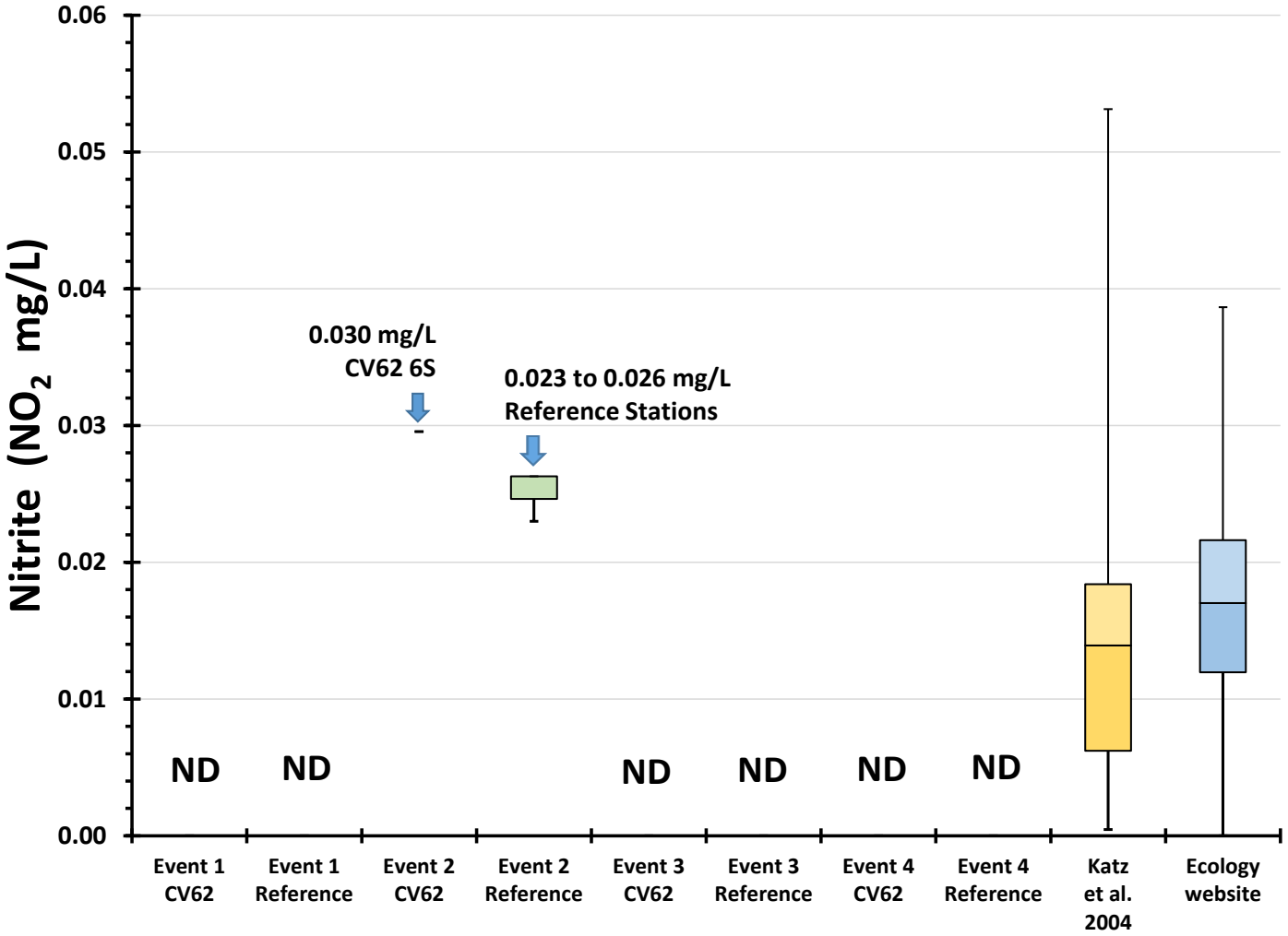


Figure 28. Comparison of the range in nitrite (NO_2) measured (mg/L) in this effort to those from Katz et al. (2004) and data reported for one station in Sinclair Inlet (SIN001_0) monitored by the State of Washington Department of Ecology (**fortress.wa.gov/ecy/eap/marinewq/mwdataset.asp?stalD=129**, accessed 15 May 2017).(Ecology,2017a) ND is non-detect.

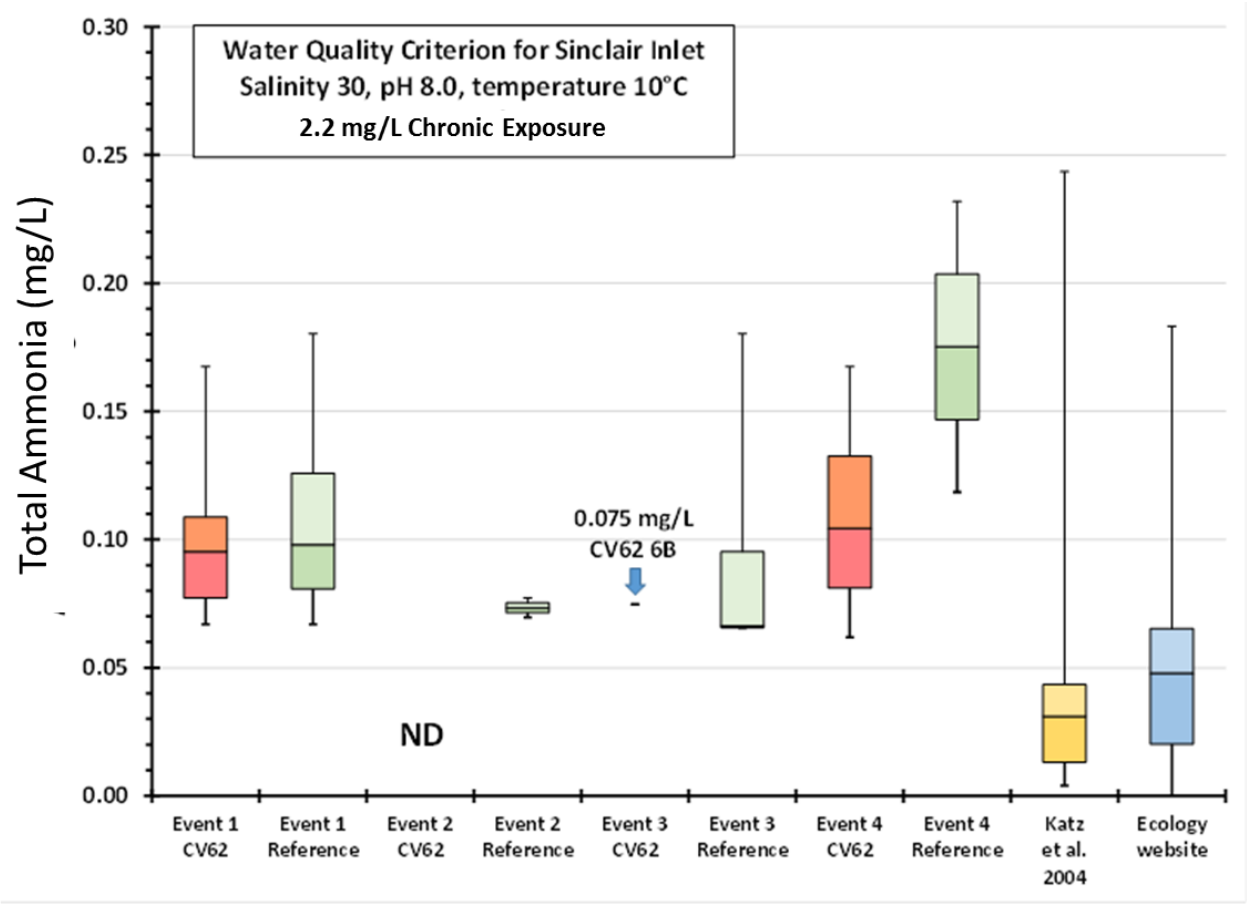


Figure 29. Comparison of the range in total ammonia measured (mg/L) in this effort to those from Katz et al. (2004) and data reported for one station in Sinclair Inlet (SIN001_0) monitored by the State of Washington Department of Ecology (**fortress.wa.gov/ecy/eap/marinewq/mwdataset.asp?stalD=129**, accessed 15 May 2017). ND is non-detect.

6.5 ORGANIC MATTER

6.5.1 Dissolved Organic Carbon (DOC)

Low concentrations of DOC were measured in this effort (Figure 30). There were only a few samples with DOC levels quantified above the 0.2 mg/L MDL for DOC, however, they were in a range (0.2 to 0.8 mg/L) that were below values reported by Rosen et al. (2009) of 0.85 to 1.51 mg/L. It should be noted, DOC data from Rosen et al. (2009) for laboratory water and Dyes Inlet were excluded from comparison. Only the Rosen et al. (2009) data for winter (31 March 2004 and 9 February 2005) were used in the DOC and copper comparisons. DOC concentrations were measured during Event 2 and Event 3, and show the cumulative effect of the biofouling removal of the ex-INDEPENDENCE combined with the effect of runoff during Event 3. DOC concentrations are highly influenced by plankton growth and other processes occurring in the water column. Plankton growth is limited during the winter months with low temperatures and less sun light available for photosynthesis. Regardless, the cumulative effect is still very low, with similar concentrations at both the ex-INDEPENDENCE and the reference stations for the detected concentrations.

6.5.2 Biological Oxygen Demand (BOD)

All the sample quantifications for BOD were below the non-detect (ND) level, with a MDL of 2.0 mg/L. This means that the method was not capable of detecting any changes in BOD below 2.0 mg/L, and it also indicates that there was not an increase in BOD to values over 2.0 mg/L throughout the sampling events. Therefore, the organic matter released during the biofouling removal from the ex-INDEPENDENCE did not impact BOD at detectable levels during the study.

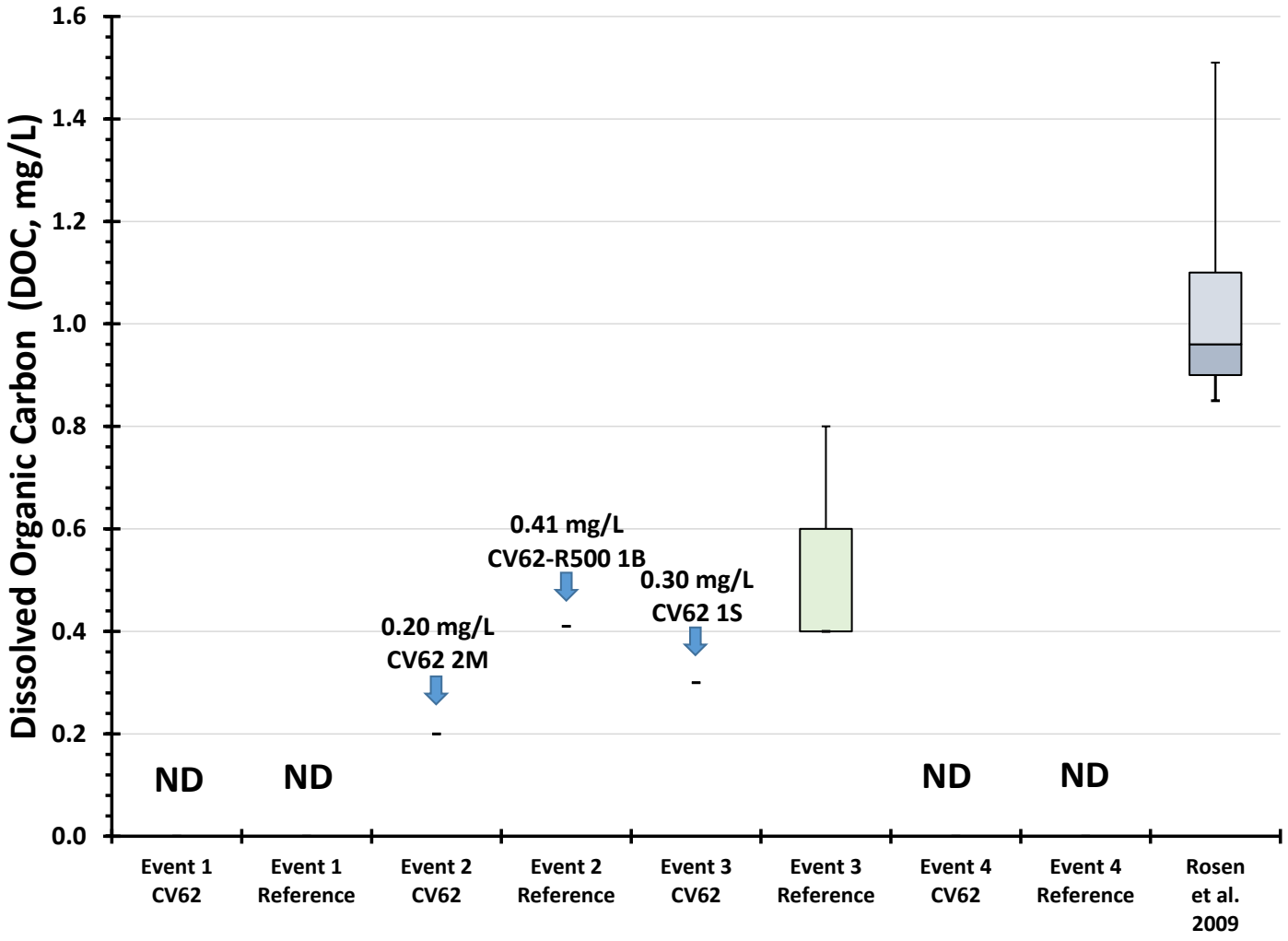


Figure 30. Comparison of the range in dissolved organic carbon (DOC) measured (mg/L) in this effort to those from Rosen et al. (2009) measured in winter. ND is non-detect (MDL 0.2 mg/L

6.6 IMPACT FROM BIOFOULING REMOVAL

Concentrations of copper and zinc measured throughout this study were at or near the instrument and method detection limits making these values difficult to precisely and accurately quantify. The trace metal data presented herein were obtained by strict adherence to QA/QC requirements which established how everything was accomplished from the type of sampling equipment that was utilized all the way through sampling procedures, laboratory processing, and data quality analysis. All of these QA/QC procedures provide a high level of confidence in the results presented.

All measured concentrations were well below the water quality standards for dissolved copper and zinc, including maximum levels measured during Event 2 and Event 3. Elevated dissolved and total copper concentrations were measured at the Ship site during Event 2 and Event 3, however the average dissolved copper concentrations for Event 2 (0.77 μg/L) and Event 3 (0.46 μg/L) were ≤ 25% and ≤ 15% of chronic WQS of 3.1 μg/L, and ≤ 16% and ≤ 10 of the acute WQS of 4.8 μg/L. By Event 4 all the measured dissolved copper concentrations were similar to Reference sites and Baseline levels (Figure 24).

A decision matrix was used to formalize conclusions about potential impacts to water quality resulting from the biofouling removal from the ex-INDEPENDENCE (Table 11). The assessment was based on statistical significance of changes to water quality parameters during and after the biofouling removal, the magnitude of any effects, and the potential of exceeding water quality standards. Conclusions were:

- No negative impacts from total and dissolved zinc, dissolved oxygen, nitrite, ammonia, DOC, and BOD were found
- Statistically significant increases of total and dissolved copper, turbidity, and nitrate were measured
- Dissolved copper and zinc, dissolved oxygen, and turbidity did not exceed Water Quality Standards and were ≤ 25% of the threshold range
- All parameters returned to baseline levels and were similar to reference conditions within 40 days after biofouling removal was completed

Table 11. Outcome of the decision matrix for determining the impact of biofouling removal from the hull of the ex-INDEPENDENCE on water quality in Sinclair Inlet, WA.

			Potential of Exceeding Benchmark or Standard					
			% of Threshold Range					
			≤25%	>25% and <50%	>50% and <75%	>75% and <100%	>100% and <150%	>150%
	Magnitude of Difference		Very Low	Low	Medium	High	Very High	Adverse
Statistical Difference from Reference	No Difference or Better than Reference	**None** **Total Zn, NO2, Ammonia, DOC, BOD**	**No Impact** **Dissolved Zn, DO**					
	≤2x Reference	**Sightly Different** **NO3**	**Negligible Impact** **Dissolved Cu, Turb**					
	>2x and <5x Reference	**Different** **Total Cu**						
	≥5x and <10x Reference							
	>10x Reference							

Note: The conclusion (shown in Table cells) is based on the magnitude of statistical differences between the ship and reference sites (Table rows) combined with the potential of exceeding a regulatory benchmark or standard (Table columns).

7. SUMMARY AND CONCLUSIONS

Biofouling removal from the ex-INDEPENDENCE was conducted from January 6 to 27, 2017, at Mooring G at Puget Sound Naval Shipyard (PSNS). This study was conducted to monitor and evaluate key water quality parameters at six sites located near the ship (area of influence) and four reference sites within western Sinclair Inlet. Four sampling events were conducted, which included *before removal* (Event 1, Baseline, November 9 to 10, 2016), *during removal* (Event 2, During-removal, January 10, 2017), at the *end of removal* (Event 3, Week-post-removal, January 31, 2017), and *40 days after removal was completed* (Event 4, Month-post-removal, March 7, 2017). Each sampling event consisted of collecting discrete water samples from the surface, mid-depth, and near bottom strata of the water column. Water samples were analyzed for dissolved and total Cu and Zn, nutrients, DOC and BOD. In addition, in-situ sensors were utilized during the study to provide continuous monitoring of temperature, salinity, pH, DO, and turbidity within the water column at each sampling site.

The results of study were analyzed to determine if there were statistically significant differences in the water quality parameters between the Ship and Reference sites, and if there was a persistent difference between Event 1 conditions at the ship and subsequent sampling events. Potential impacts to water quality were evaluated by comparing the concentrations observed to US EPA and Washington State water quality standards as well as other previous and ongoing water quality monitoring efforts in Sinclair Inlet.

Statistical tests indicated elevated levels of total or dissolved copper and nutrient concentrations that were small in magnitude and temporary. This indicates that the study design was sensitive enough to discern potential changes in the environment associated with biofouling removal but does not indicate untoward environmental impacts. Given that these levels did not exceed water quality standards; it was concluded that there was no impact associated with these potential effects. Overall, levels of turbidity, DO, dissolved copper, and dissolved zinc did not exceed water quality standards during the study and small differences between the Ship and Reference sites were detected for DO, turbidity, nutrients, and dissolved and total Cu and Zn. There was no evidence of any parameter exceeding a regulatory threshold established by USEPA and Department of Ecology, and no evidence of a persistent impact. Finally, the observed differences in water quality indicators returned to near baseline levels within 40 days after the biofouling removal was completed.

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APPENDICES

Figures

Tables

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APPENDIX A
FIELD DATA COLLECTIONS

OVERVIEW

Appendix A contains details of four events and data links. For printed versions of the report there is a CD included that contains content shown here as hyperlinks. Also included on the CD are: TROLL_DATA, wqdTA5, Bremerton_data, and Bremerton_Plots.

EVENTS

A.1 EVENT 1

EVENT 1, Baseline Nov 9-10, 2016

\Data\CV2016_11_09-10\
- Chain of Custody Sheets
- Field Logs
- Raw Data Files
- In-situ Troll 9500 Profile Plots

ProfilePlots_CV2016_Baseline.xlxs

A.2 EVENT 2

EVENT 2, During-Removal Jan 10, 2017

\Data\CV2017_01_10\
- Chain of Custody Sheets
- Field Logs
- Raw Data Files
- In-situ Troll 9500 Profile Plots

ProfilePlots_CV2017_BeginingCleaning.xlxs

A.3 EVENT 3

EVENT 3, Week-Post-Removal Jan 31, 2017

\Data\CV2017_01_31\
- Chain of Custody Sheets
- Field Logs
- Raw Data Files
- In-situ Troll 9500 Profile Plots

ProfilePlots_CV2017_EndCleaning.xlxs

A.4 EVENT 4

EVENT 4, Month-Post-Removal Mar 7, 2017

\Data\CV2017_03_07\
- Chain of Custody Sheets
- Field Logs
- Raw Data Files
- In-situ Troll 9500 Profile Plots

ProfilePlots_CV2017_AfterCleaning.xlxs

DATA LINKS

There are three data links in this section, In-situ Troll 9500 data, Statistics Data File, and ADCP data.

A.5 IN-SITU TROLL 9500 DATA

\Data\TROLL_DATA_master4.xlsx

A.6 STATISTICS DATA FILE

Data\WQData5.xls

A.7 ADCP DATA

Data\ADCP\ADCP_2017_CV62_Bremerton_data.xlsx

Data\ADCP\ADCP_2017_CV62_Bremerton_Plots.pptx

APPENDIX B
ANALYITICAL CHEMISTRY, QA/QC NARRATIVE AND RAW DATA

OVERVIEW

Appendix B contains two parts B1. Analytical Chemistry QA/QC Narrative and B.2 SSC Pacific RAW Data Tables.

B.1 ANALYTICAL CHEMISTRY QA/QC NARRATIVE

PROJECT: Water Quality Monitoring of Biofouling Removal from the ex-INDEPENDENCE

PARAMETER: Total and Dissolved Metals – Cu, Zn

Nitrite (NO_2-N), Nitrate (NO_3-N), Ammonia (NH_3-N) Dissolved Organic Carbon (DOC), and Biological Oxygen Demand (BOD)

LABORATORY: EnviroMatrix, ALS, SPAWAR Systems Center Pacific

MATRIX: Seawater

SAMPLE CUSTODY AND PROCESSING:

Samples were collected from reference stations and in the vicinity of the ex-INDEPENDENCE during four different time intervals relative to the vessels hull cleaning. Baseline samples were collected on 11/10/2016, during the cleaning event on 1/10/2017, one-week post cleaning on 1/31/2017, and one month post cleaning on 3/7/2017. Samples were collected by SPAWAR Systems Center Pacific (SSC Pacific) scientist on board a U.S. Navy small boat.

Samples were stored on ice in a cooler until shipment to the various laboratories. Coolers were shipped overnight to EnviroMatrix for nutrient analysis, to ALS for BOD and DOC, and to SSC Pacific for metals analysis. All samples were received in good condition and below 4° C.

There were a few issues related to the Chain of Custody (COC). The COC for BOD sent to ALS on 11/10/2016 was not signed. The COC for ALS on the 1/10/2017 sampling date was missing page 1 of 3 for the BOD samples, however, sample IDs and times were written on the bottles and corresponded to the sample times and IDs on the COC for DOC samples, which were collected at the same time as the BOD samples.

There were a few discrepancies between bottle labels and COC for samples collected during 3/7/2017. Two bottles were labeled CV62-2B, no sample bottle for CV62-2B Dup (the "Dup" was left of the second CV62-2B bottle), while there was only one sample collected for CV62-1B, which was erroneously labeled with the "Dup" extension.

The following lists information on sample receipt and processing activities:

Table B-1. Sample Chain of Custody report.

Sample Type	Lab	Collected	Receipt	Analysis
"Baseline" BOD/DOC	ALS	11/10/2016	11/11/2016	11/11/2016
"During-Cleaning" BOD DOC	ALS	 1/10/2017 1/10/2017	 1/12/2017 1/12/2017	 1/13/2017 1/18/2017
"Week-Post-Cleaning" BOD DOC	ALS	 1/31/2017 1/31/2017	 2/1/2017 2/1/2017	 2/1/2017 2/6/2017
"Month-Post-Cleaning" BOD/DOC	ALS	3/7/2017	3/9/2017	
"Baseline" Nutrients Nitrite Nitrate Ammonia	EnviroMatrix	 11/9 and 11/10, 2 11/9 and 11/10, 2 11/9 and 11/10, 2	 11/10 and 11/11, 2 11/10 and 11/11, 2 11/17, 2016	 11/10 and 11/11, 2 11/19 and 11/21, 2 11/21, 2016
"During-Cleaning" Nutrients Nitrite Nitrate Ammonia	EnviroMatrix	 1/10/2017 1/10/2017 1/10/2017	 1/11/2017 1/11/2017 1/17/2017	 1/11/2017 1/25/2017 1/24/2017
"Week-Post-Cleaning" Nutrients Nitrite Nitrate Ammonia	EnviroMatrix	 1/31/2017 1/31/2017 1/31/2017	 2/1/2017 2/1/2017 2/3/2017	 2/1/2017 2/11/2017 2/10/2017
"Month-Post-Cleaning" Nutrients Nitrite Nitrate Ammonia	EnviroMatrix	 3/7/2017 3/7/2017 3/7/2017	 3/9/2017 3/9/2017 3/10/2017	 3/9/2017 3/20/2017 3/17/2017
"Baseline" Metals	SSC Pacific	11/10/2016	11/12/2016	11/30/2016
"During-Cleaning" Meta	SSC Pacific	1/10/2017	1/12/2017	2/24/2017
"Week-Post-Cleaning" Metals	SSC Pacific	1/31/2017	2/2/2017	2/28/2017
"Month-Post-Cleaning" Metals	SSC Pacific	3/7/2017	3/9/2017	4/4/2017

Table B-2. QA/QC data quality objectives for seawater samples.

				MS	SRM			
Analyte	Analytical Method for Seawater			Range of Recovery	Percent Difference	Replicate Precision	Method Detection Limits	Reporting Limits
DOC	SM5310 C			NA	≤20%	≤20%	0.2 mg/L	1.0 mg/L
BOD	SM5210B			80-120%	≤20%	≤20%	2.0 mg/L	4.0 mg/L
Nitrite	SM4500 NO2 B			80-120%	≤20%	≤20%	0.007 mg/L	0.05 mg/L
Nitrate	SM4500 NO3 E			80-120%	≤20%	≤20%	0.009 mg/L	0.05 mg/L
Ammonia	EPA 350.1			80-120%	≤20%	≤20%	0.048 mg/L	0.10 mg/L
Copper	ICP-MS			70-130%	≤30%	≤30%	0.47 µg/L	1.55 µg/L
Zinc	ICP-MS			70-100%	≤30%	≤30%	0.66 µg/L	2.20 µg/L

METHODS:

Prior to analysis, all samples were acidified to pH ≤2 with quartz still grade nitric acid (Q-HNO_3) in a HEPA class-100 all polypropylene working area. Copper and zinc concentrations in the samples were measured with a Perkin-Elmer SCIEX ELAN DRC II inductively coupled plasma mass spectrometer (ICP-MS; US EPA, 1994).

Metal concentration in seawater samples were quantified by flow injection analysis. An on-line Perkin-Elmer Flow Injection for Atomic Spectroscopy (FIAS) 400 was used for pre-concentration and salt matrix removal using TOYOPEARL AF-Chelate-650M from Tosoh Corp. The FIAS 400 is coupled with an Autosampler 100 and set to inject the treated sample directly into the ICP-MS. Analytical standards were made with Perkin-Elmer multi-element standard solution (PEMES-3) diluted in 0.45 µm filtered and acidified (pH ≤2 with Q-HNO_3) seawater collected outside San Diego Bay in September 1999 (SDBSW), to match the salinity of the test samples. Standards were analyzed at the beginning and end of the run with acceptable calibration curves with R ≥0.999. Blanks made up of SDBSW were analyzed every five samples. The ICP-MS data were reported in units of µg/L

HOLDING TIMES:

All samples were analyzed within the established holding times except for those noted below:

ALS

For the "one month" samples collected on 3/7/2017, Samples #1 R500-1B, #4 R500-2B, #7 R1000-1B and #10 R1000-2B were received with insufficient holding time remaining. Samples were analyzed at 1300 on 3/9/2017, with the above four samples being collected between 1007 and 1140 on 3/7/2017, up to three hours after the 48 hour holding time window. The analysis was performed as soon as possible after receipt by the laboratory. The data was flagged to indicate the holding time violation. In spite of the time violation, all samples collected this day and all previous sampling events were non-detects (ND U), below the MRL/MDL.

EnviroMatrix

The nitrate samples from all of the sampling events had the following listed in notes "W-02", where W-02 means, "The sample for nitrate analysis was preserved with H2SO4 after the nitrite portion of the analysis was completed, extending the timeframe any given sample may be held before it must be analyzed. Nitrate results are corrected for the nitrite contribution per this method". Samples were then analyzed within the allowable holding time following preservation.

All nitrite samples collected on 3/17/2017 were designated "HT-13." HT-13 samples were received with limited processing time remaining prior to the deadline for analysis. Samples were collected between 1007 and 1616 on 3/7/2017, and were received by the laboratory on 3/9/2017 at 0923. Time of analysis was not provided in the data deliverable, but a small portion of the samples collected in the morning of 3/7/2017 may have been analyzed outside of the 48 hour holding time window. All nitrite results from 3/7/2017 were non-detect (ND) which is consistent with the nitrite results from all other sampling periods except for those collected on 1/10/2017, where eight samples had nitrite concentrations at or slightly above the MDL, but below the MRL.

DETECTION LIMITS:

The data are evaluated and flagged as follows:

* The result is an outlier. See case narrative.

The control limit criteria is not applicable. See case narrative.

B The analyte was found in the associated method blank at a significant level relative to sample result levels defined by the DOD or NELAC standards.

E The result is an estimated amount because the value exceeded the instrument calibration range.

J The result is an estimated value.

U After thorough analysis, the analyte was not detected ("Non-detect") at or above the MRL/MDL. DOD-QSM 4.2 definition: Analyte was not detected and is reported as less than the LOD or as defined by the project. The detection limit is adjusted for dilution.

i The MRL/MDL or LOQ/LOD is elevated due to a matrix interference.

X See case narrative.

Q See case narrative. One or more quality control criteria was outside the limits.

H The holding time for this test

METHOD BLANKS:

A minimum of three method blanks were analyzed by each instrument with each analytical batch for metals. The average method blank for each batch was less than the RL for all constituents.

LABORATORY CONTROL SAMPLES:

A minimum of one LCS (OPR or blank spike) was prepared and analyzed with each analytical batch of 20 or fewer samples for metals. Percent recoveries for LCS samples were within the QC acceptance criterion of 80% to 120% for all constituents.

MATRIX SPIKE ACCURACY:

A minimum of one set of duplicate matrix spikes (MS/MSD) was prepared and analyzed with each analytical batch of 20 or fewer samples for metals. Percent recoveries for matrix spikes were within the QC limits of 80% to 120% for BOD, DOC, and nutrients, and within the QC limits of 70% to 130% for metals, except as noted below.

ALS

11/10/2017

The matrix spike recoveries for samples CV62R-1000-1B and CV62-2B were outside control criteria (~55% recovery) because of suspected matrix interference. As a result of the interference, the results for this analyte contained a potential low bias. No further corrective action was taken.

1/10/2017

The matrix spike recoveries for samples 500-1-B and CV62-4-M were outside control criteria (~50% recovery) believed to be the result of matrix interference As a result of the interference, the results for this analyte contained a potential low bias. No further corrective action was taken.

1/31/2017

The matrix spike recoveries for samples R1000-1-B and CV62-3-S were outside control criteria (~50% recovery) because of suspected matrix interference. As a result of the interference, the results for this analyte contained a potential low bias. No further corrective action was taken.

3/7/2017

The matrix spike recoveries for samples #2 R500-1m and #22 CV62-4b were outside control criteria because of suspected matrix interference. As a result of the interference, the results for this analyte contained a potential low bias. No further corrective action was taken.

SPAWAR

3/7/2017

Matrix spikes for copper were registering below the 70% recovery range are as follows: CV62 2M spike concentration 1.85 µg/L, measured concentration 1.12 µg/L; 61% recovery. CV62R 500 2S spike concentration 1.85 µg/L, measured concentration 1.16 µg/L; 62% recovery. CV62 6B spike concentration 1.63 µg/L, measured concentration 0.91 µg/L; 56% recovery

Matrix spikes for zinc were registering below the 70% recovery range are as follows: CV62 2M spike concentration 1.85 µg/L measured concentration 1.12 µg/L; 61% recovery. CV62R 500 2S spike concentration 1.85 µg/L measured concentration 1.16 µg/L; 62% recovery. CV62 6B spike concentration 1.63 µg/L measured concentration 0.91 µg/L; 56% recovery

REPLICATE PRECISION:

Laboratory precision was expressed as the relative percent difference (RPD) between laboratory duplicates. The RPD values for the laboratory duplicates were within the QC acceptance criterion of ±20% for all parameters detected above the RL.

ALS

1/10/2017

The Relative Percent Difference (RPD) criterion for the replicate analysis in sample 500-1-B was not applicable because the analyte concentration was not significantly greater than the Method Reporting Limit (MRL). Analytical values derived from measurements close to the detection limit are not subject to the same accuracy and precision criteria, as it is for results derived from measurements higher on the calibration range for the method.

1/31/2017

The Relative Percent Difference (RPD) criterion for the replicate analysis in samples R1000-1-B and R1000-1-M were not applicable as the analyte concentration was not significantly greater than the Method Reporting Limit (MRL). Analytical values derived from measurements close to the detection limit are not subject to the same accuracy and precision criteria, as results derived from measurements higher on the calibration range for the method.

STANDARD REFERENCE MATERIAL ACCURACY:

Certified reference materials (CRM) CASS-6, Nearshore Seawater Certified Reference Material for Trace Metals and other Constituents, from the National Research Council from Canada was analyzed with each analytical batch at a minimum frequency of 1 per 20 or fewer samples. Analytical accuracy was expressed as the percent recovery (PR) between the measured and the certified value.

CASS-6 is certified to 0.530 ± 0.032 µg/L for copper and 1.27 ± 0.18 µg/L zinc. The actual recovery for CASS6 was 89 ± 14 % for copper and 92 ± 17 % for zinc.

B.3 SSC PACIFIC RAW DATA TABLES

Table B-3. Data for Event 1, Baseline, sampled on 9 to 10 November 2016.

Sample ID	NO_3 (mg/L)	NO_2 (mg/L)	Ammonia (mg/L)	BOD (mg/L)	DOC (mg/L)	Diss. Cu (µg/L)	Total Cu (µg/L)	Diss. Zn (µg/L)	Total Zn (µg/L)
CV62-1B	2.30	ND	0.095	ND	ND	0.65	0.73	17.48*	1.14
CV62-1M	1.81	ND	0.072		ND	1.08	1.13	2.16*	1.76
CV62-1S	1.64	ND	0.098		ND	0.93	0.82	3827*	1.96
CV62-2B	2.12	ND	0.109	ND	ND	0.48	0.81	1.25	1.60
CV62-2M	1.77	ND	0.081		ND	0.70	0.69	0.83	1.10
CV62-2S						1.14	1.69	63.68*	5.40
CV62-3B	1.86	ND	0.108	ND	ND	0.78	0.95	2.85*	1.30
CV62-3M	1.68	ND	0.076		ND	0.57	0.64	1.79*	1.11
CV62-3S					ND	0.82	0.93	1.92	2.59
CV62-4B	1.73	ND	0.079	ND	ND	0.69	0.81	1.14	1.62
CV62-4M	1.77	ND	0.070		ND	0.57	0.49	1.20	1.81
CV62-4S	1.64	ND	0.103		ND	0.92	0.97	14.52*	3.02
CV62-5B	1.77	ND	0.093	ND	ND	0.59	0.82	41.89*	1.56
CV62-5M	1.59	ND	0.067		ND	0.62	0.58	5.32*	0.94
CV62-5S					ND	0.51	0.83	0.92	2.50
CV62-6B	1.81	ND	0.167	ND		0.57	0.90	12.41*	2.18
CV62-6M	1.90	ND	0.142		ND	0.62	1.00	1.41	2.05

Diss. Means dissolved, blank boxes represent uncollected samples, ND is Non-Detect, shaded yellow cell indicate ND value was substituted for half the Detection Limit (DL/2), * is for dissolved Zinc samples considered contaminated during sample collection and not included in the statistics here, or in the discussion in the report.

Table 3. Data for Event 1, Baseline, sampled on 9 to 10 November 2016. (Continued)

Sample ID	NO3 (mg/L)	NO2 (mg/L)	Ammonia (mg/L)	BOD (mg/L)	DOC (mg/L)	Diss. Cu (µg/L)	Total Cu (µg/L)	Diss. Zn (µg/L)	Total Zn (µg/L)
CV62-6S	1.77	ND	0.109		ND	0.69	0.84	1.29	2.13
CV62-R500 1B	1.95	ND	0.088	ND	ND	0.43	0.70	8.10*	2.14
CV62-R500 1M	1.90	ND	0.093		ND	0.47	0.69	1.60	1.79
CV62-R500 1S	1.42	ND	ND		ND	0.49	0.65	3.10*	1.57
CV62-R500 2B	1.95	ND	0.121	ND	ND	0.48	0.91	0.85	0.98
CV62-R500 2M	1.90	ND	0.067			0.56	0.66	0.86	0.72
CV62-R500 2S	1.51	ND	0.075		ND	0.54	0.78	1.08	2.46
CV62-R1000 1B	1.73	ND	0.127	ND	ND	0.49	0.58	1.66	1.12
CV62-R1000 1M	1.81	ND	0.167			0.46	0.71	1.77	1.67
CV62-R1000 1S	1.46	ND	0.079		ND	0.61	0.94	1.71	2.93
CV62-R1000 2B	1.81	ND	0.180	ND	ND	0.43	0.55	1.49*	0.10
CV62-R1000 2M	1.95	ND	ND			0.51	0.51	1.83	0.97
CV62-R1000 2S	1.42	ND	0.103		ND	0.38	0.86	1.89	2.57

Diss. Means dissolved, blank boxes represent uncollected samples, ND is Non-Detect, shaded yellow cell indicate ND value was substituted for half the Detection Limit (DL/2), * is for dissolved Zinc samples considered contaminated during sample collection and not included in the statistics here, or in the discussion in the report.

Table B-4. Data for Event 2, During-removal, sampled on 10 January 2017.[2]

Sample ID	NO3 (mg/L)	NO2 (mg/L)	Ammonia (mg/L)	BOD (mg/L)	DOC (mg/L)	Diss. Cu (µg/L)	Total Cu (µg/L)	Diss. Zn (µg/L)	Total Zn (µg/L)
CV62-1B	2.12	ND	ND	ND	ND	0.49	2.75	0.10	0.89
CV62-1M	1.90	ND	ND		ND	0.66	3.30	0.10	1.32
CV62-1S	2.17	ND	ND		ND	0.82	2.07	0.10	0.63
CV62-2B	1.95	ND	ND	ND	ND	0.68	6.64	0.10	1.52
CV62-2M	1.81	ND	ND		0.20	0.61	3.53	0.10	1.43
CV62-2S	1.81	ND	ND		ND	1.43	8.84	0.66	3.82
CV62-3B	2.04	ND	ND	ND	ND	0.40	1.40	0.10	1.48
CV62-3M	1.99	ND	ND		ND	0.42	4.91	0.10	1.39
CV62-3S	1.99	ND	ND		ND	0.78	6.77	0.17	1.67
CV62-4B	2.04	ND	ND	ND	ND	0.38	1.27	0.27	1.39
CV62-4M	1.86	ND	ND		ND	0.45	0.99	0.31	1.58
CV62-4S	2.21	ND	ND		ND	0.96	2.11	0.71	1.62
CV62-5B	2.08	ND	ND	ND	ND	0.61	1.22	0.50	1.37
CV62-5M					ND	0.60	1.57	0.48	1.65
CV62-5S	2.12	ND	ND		ND	0.83	1.52	0.63	1.75
CV62-6B	1.95	ND	ND	ND	ND	1.14	10.21	0.73	2.00
CV62-6M	1.86	ND	ND		ND	1.08	3.20	0.79	1.44
CV62-6S	2.04	0.030	ND		ND	1.58	3.33	1.03	0.10
CV62-R500 1B	2.21	ND	ND	ND	0.41	0.28	0.93	0.66	1.54

[2] Shaded yellow cell indicate ND value was substituted for half the Detection Limit (DL/2)

Table B-4. Data for Event 2, During-removal, sampled on 10 January 2017.[2] (Continued)

Sample ID	NO3 (mg/L)	NO2 (mg/L)	NH3 (mg/L)	BOD (mg/L)	DOC (mg/L)	Diss. Cu (µg/L)	Total Cu (µg/L)	Diss. Zn (µg/L)	Total Zn (µg/L)
CV62-R500 1M	2.04	0.026	ND		ND	0.05	0.97	0.10	1.83
CV62-R500 1S	1.90	0.026	0.070		ND	0.82	1.36	1.16	1.02
CV62-R500 2B	1.95	ND	ND	ND	ND	0.33	0.73	0.87	1.60
CV62-R500 2M	2.04	ND	ND		ND	0.54	0.87	0.83	1.66
CV62-R500 2S	1.95	0.026	ND		ND	0.71	1.31	0.97	1.74
CV62-R1000 1B	2.12	ND	ND	ND	ND	0.29	0.78	0.75	1.91
CV62-R1000 1M	1.90	ND	ND			0.24	0.45	0.70	1.13
CV62-R1000 1S	2.08	0.026	0.077		ND	0.81	1.25	1.38	2.02
CV62-R1000 2B	1.86	0.023	ND	ND	ND	0.39	0.84	1.13	1.85
CV62-R1000 2M	1.99	0.026	ND			0.41	0.81	1.17	1.72
CV62-R1000 2S	2.08	0.023	ND		ND	0.78	0.91	1.25	1.29

[2] Shaded cell indicate ND value was substituted for half the Detection Limit (DL/2)

Table B-5. Data for Event 3, Week-post-removal, sampled on 31 January 2017.[2]

Sample ID	**NO_3 (mg/L)**	**NO_2 (mg/L)**	**NH_3 (mg/L)**	**BOD (mg/L)**	**DOC (mg/L)**	**Diss. Cu (µg/L)**	**Total Cu (µg/L)**	**Diss. Zn (µg/L)**	**Total Zn (µg/L)**
CV62-1B	2.21	ND	ND	ND	ND	0.05	3.69	1.52	1.00
CV62-1M	2.43	ND	ND		ND	0.05	1.71	2.38	1.19
CV62-1S	2.39	ND	ND		0.3	0.05	2.40	1.91	1.17
CV62-2B	2.61	ND	ND	ND	ND	0.05	7.16	1.91	1.55
CV62-2M	2.52	ND	ND		ND	0.46	2.61	2.56	1.23
CV62-2S	2.43	ND	ND		ND	0.34	2.25	2.06	1.14
CV62-3B	2.52	ND	ND	ND	ND	0.44	1.54	1.38	0.88

[2] Shaded cell indicate ND value was substituted for half the Detection Limit (DL/2)

Table B-5. Data for Event 3, Week-post-removal, sampled on 31 January 2017.2 (Continued)

Sample ID	NO3 (mg/L)	NO2 (mg/L)	NH3 (mg/L)	BOD (mg/L)	DOC (mg/L)	Diss. Cu (µg/L)	Total Cu (µg/L)	Diss. Zn (µg/L)	Total Zn (µg/L)
CV62-3M	2.43	ND	ND		ND	0.28	2.39	1.70	0.98
CV62-3S	2.39	ND	ND		ND	0.75	2.82	2.35	1.43
CV62-4B	2.48	ND	ND	ND	ND	0.05	1.04	1.43	1.00
CV62-4M					ND	0.62	1.95	2.30	1.54
CV62-4S	2.61	ND	ND		ND	0.93	2.41	2.08	1.12
CV62-5B	2.39	ND	ND	ND	ND	0.05	1.44	1.41	0.91
CV62-5M	2.39	ND	ND		ND	0.09	1.83	1.74	0.84
CV62-5S	2.48	ND	ND		ND	1.08	1.58	1.86	0.93
CV62-6B	2.52	ND	0.075	ND	ND	0.83	6.10	1.30	1.04
CV62-6M	2.35	ND	ND		ND	0.70	2.54	1.40	1.01
CV62-6S	2.35	ND	ND		ND	1.54	2.92	1.81	1.61
CV62-R500 1B	2.21	ND	ND	ND	ND	0.05	1.44	1.74	1.33
CV62-R500 1M	2.21	ND	ND		ND	0.18	0.88	1.92	1.07
CV62-R500 1S	2.57	ND	0.180		ND	0.16	0.94	1.94	1.22
CV62-R500 2B	2.39	ND	ND	ND	ND	0.36	0.38	1.79	1.03
CV62-R500 2M	2.52	ND	0.066		ND	0.26	0.73	2.01	1.16
CV62-R500 2S	2.30	ND	0.066		ND	0.05	0.60	1.95	1.25
CV62-R1000 1B	1.95	ND	ND	ND	0.8	0.05	0.62	1.48	2.15
CV62-R1000 1M	2.35	ND	0.067		0.4	0.12	0.39	1.71	1.84
CV62-R1000 1S	2.43	ND	ND		ND	0.15	0.97	1.64	1.61
CV62-R1000 2B	2.26	ND	ND	ND	ND	0.05	0.45	1.41	1.08
CV62-R1000 2M	2.21	ND	ND		0.4	0.14	1.01	1.59	1.45
CV62-R1000 2S	2.39	ND	ND		ND	0.16	0.90	1.82	1.53

[2] Shaded yellow cell indicate ND value was substituted for half the Detection Limit (DL/2)

Table B-6. Data for Event 4, Month-post-removal, sampled on 7 March 2017.

Sample ID	NO_3 (mg/L)	NO_2 (mg/L)	NH_3 (mg/L)	BOD (mg/L)	DOC (mg/L)	Diss. Cu (µg/L)	Total Cu (µg/L)	Diss. Zn (µg/L)	Total Zn (µg/L)
CV62-1B	2.04	ND	ND	ND	ND	0.52	0.88	1.03	1.37
CV62-1M	1.95	ND	ND		ND	0.64	0.72	1.14	1.22
CV62-1S	2.08	ND	0.062		ND	0.65	0.94	1.15	1.42
CV62-2B	2.04	ND	ND	ND	ND	0.50	0.88	1.01	1.37
CV62-2M	2.17	ND	ND		ND	0.68	0.97	1.17	1.45
CV62-2S	2.08	ND	ND		ND	0.72	0.84	1.21	1.33
CV62-3B	2.17	ND	ND	ND	ND	0.50	0.81	1.00	1.30
CV62-3M					ND	0.61	0.80	1.11	1.29
CV62-3S	2.17	ND	0.121		ND	0.74	0.91	1.23	1.40
CV62-4B	2.30	ND	ND	ND	ND	0.55	0.73	1.05	1.22
CV62-4M					ND	0.59	0.63	1.09	1.12
CV62-4S	2.26	ND	ND		ND	0.65	0.60	1.14	1.10
CV62-5B	2.30	ND	ND	ND	ND	0.50	0.63	1.01	1.13
CV62-5M					ND	0.60	0.64	1.10	1.13
CV62-5S	2.35	ND	0.167		ND	0.67	0.67	1.17	1.17
CV62-6B	2.30	ND	ND	ND	ND	0.50	0.83	1.00	1.32
CV62-6M	2.39	ND	ND		ND	0.57	0.64	1.07	1.14
CV62-6S	1.99	ND	0.088		ND	0.67	0.82	1.16	1.31
CV62-R500 1B	2.30	ND	ND	ND	ND	0.54	0.66	1.04	1.16
CV62-R500 1M					ND	0.55	0.63	1.05	1.12
CV62-R500 1S	2.17	ND	0.232		ND	0.59	0.65	1.08	1.15
CV62-R500 2B	2.35	ND	ND	ND	ND	0.51	0.57	1.01	1.07
CV62-R500 2M					ND	0.54	0.61	1.04	1.11

Table B-6. Data for Event 4, Month-post-removal, sampled on 7 March 2017. (Continued)

Sample ID	NO3 (mg/L)	NO2 (mg/L)	NH3 (mg/L)	BOD (mg/L)	DOC (mg/L)	Diss. Cu (µg/L)	Total Cu (µg/L)	Diss. Zn (µg/L)	Total Zn (µg/L)
CV62-R500 2S	2.12	ND	0.118		ND	0.56	0.64	1.06	1.14
CV62-R1000 1B	2.21	ND	ND	ND	ND	0.55	0.69	1.05	1.19
CV62-R1000 1M					ND	0.57	0.59	1.07	1.09
CV62-R1000 1S					ND	0.54	0.65	1.05	1.15
CV62-R1000 2B	2.52	ND	ND	ND	ND	0.54	0.70	1.04	1.19
CV62-R1000 2M					ND	0.60	0.59	1.10	1.09
CV62-R1000 2S	2.04	ND	ND		ND	0.59	0.58	1.09	1.08

APPENDIX C
STATISTICAL RESULTS

A.1 OVERVIEW

Descriptive statistics were compiled for the data by *Event* (1-Baseline, 2-During Removal, 3-End of Removal, and 4-After Removal), *Type* (Ship and Reference), and *Strata* (Surface, Middle, and Bottom) for the in-situ and discrete data Table C-1.

To visualize the data, a series of x-y plots were generated for the salient variables, where x=days since 11/8/2016 (start of the study) and y = variable of interest (Figure C-1 to Figure C-7). In these figures, the two vertical dashed lines on the x-axis depict the period from the start of biofouling removal (Day = 59.33, 1/6/2017 0800) to the end of biofouling removal (Day = 80.67, 1/27/2017 1600). All data from each sampling event are presented by denoting Ship (red circles) and Reference (blue triangles). If a regulatory threshold was applicable, a green horizontal dotted line is shown.

Table C-2 presents a summary of statistical analyses for hypothesis testing for water quality parameters of interest.

Figure C-167 show box and whisker plots for the parameters measured across the four events by *Type* (Ship or Reference) and water column *Strata* (S = surface, M = middle, and B = bottom).

See Section 4.6 in Methods for details of the statistical analysis.

Note about nutrients: The contract laboratory reported the analytical results for nutrients as nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), and ammonia-nitrogen (NH_4-N). These values are displayed in Figure C-7 and Figure C-16. Total nitrogen concentration can be calculated as:

$$\text{Total N} = NO_3\text{-N} + NO_2\text{-N} + NH_4\text{-N}$$

Note that the document text (e.g. Section 5.9 Nutrients) reports concentration (mg/L) of NO_3, NO_2, and NH_4 to facilitate reporting and discussion (e.g. comparison to historical nutrient concentrations). Conversion factors were calculated using the atomic mass (u) of the elements involved.

Element	Atomic Mass		(Pilson 1998)	
N	14.007	u		
O	15.999	u		
H	1.0079	u		

Compound			Conversion ratio	CR
NO_3	62.004	u	NO_3-N	4.4266
NO_2	46.005	u	NO_2-N	3.2844
NH_4	18.0386	u	NH_4-N	1.2878

Where

$NO_3 = NO_3\text{-N} \times 4.4266$
$NO_2 = NO_2\text{-N} \times 3.2844$
$NH_4 = NH_4\text{-N} \times 1.2878$

There is no difference in statistical tests using either form of nutrient data.

Table C-1, Summary of water quality parameters measured during the study by Event, Type, and Strata (A), by Event and Type (B), and by Event (C).

A.			**In-situ Temperature (°C, iTemp)**					**In-situ Salinity (psu. iSal)**					**In-situ DO Saturation (%, DO-Sat)**				
Event	***Type***	***Strata***	***n***	***mean***	***sdev***	***min***	***max***	***n***	***mean***	***sdev***	***min***	***max***	***n***	***mean***	***sdev***	***min***	***max***
1-Baseline	Ship	Surface	6	12.88	0.19	12.71	13.21	6	28.43	0.73	27.73	29.55	6	112.1	10.4	95.8	128.3
		Middle	6	12.64	0.02	12.61	12.67	6	29.89	0.24	29.63	30.35	6	79.9	7.8	64.8	87.5
		Bottom	6	12.63	0.01	12.62	12.65	6	30.09	0.16	29.88	30.37	6	71.6	4.9	63.3	76.4
	Reference	Surface	4	12.82	0.05	12.77	12.88	4	29.53	0.11	29.41	29.65	4	116.9	2.5	114.4	119.2
		Middle	4	12.68	0.03	12.65	12.70	4	29.99	0.08	29.92	30.10	4	84.8	11.8	68.1	93.7
		Bottom	4	12.63	0.02	12.61	12.66	4	30.31	0.11	30.16	30.41	4	65.6	10.4	52.5	77.9
2-During Removal	Ship	Surface	6	8.08	0.08	7.97	8.17	6	29.79	0.07	29.68	29.86	6	102.2	1.4	100.2	104.2
		Middle	6	8.23	0.04	8.17	8.27	6	29.94	0.11	29.79	30.06	6	100.5	1.3	99.3	102.3
		Bottom	6	8.31	0.03	8.26	8.35	6	30.05	0.10	29.94	30.19	6	99.1	0.8	97.8	99.9
	Reference	Surface	4	7.50	0.26	7.16	7.79	4	30.02	0.28	29.73	30.40	4	105.4	0.4	105.0	106.0
		Middle	4	8.15	0.17	7.90	8.28	4	30.54	0.17	30.30	30.68	4	101.6	1.8	99.0	103.3
		Bottom	4	8.41	0.09	8.28	8.46	4	30.72	0.18	30.54	30.95	4	99.2	0.4	98.7	99.6
3-End of Removal	Ship	Surface	6	8.50	0.03	8.47	8.54	6	29.92	0.03	29.87	29.96	6	98.9	0.5	98.4	99.5
		Middle	6	8.53	0.03	8.50	8.59	6	29.98	0.03	29.94	30.02	6	96.7	1.9	93.6	99.0
		Bottom	6	8.61	0.01	8.59	8.62	6	30.09	0.02	30.07	30.12	6	92.3	1.5	90.7	93.9
	Reference	Surface	4	8.24	0.07	8.14	8.30	4	30.10	0.31	29.80	30.37	4	103.3	0.8	102.1	103.9
		Middle	4	8.39	0.07	8.31	8.47	4	30.20	0.35	29.89	30.60	4	100.5	0.7	99.9	101.5
		Bottom	4	8.61	0.03	8.59	8.66	4	30.54	0.35	30.18	30.97	4	94.7	1.5	93.8	96.9
4-After Removal	Ship	Surface	6	7.86	0.13	7.68	8.06	6	29.47	0.34	29.01	29.93	6	109.0	0.7	108.2	109.9
		Middle	6	8.16	0.02	8.12	8.18	6	30.25	0.04	30.18	30.31	6	104.5	1.5	103.3	107.3
		Bottom	6	8.17	0.01	8.15	8.18	6	30.35	0.02	30.33	30.38	6	101.7	0.4	101.0	102.1
	Reference	Surface	4	7.92	0.10	7.84	8.06	4	30.00	0.16	29.83	30.21	4	109.9	0.6	109.1	110.5
		Middle	4	8.10	0.08	8.02	8.21	4	30.21	0.11	30.10	30.31	4	107.8	1.3	106.0	108.7
		Bottom	4	8.17	0.03	8.13	8.21	4	30.50	0.08	30.42	30.57	4	103.4	1.4	102.1	105.3
B. **Summary by Event and Type**																	
1-Baseline		Ship	18	12.72	0.16	12.61	13.21	18	29.47	0.87	27.73	30.37	18	87.86	19.49	63.29	128.26
		Reference	12	12.71	0.09	12.61	12.88	12	29.94	0.35	29.41	30.41	12	89.10	23.60	52.45	119.19
2-During		Ship	18	8.21	0.11	7.97	8.35	18	29.93	0.14	29.68	30.19	18	100.60	1.71	97.82	104.23
		Reference	12	8.02	0.44	7.16	8.46	12	30.43	0.36	29.73	30.95	12	102.05	2.85	98.74	105.95
3-End of Removal		Ship	18	8.55	0.05	8.47	8.62	18	29.99	0.08	29.87	30.12	18	95.99	3.14	90.69	99.51
		Reference	12	8.41	0.17	8.14	8.66	12	30.28	0.36	29.80	30.97	12	99.49	3.86	93.81	103.89
4-After Removal		Ship	18	8.06	0.17	7.68	8.18	18	30.02	0.44	29.01	30.38	18	105.03	3.23	101.00	109.89
		Reference	12	8.06	0.13	7.84	8.21	12	30.24	0.24	29.83	30.57	12	107.02	2.99	102.08	110.49
C. **Summary by Event**																	
1-Baseline			30	12.71	0.13	12.61	13.21	30	29.66	0.74	27.73	30.41	30	88.35	20.84	52.45	128.26
2-During Removal			30	8.13	0.30	7.16	8.46	30	30.13	0.35	29.68	30.95	30	101.18	2.30	97.82	105.95
3-End of Removal			30	8.49	0.13	8.14	8.66	30	30.11	0.27	29.80	30.97	30	97.39	3.80	90.69	103.89
4-After Removal			30	8.06	0.15	7.68	8.21	30	30.11	0.39	29.01	30.57	30	105.82	3.24	101.00	110.49

Table C-1 Continued.

A			In-situ Turbidity (ntu)					Discrete Turbidity (ntu)					Dissolved Cu (µg/L)				
Event	*Type*	*Strata*	*n*	*mean*	*sdev*	*min*	*max*	*n*	*mean*	*sdev*	*min*	*max*	*n*	*mean*	*sdev*	*min*	*max*
1-Baseline	Ship	Surface	6	0.07	0.05	0.02	0.15	6	0.01	0.00	0.01	0.01	6	0.84	0.22	0.51	1.14
		Middle	6	0.03	0.02	0.01	0.06	6	0.52	0.97	0.01	2.42	6	0.69	0.20	0.57	1.08
		Bottom	6	0.06	0.03	0.02	0.10	6	1.25	0.86	0.39	2.47	6	0.63	0.10	0.48	0.78
	Reference	Surface	4	0.15	0.13	0.04	0.31	4	0.49	0.96	0.01	1.93	4	0.51	0.10	0.38	0.61
		Middle	4	0.04	0.02	0.02	0.07	4	0.02	0.02	0.01	0.05	4	0.50	0.05	0.46	0.56
		Bottom	4	0.05	0.04	0.01	0.11	4	0.12	0.22	0.01	0.45	4	0.46	0.03	0.43	0.49
2-During Removal	Ship	Surface	6	0.04	0.01	0.02	0.06						6	1.07	0.35	0.78	1.58
		Middle	6	0.06	0.03	0.03	0.11						6	0.64	0.24	0.42	1.08
		Bottom	6	0.11	0.02	0.08	0.13						6	0.62	0.28	0.38	1.14
	Reference	Surface	4	0.04	0.03	0.02	0.08						4	0.78	0.05	0.71	0.82
		Middle	4	0.03	0.01	0.02	0.05						4	0.31	0.21	0.05	0.54
		Bottom	4	0.08	0.01	0.07	0.10						4	0.32	0.05	0.28	0.39
3-End of Removal	Ship	Surface	6	0.04	0.01	0.02	0.06	6	0.61	0.36	0.01	1.03	6	0.78	0.53	0.05	1.54
		Middle	6	0.07	0.03	0.04	0.13	6	1.89	0.98	0.84	3.46	6	0.37	0.27	0.05	0.70
		Bottom	6	0.20	0.07	0.12	0.30	6	1.19	1.63	0.28	4.42	6	0.25	0.33	0.05	0.83
	Reference	Surface	4	0.03	0.02	0.02	0.06	4	0.57	0.38	0.07	0.92	4	0.13	0.05	0.05	0.16
		Middle	4	0.03	0.01	0.02	0.03	4	0.68	0.57	0.12	1.33	4	0.18	0.06	0.12	0.26
		Bottom	4	0.06	0.01	0.04	0.07	4	1.37	0.46	0.97	1.84	4	0.13	0.16	0.05	0.36
4-After Removal	Ship	Surface	6	0.05	0.03	0.02	0.10	6	0.55	0.76	0.01	1.67	6	0.68	0.04	0.65	0.74
		Middle	6	0.04	0.01	0.02	0.06	6	0.04	0.08	0.01	0.20	6	0.62	0.04	0.57	0.68
		Bottom	6	0.12	0.03	0.09	0.16	6	0.87	0.85	0.16	2.38	6	0.51	0.02	0.50	0.55
	Reference	Surface	4	0.04	0.01	0.03	0.06	3	0.54	0.93	0.01	1.62	4	0.57	0.02	0.54	0.59
		Middle	4	0.04	0.01	0.03	0.04	4	0.18	0.36	0.01	0.72	4	0.57	0.03	0.54	0.60
		Bottom	4	0.09	0.04	0.06	0.15	4	0.35	0.46	0.01	0.99	4	0.54	0.02	0.51	0.55
B																	
1-Baseline		Ship	18	0.05	0.04	0.01	0.15	18	0.59	0.88	0.01	2.47	18	0.72	0.19	0.48	1.14
		Reference	12	0.08	0.09	0.01	0.31	12	0.21	0.56	0.01	1.93	12	0.49	0.06	0.38	0.61
2-During		Ship	18	0.07	0.04	0.02	0.13						18	0.77	0.35	0.38	1.58
		Reference	12	0.05	0.03	0.02	0.10						12	0.47	0.26	0.05	0.82
3-End of Removal		Ship	18	0.11	0.08	0.02	0.30	18	1.23	1.18	0.01	4.42	18	0.46	0.44	0.05	1.54
		Reference	12	0.04	0.02	0.02	0.07	12	0.87	0.57	0.07	1.84	12	0.14	0.09	0.05	0.36
4-After Removal		Ship	18	0.07	0.04	0.02	0.16	18	0.48	0.71	0.01	2.38	18	0.60	0.08	0.50	0.74
		Reference	12	0.06	0.04	0.03	0.15	11	0.34	0.55	0.01	1.62	12	0.56	0.03	0.51	0.60
C																	
1-Baseline			30	0.06	0.07	0.01	0.31	30	0.44	0.78	0.01	2.47	30	0.63	0.19	0.38	1.14
2-During Removal			30	0.06	0.03	0.02	0.13						30	0.65	0.34	0.05	1.58
3-End of Removal			30	0.08	0.07	0.02	0.30	30	1.09	0.99	0.01	4.42	30	0.34	0.38	0.05	1.54
4-After Removal			30	0.07	0.04	0.02	0.16	29	0.43	0.65	0.01	2.38	30	0.58	0.07	0.50	0.74

Table C-1 Continued.

A			Total Cu (µg/L)					Dissolved Zn (µg/L)					Total Zn (µg/L)				
Event	*Type*	*Strata*	*n*	*mean*	*sdev*	*min*	*max*	*n*	*mean*	*sdev*	*min*	*max*	*n*	*mean*	*sdev*	*min*	*max*
1-Baseline	Ship	Surface	6	1.01	0.34	0.82	1.69	3	1.38	0.51	0.92	1.92	6	2.93	1.26	1.96	5.40
		Middle	6	0.76	0.25	0.49	1.13	3	1.15	0.29	0.83	1.41	6	1.46	0.47	0.94	2.05
		Bottom	6	0.84	0.08	0.73	0.95	2	1.20	0.08	1.14	1.25	6	1.57	0.36	1.14	2.18
	Reference	Surface	4	0.81	0.12	0.65	0.94	3	1.56	0.43	1.08	1.89	4	2.38	0.58	1.57	2.93
		Middle	4	0.64	0.09	0.51	0.71	3	1.41	0.48	0.86	1.77	4	1.29	0.52	0.72	1.79
		Bottom	4	0.69	0.16	0.55	0.91	2	1.26	0.57	0.85	1.66	4	1.09	0.84	0.10	2.14
2-During Removal	Ship	Surface	6	4.11	3.00	1.52	8.84	5	0.45	0.29	0.10	0.71	6	1.60	1.28	0.10	3.82
		Middle	6	2.92	1.42	0.99	4.91	6	0.31	0.28	0.10	0.79	6	1.47	0.12	1.32	1.65
		Bottom	6	3.92	3.71	1.22	10.21	6	0.30	0.26	0.10	0.73	6	1.44	0.36	0.89	2.00
	Reference	Surface	4	1.21	0.20	0.91	1.36	4	1.19	0.17	0.97	1.38	4	1.52	0.45	1.02	2.02
		Middle	4	0.78	0.23	0.45	0.97	4	0.70	0.45	0.10	1.17	4	1.59	0.31	1.13	1.83
		Bottom	4	0.82	0.09	0.73	0.93	4	0.85	0.20	0.66	1.13	4	1.73	0.18	1.54	1.91
3-End of Removal	Ship	Surface	6	2.40	0.48	1.58	2.92	6	0.38	0.65	0.10	1.71	6	0.36	0.39	0.03	0.98
		Middle	6	2.17	0.39	1.71	2.61	6	0.42	0.78	0.10	2.02	6	0.19	0.31	0.01	0.81
		Bottom	6	3.50	2.62	1.04	7.16	6	0.28	0.30	0.10	0.83	6	0.32	0.46	0.10	1.26
	Reference	Surface	4	0.85	0.17	0.60	0.97	4	0.11	0.02	0.10	0.14	4	0.72	0.63	0.10	1.30
		Middle	4	0.75	0.27	0.39	1.01	4	0.10	0.00	0.10	0.10	4	0.93	1.00	0.10	2.13
		Bottom	4	0.72	0.49	0.38	1.44	4	0.17	0.13	0.10	0.36	4	1.43	0.96	0.42	2.54
4-After Removal	Ship	Surface	6	0.80	0.13	0.60	0.94	6	1.18	0.04	1.14	1.23	6	1.29	0.13	1.10	1.42
		Middle	6	0.73	0.13	0.63	0.97	6	1.11	0.04	1.07	1.17	6	1.23	0.13	1.12	1.45
		Bottom	6	0.79	0.10	0.63	0.88	6	1.02	0.02	1.00	1.05	6	1.29	0.09	1.13	1.37
	Reference	Surface	4	0.63	0.03	0.58	0.65	4	1.07	0.02	1.05	1.09	4	1.13	0.03	1.08	1.15
		Middle	4	0.61	0.02	0.59	0.63	4	1.07	0.03	1.04	1.10	4	1.10	0.02	1.09	1.12
		Bottom	4	0.66	0.06	0.57	0.70	4	1.04	0.02	1.01	1.05	4	1.15	0.06	1.07	1.19
B																	
1-Baseline		Ship	18	0.87	0.26	0.49	1.69	8	1.25	0.33	0.83	1.92	18	1.99	1.02	0.94	5.40
		Reference	12	0.71	0.14	0.51	0.94	8	1.43	0.43	0.85	1.89	12	1.59	0.84	0.10	2.93
2-During		Ship	18	3.65	2.75	0.99	10.21	17	0.35	0.27	0.10	0.79	18	1.50	0.73	0.10	3.82
		Reference	12	0.93	0.26	0.45	1.36	12	0.91	0.35	0.10	1.38	12	1.61	0.31	1.02	2.02
3-End of Removal		Ship	18	2.69	1.58	1.04	7.16	18	0.36	0.58	0.10	2.02	18	0.29	0.38	0.01	1.26
		Reference	12	0.78	0.31	0.38	1.44	12	0.13	0.07	0.10	0.36	12	1.03	0.86	0.10	2.54
4-After Removal		Ship	18	0.77	0.12	0.60	0.97	18	1.10	0.07	1.00	1.23	18	1.27	0.11	1.10	1.45
		Reference	12	0.63	0.04	0.57	0.70	12	1.06	0.02	1.01	1.10	12	1.13	0.04	1.07	1.19
C																	
1-Baseline			30	0.81	0.23	0.49	1.69	16	1.34	0.38	0.83	1.92	30	1.83	0.96	0.10	5.40
2-During Removal			30	2.56	2.51	0.45	10.21	29	0.58	0.41	0.10	1.38	30	1.55	0.59	0.10	3.82
3-End of Removal			30	1.92	1.55	0.38	7.16	30	0.26	0.46	0.10	2.02	30	0.58	0.70	0.01	2.54
4-After Removal			30	0.72	0.12	0.57	0.97	30	1.08	0.06	1.00	1.23	30	1.21	0.11	1.07	1.45

Table C-1 Continued.

A			Nitrate N03-N (mg/L)					Nitrate NO2-N (mg/L)					Ammonia NH4 (mg/L)				
Event	*Type*	*Strata*	*n*	*mean*	*sdev*	*min*	*max*	*n*	*mean*	*sdev*	*min*	*max*	*n*	*mean*	*sdev*	*min*	*max*
1-Baseline	Ship	Surface	3	0.380	0.017	0.370	0.400	3	0.025		0.025	0.025	3	0.080	0.005	0.076	0.085
		Middle	6	0.397	0.024	0.360	0.430	6	0.025		0.025	0.025	6	0.066	0.022	0.052	0.110
		Bottom	6	0.437	0.052	0.390	0.520	6	0.025		0.025	0.025	6	0.084	0.024	0.061	0.130
	Reference	Surface	4	0.328	0.010	0.320	0.340	4	0.025		0.025	0.025	4	0.062	0.013	0.050	0.080
		Middle	4	0.428	0.013	0.410	0.440	4	0.025		0.025	0.025	4	0.076	0.037	0.050	0.130
		Bottom	4	0.420	0.024	0.390	0.440	4	0.025		0.025	0.025	4	0.100	0.030	0.068	0.140
2-During Removal	Ship	Surface	6	0.465	0.033	0.410	0.500	6	0.022	0.007	0.009	0.025	6	0.050		0.050	0.050
		Middle	5	0.426	0.015	0.410	0.450	5	0.025		0.025	0.025	5	0.050		0.050	0.050
		Bottom	6	0.458	0.016	0.440	0.480	6	0.025		0.025	0.025	6	0.050		0.050	0.050
	Reference	Surface	4	0.453	0.021	0.430	0.470	4	0.008	0.001	0.007	0.008	4	0.054	0.005	0.050	0.060
		Middle	4	0.450	0.014	0.430	0.460	4	0.017	0.010	0.008	0.025	4	0.050		0.050	0.050
		Bottom	4	0.460	0.037	0.420	0.500	4	0.021	0.009	0.007	0.025	4	0.050		0.050	0.050
3-End of Removal	Ship	Surface	6	0.552	0.021	0.530	0.590	6	0.025		0.025	0.025	6	0.050		0.050	0.050
		Middle	5	0.548	0.015	0.530	0.570	5	0.025		0.025	0.025	5	0.050		0.050	0.050
		Bottom	6	0.555	0.031	0.500	0.590	6	0.025		0.025	0.025	6	0.051	0.003	0.050	0.058
	Reference	Surface	4	0.548	0.025	0.520	0.580	4	0.025		0.025	0.025	4	0.073	0.045	0.050	0.140
		Middle	4	0.525	0.033	0.500	0.570	4	0.025		0.025	0.025	4	0.051	0.001	0.050	0.052
		Bottom	4	0.498	0.042	0.440	0.540	4	0.025		0.025	0.025	4	0.050		0.050	0.050
4-After Removal	Ship	Surface	6	0.487	0.029	0.450	0.530	6	0.025		0.025	0.025	6	0.073	0.033	0.048	0.130
		Middle	3	0.490	0.050	0.440	0.540	3	0.025		0.025	0.025	3	0.050		0.050	0.050
		Bottom	6	0.495	0.029	0.460	0.520	6	0.025		0.025	0.025	6	0.050		0.050	0.050
	Reference	Surface	3	0.477	0.015	0.460	0.490	3	0.025		0.025	0.025	3	0.107	0.066	0.050	0.180
		Middle															
		Bottom	4	0.530	0.029	0.500	0.570	4	0.025		0.025	0.025	4	0.050		0.050	0.050
B																	
1-Baseline		Ship	15	0.409	0.042	0.360	0.520	15	0.025		0.025	0.025	15	0.076	0.022	0.052	0.130
		Reference	12	0.392	0.050	0.320	0.440	12	0.025		0.025	0.025	12	0.080	0.031	0.050	0.140
2-During		Ship	17	0.451	0.028	0.410	0.500	17	0.024		0.009	0.025	17	0.050	0.000	0.050	0.050
		Reference	12	0.454	0.024	0.420	0.500	12	0.015	0.009	0.007	0.025	12	0.051	0.003	0.050	0.060
3-End of Removal		Ship	17	0.552	0.023	0.500	0.590	17	0.025		0.025	0.025	17	0.050	0.002	0.050	0.058
		Reference	12	0.523	0.037	0.440	0.580	12	0.025		0.025	0.025	12	0.058	0.026	0.050	0.140
4-After Removal		Ship	15	0.491	0.032	0.440	0.540	15	0.025		0.025	0.025	15	0.059	0.023	0.048	0.130
		Reference	7	0.507	0.036	0.460	0.570	7	0.025		0.025	0.025	7	0.075	0.049	0.050	0.180
C																	
1-Baseline			27	0.401	0.046	0.320	0.520	27	0.025		0.025	0.025	27	0.078	0.025	0.050	0.140
2-During Removal			29	0.452	0.026	0.410	0.500	29	0.020	0.008	0.007	0.025	29	0.050	0.002	0.050	0.060
3-End of Removal			29	0.540	0.032	0.440	0.590	29	0.025		0.025	0.025	29	0.054	0.017	0.050	0.140
4-After Removal			22	0.496	0.033	0.440	0.570	22	0.025		0.025	0.025	22	0.064	0.033	0.048	0.180

Table C-2. Secci Disk Depths Measured During Sampling Events.

		Secchi Disk Depth (ft)				
Event	***Strata***	***n***	***mean***	***sdev***	***min***	***max***
B						
1-Baseline	Ship	5	3.7	3.7	12.0	22.0
	Reference	4	4.9	4.9	6.0	16.0
2-During	Ship					
	Reference					
3-End of Removal	Ship	5	2.3	2.3	13.0	19.0
	Reference	4	1.7	1.7	18.0	22.0
4-After Removal	Ship	6	2.5	2.5	14.0	20.0
	Reference	4	0.5	0.5	16.0	17.0
C						
1-Baseline		9	13.3	5.1	6.0	22.0
2-During Removal						
3-End of Removal		9	17.3	3.4	13.0	22.0
4-After Removal		10	16.8	1.9	14.0	20.0

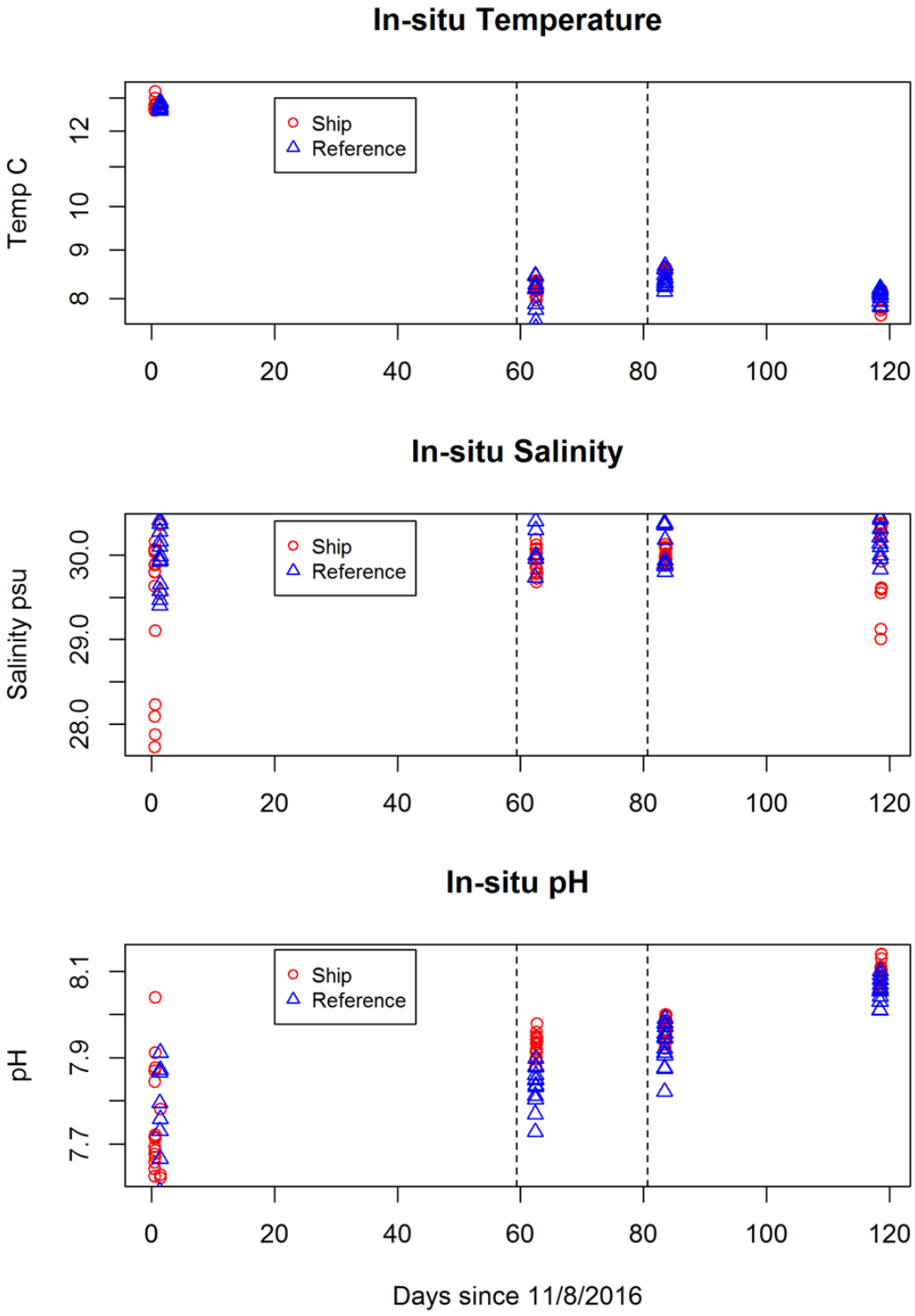

Figure C-1. Results for in-situ temperature (°C), in-situ salinity (psu), and in-situ pH measured at Ship and Reference sites during the study. The vertical dashed lines denote the beginning and end of biofouling removal.

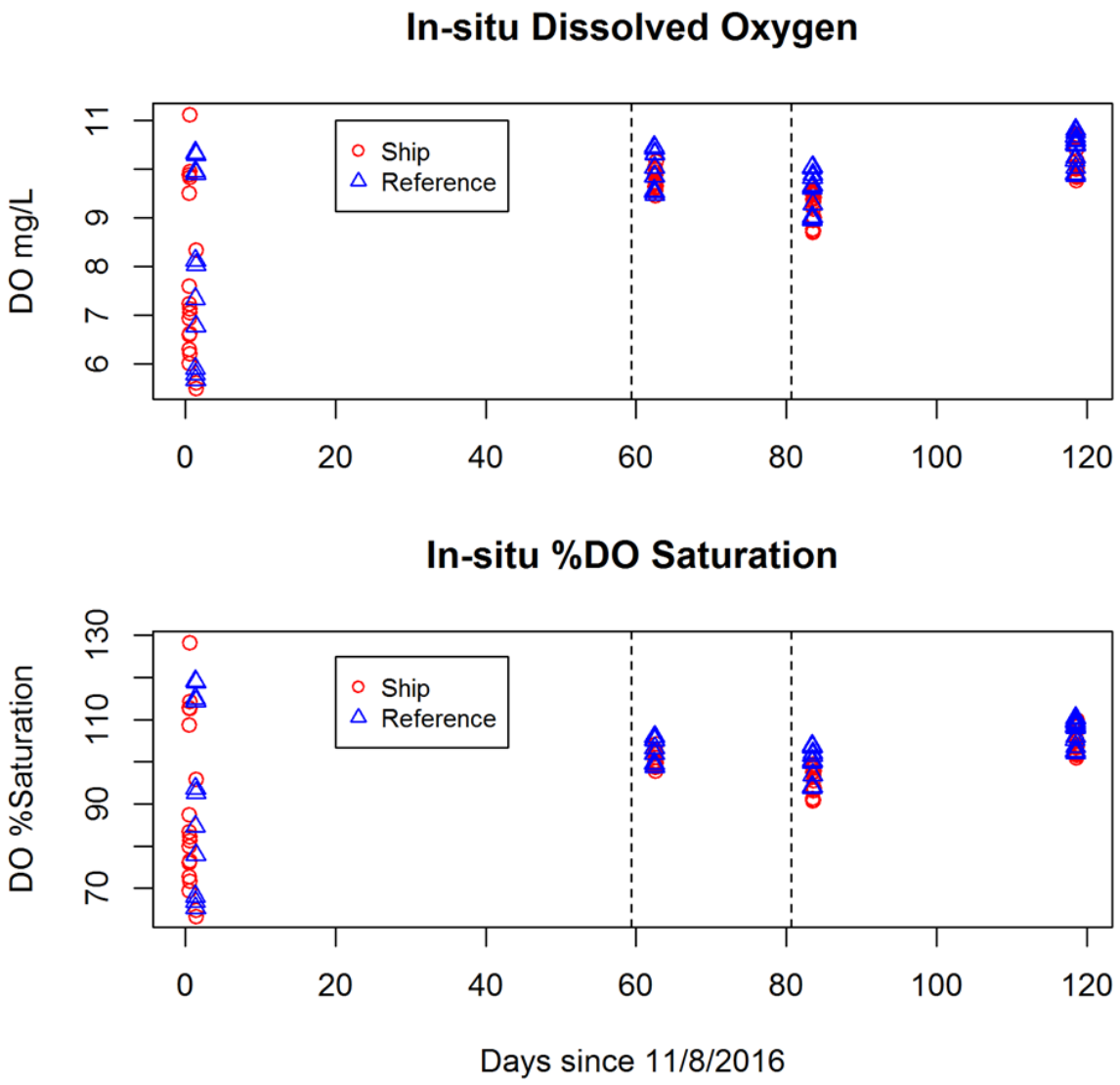

Figure C-2. Results for in-situ dissolved oxygen (mg/L) and in-situ percent dissolved oxygen saturation measured at Ship and Reference sites during the study. The vertical dashed lines denote the beginning and end of biofouling removal.

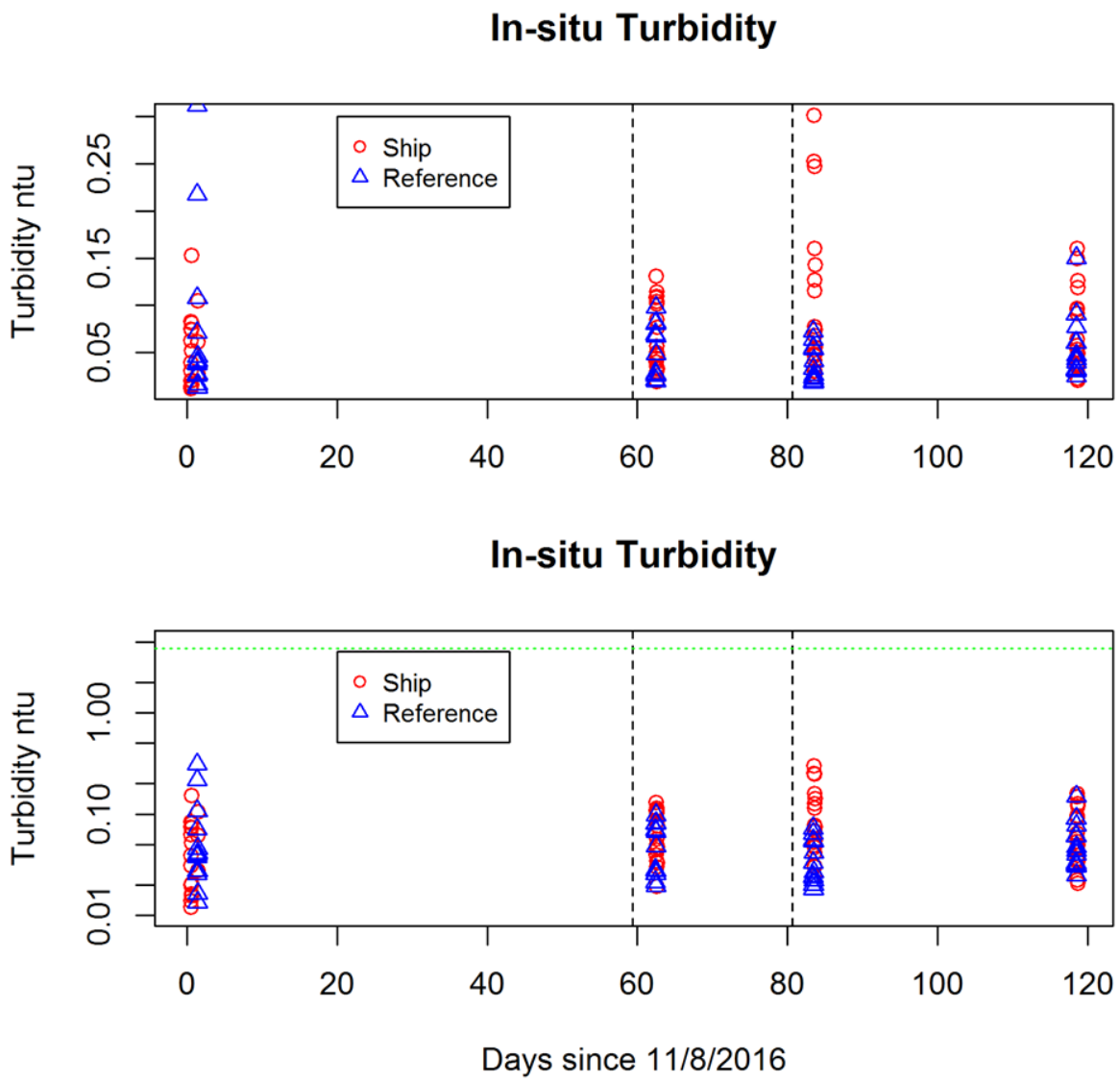

Figure C-3. Results for in-situ turbidity (NTU, log scale) measured at Ship and Reference sites during the study, with the lower panel showing the data scaled to show the regulatory threshold of 5 NTU above background (green dotted line). The vertical dashed lines denote the beginning and end of biofouling removal.

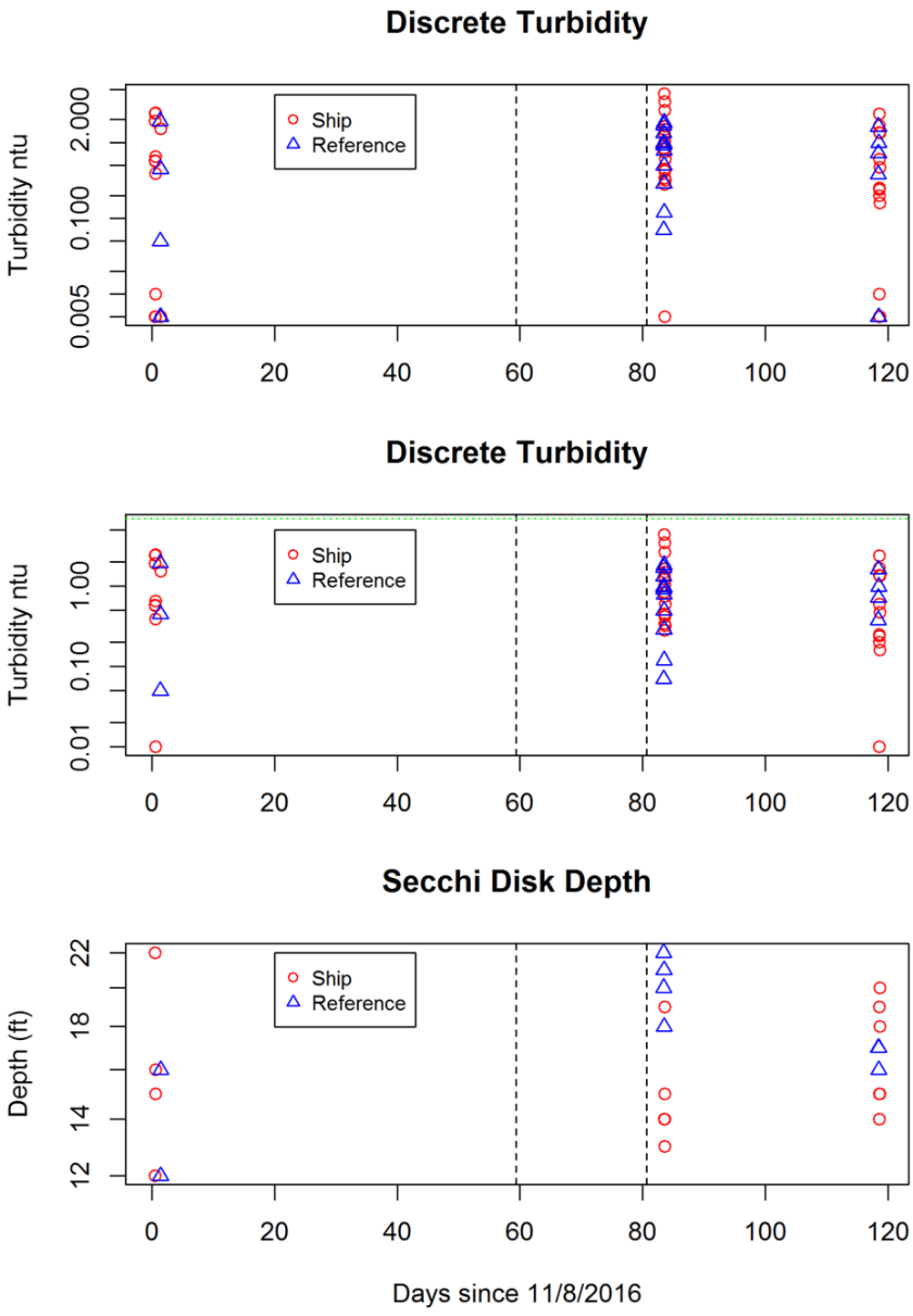

Figure C-4. Results for discrete turbidity (NTU, log scale) and Secchi disk depth (ft) measured at Ship and Reference sites during the study. The middle panel shows the data scaled to the regulatory threshold of 5 NTU above background (green dotted line). The vertical dashed lines denote the beginning and end of biofouling removal.

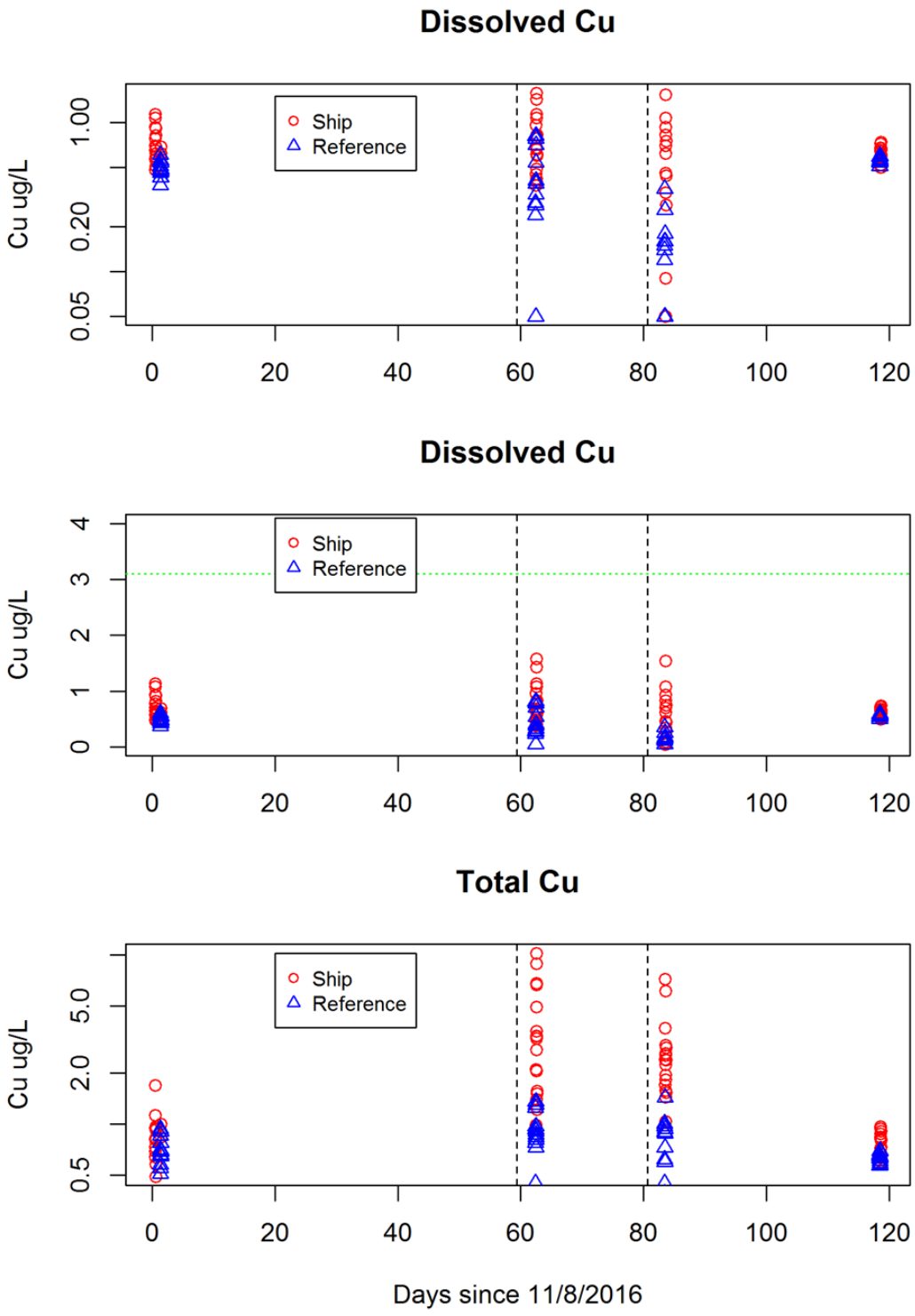

Figure C-5. Results for dissolved and total copper (µg/L) measured at Ship and Reference sites during the study. The middle panel has the data scaled to show chronic water quality standard (green dotted line) and the vertical dashed lines denote the beginning and end of biofouling removal.

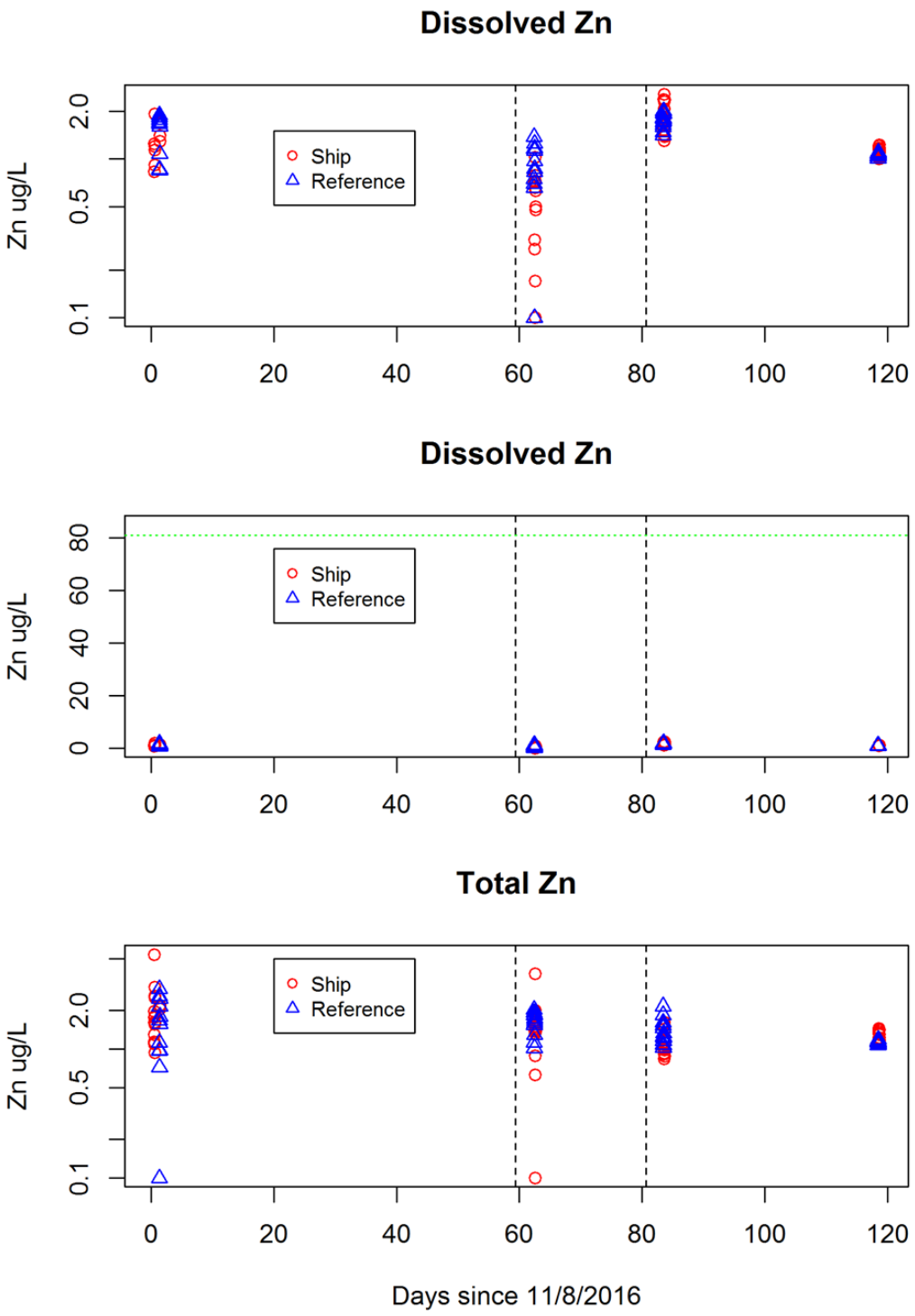

Figure C-6. Results for dissolved zinc (µg/L) measured at Ship and Reference sites during the study. The data are scaled to show chronic water quality standard (green dotted line) and the vertical dashed lines denote the beginning and end of biofouling removal.

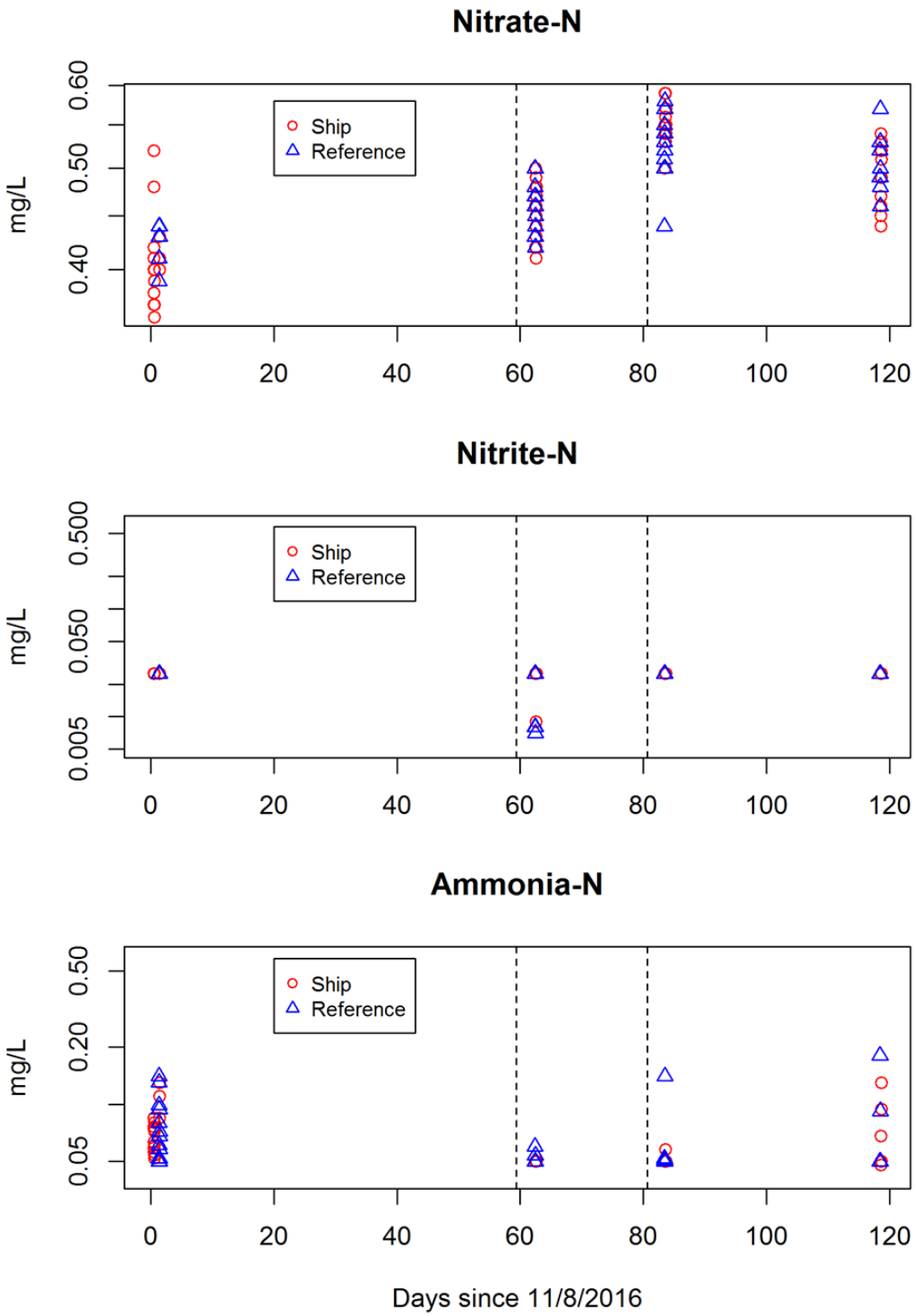

Figure C-7. Results for nitrate, nitrite, and ammonia expressed as nitrogen (mg/L) measured at Ship and Reference sites during the study. The vertical dashed lines denote the beginning and end of biofouling removal.

Table C-3. Summary of statistical analysis for hypothesis testing for $H1_o$ (A) and $H2_o$ (B) for water quality parameters of interest. Bolded entries indicate that null hypothesis was rejected if $p \leq 0.05$. Difference arrows indicate the direction of Ship compared to Reference (higher or lower) and magnitude of difference.

A. Null hypothesis $H1_o$, where p(F) is probability of ANOVA F-test and p(KW) is probability of non-parametric Kruskal-Wallis statistic.

		H1o: NO DIFFERENCE BETWEEN SHIP and REFEENCE for EACH SAMPLING EVENT											
		1-Baseline			2-During Removal			3-End of Cleaning			4-After Cleaning		
variable	*unit*	*p(F)*	*p(KW)*	*direction*	*p(F)*	*p(KW)*	*direction*	*p(F)*	*p(KW)*	*direction*	*p(F)*	*p(KW)*	*direction*
Temp	C	0.8849	0.6415		0.0871	0.5534		**0.0036**	**0.0309**	+	0.9652	0.8324	
Salinity	psu	0.0893	0.1501		**0.0001**	**0.0011**	-	**0.0027**	**0.0754**	-	0.1395	0.3302	
DO %Sat	%	0.8767	0.7349		0.0916	0.2530		**0.0108**	**0.0067**	-	0.0994	0.0754	
Turbidity	ntu	0.2714	0.7032		0.1287	0.0826		**0.0117**	**0.0040**	↑	0.3391	0.3302	
Turbidity	ntu	0.1914	0.1384					0.3418	0.7670		0.5709	0.3775	
NO3	mg/L	0.3280	0.9414		0.7721	0.7721		**0.0170**	**0.0270**	+	0.2890	0.3559	
NH4	mg/L	0.7350	0.8260		0.1189	0.0867		0.2494	0.0693		0.3253	0.4725	
Cu Diss	ug/L	**0.0004**	**0.0001**	+	**0.0156**	**0.0158**	+	**0.0195**	0.1012	↑	0.0600	0.1160	
Cu Total	ug/L	0.6443	0.0514		**0.0021**	**0.0001**	↑↑	**0.0003**	**0.0001**	↑↑	**0.0004**	**0.0017**	+
Zn Diss	ug/L	0.3560	0.4622		**0.0001**	**0.0003**	-	0.1886	0.3761		**0.0499**	0.1067	+
Zn Total	ug/L	0.2687	0.3517		0.6369	0.1624		**0.0032**	**0.0048**	↓	**0.0005**	**0.0017**	+

Legend for Direction: +/- = Ship sites slightly higher/lower than Reference sites
↑/↓- = Ship sites higher/lower than Reference sites
↑↑ = Ship sites much higher/lower than Reference sites

B. Null hypothesis $H2_o$, where p(T) is probability of T-test and p(W) is probability of non-parametric Wilcoxon statistic.

		H2o: NO DIFFERENCE FOR SHIP BETWEEN BASELINE and SUBSEQUENT EVENTS:								
		2-During Removal			3-End of Removal			4-After Removal		
variable	*unit*	*p(T)*	*p(W)*	*direction*	*p(T)*	*p(W)*	*direction*	*p(T)*	*p(W)*	*direction*
Temp	C	**0.0001**	**0.0001**	↓↓	**0.0001**	**0.0001**	↓↓	**0.0001**	**0.0001**	↓↓
Salinity	psu	**0.0435**	0.2931	+	**0.0216**	**0.0315**	+	**0.0252**	**0.0205**	-
DO %Sat	%	**0.0132**	**0.0224**	↑	0.0977	**0.0435**	↑	**0.0017**	**0.0171**	↑
In-Turb	ntu	0.1957	0.1182		**0.0234**	**0.0342**	+	0.1564	0.1260	
dis-Turb	ntu				0.0741	**0.0172**	+	0.6989	0.9472	
NO3	mg/L	**0.0032**	**0.0010**	+	**0.0001**	**0.0001**	+	**0.0001**	**0.0001**	+
NH4	mg/L	**0.0003**	**0.0001**	+	**0.0004**	**0.0001**	+	**0.0487**	**0.0008**	+
Cu Diss	ug/L	0.5615	0.9747		0.0340	0.0322		**0.0270**	**0.0708**	-
Cu Total	ug/L	**0.0005**	**0.0001**	↑	**0.0001**	**0.0001**	↑	0.1730	0.2167	
Zn Diss	ug/L	**0.0001**	**0.0001**	↓	**0.0001**	**0.0008**	↓	0.2676	0.1331	
Zn Total	ug/L	0.1115	0.0738		**0.0001**	**0.0001**	↓	**0.0084**	**0.0056**	-

Legend for Direction: +/- = Ship sites during Event slightly higher/lower than Ship sites during Baseline
↑/↓- = Ship sites during Event higher/lower than Ship sites during Baseline
↑↑ = Ship sites during Event much higher/lower than Ship sites during Baseline

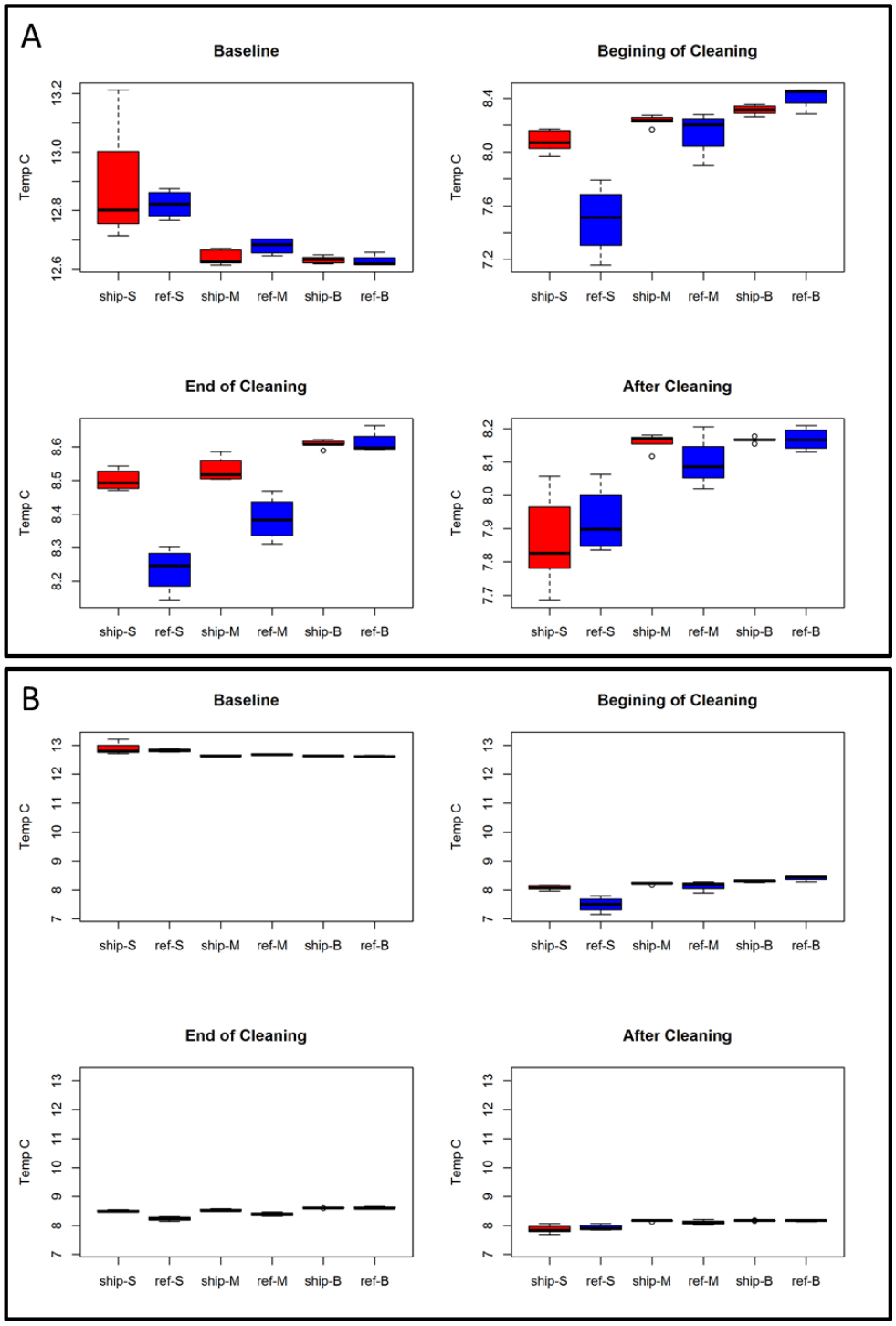

Figure C-8. Box and whisker plots for in-situ temperature (°C) by sampling event, type and strata independently scaled by event in upper panel (A) and same scale for all events in lower panel (B).

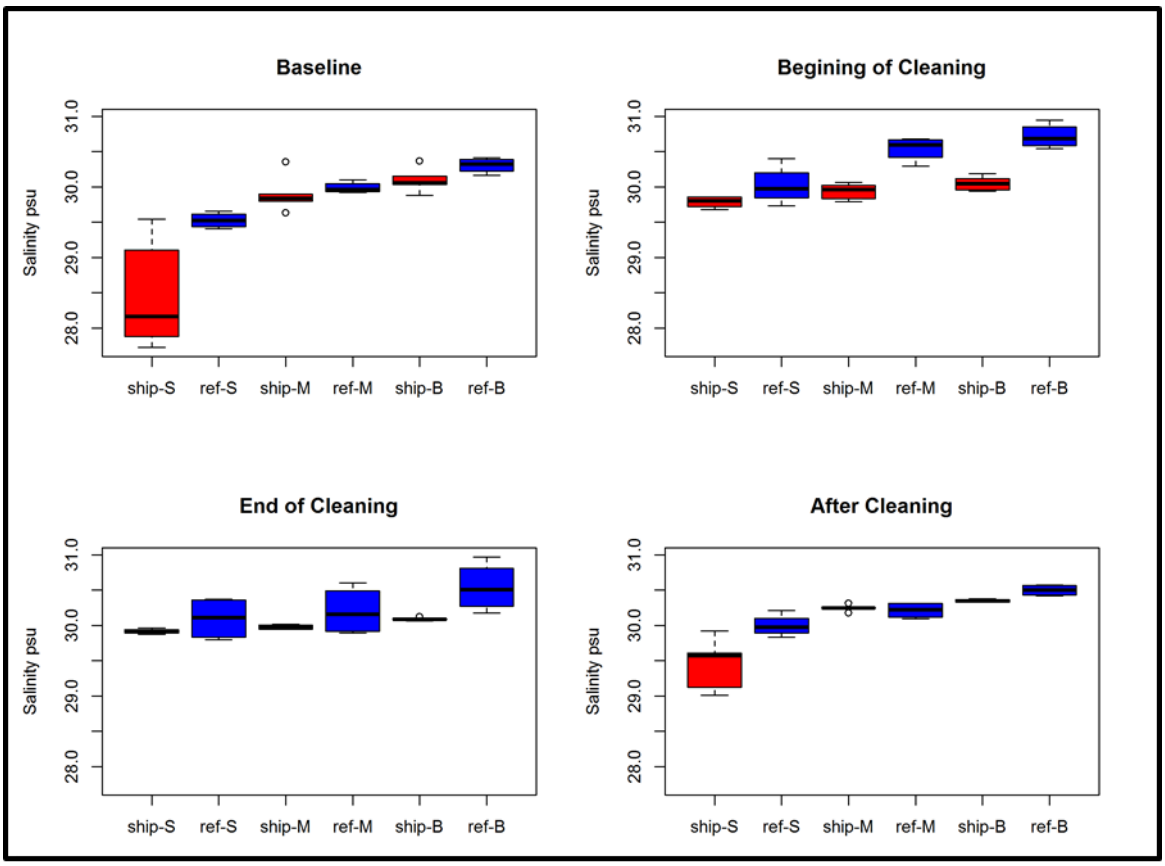

Figure C-9. Box and whisker plots for in-situ salinity (psu) by sampling event, type and strata scaled at same scale for all events.

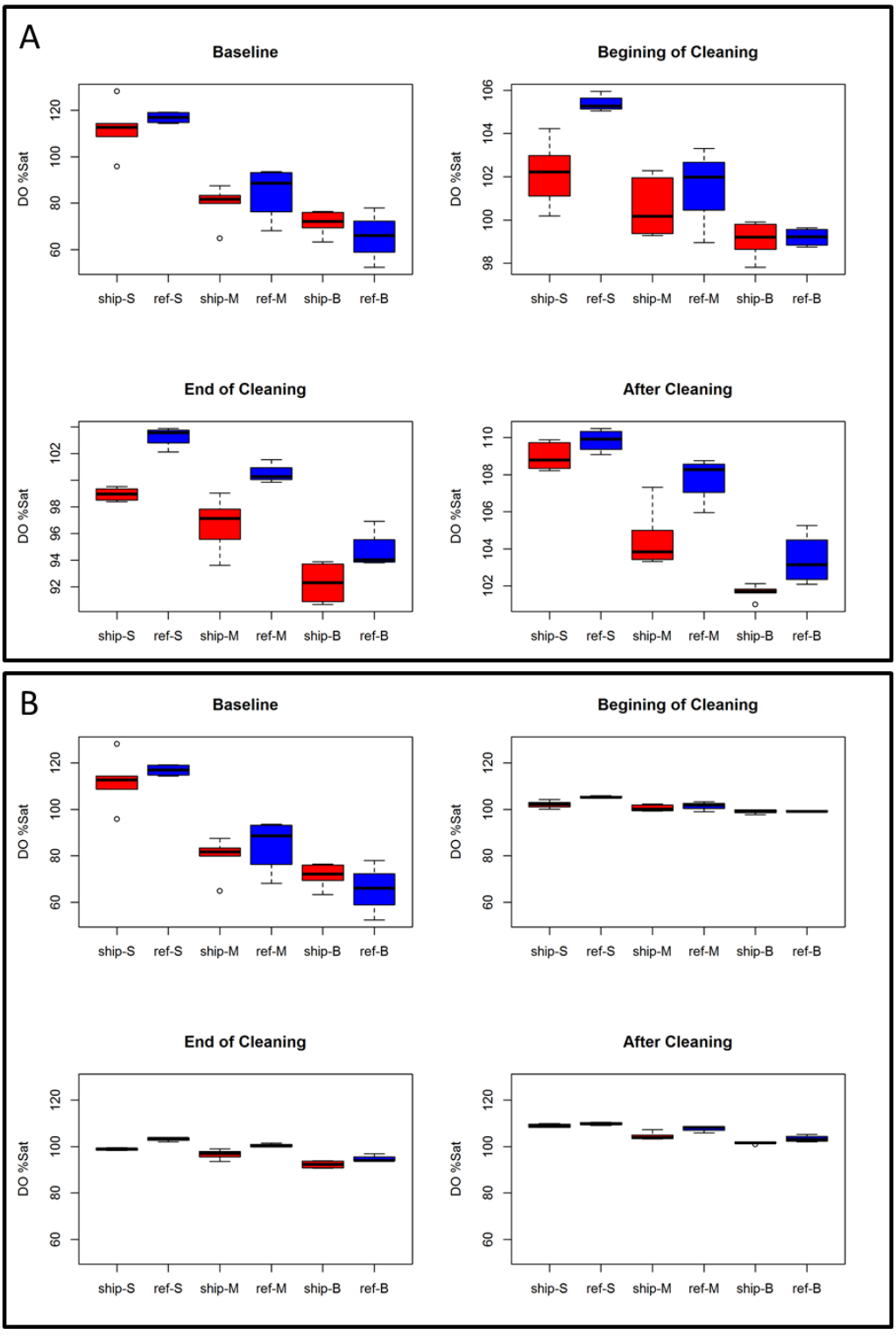

Figure C-10. Box and whisker plots for percent DO saturation by sampling event, type and strata independently scaled by event in upper panel (A) and same scale for all events in lower panel (B).

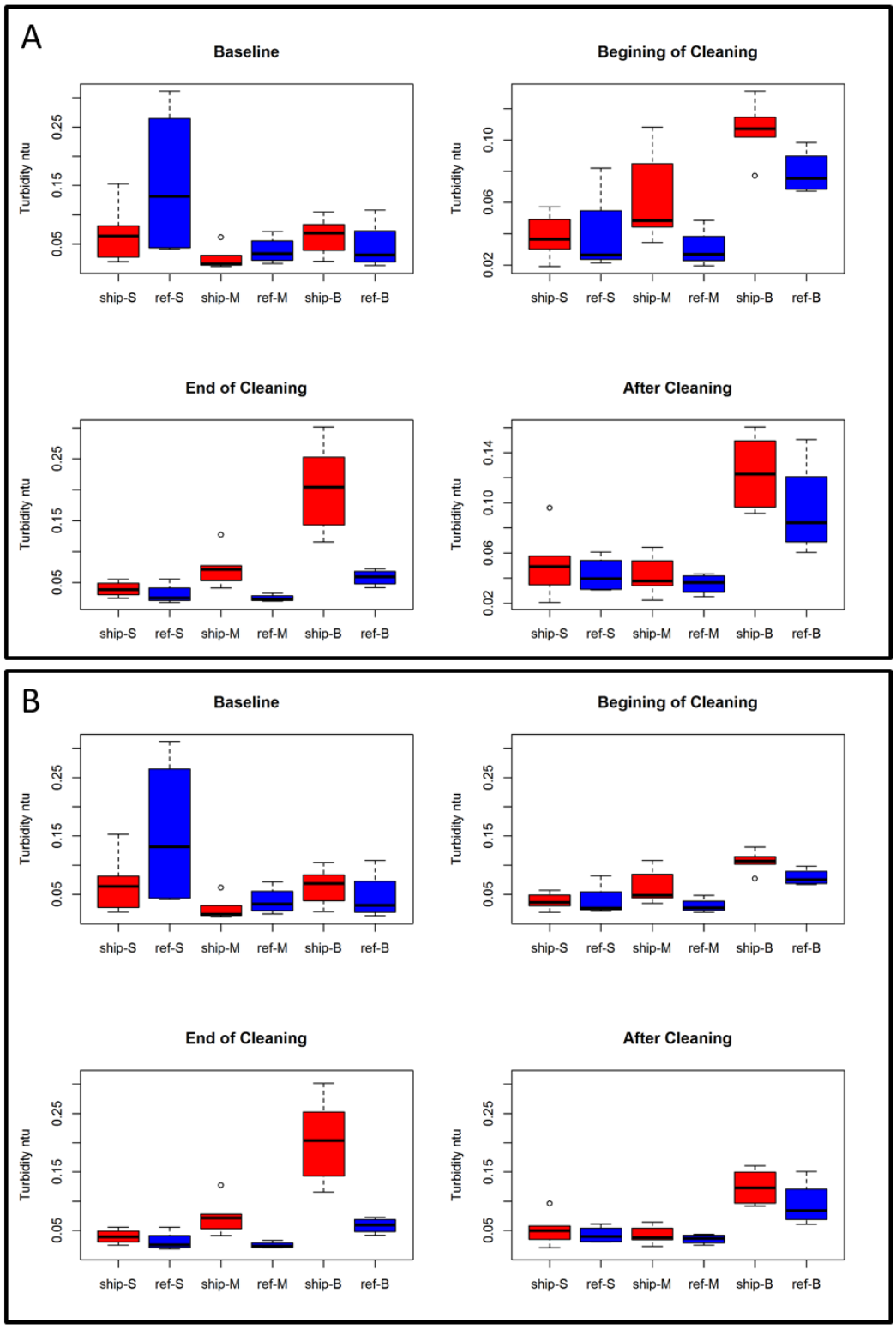

Figure C-11. Box and whisker plots for in-situ turbidity (NTU) by sampling event, type and strata independently scaled by event in upper panel (A) and same scale for all events in lower panel (B).

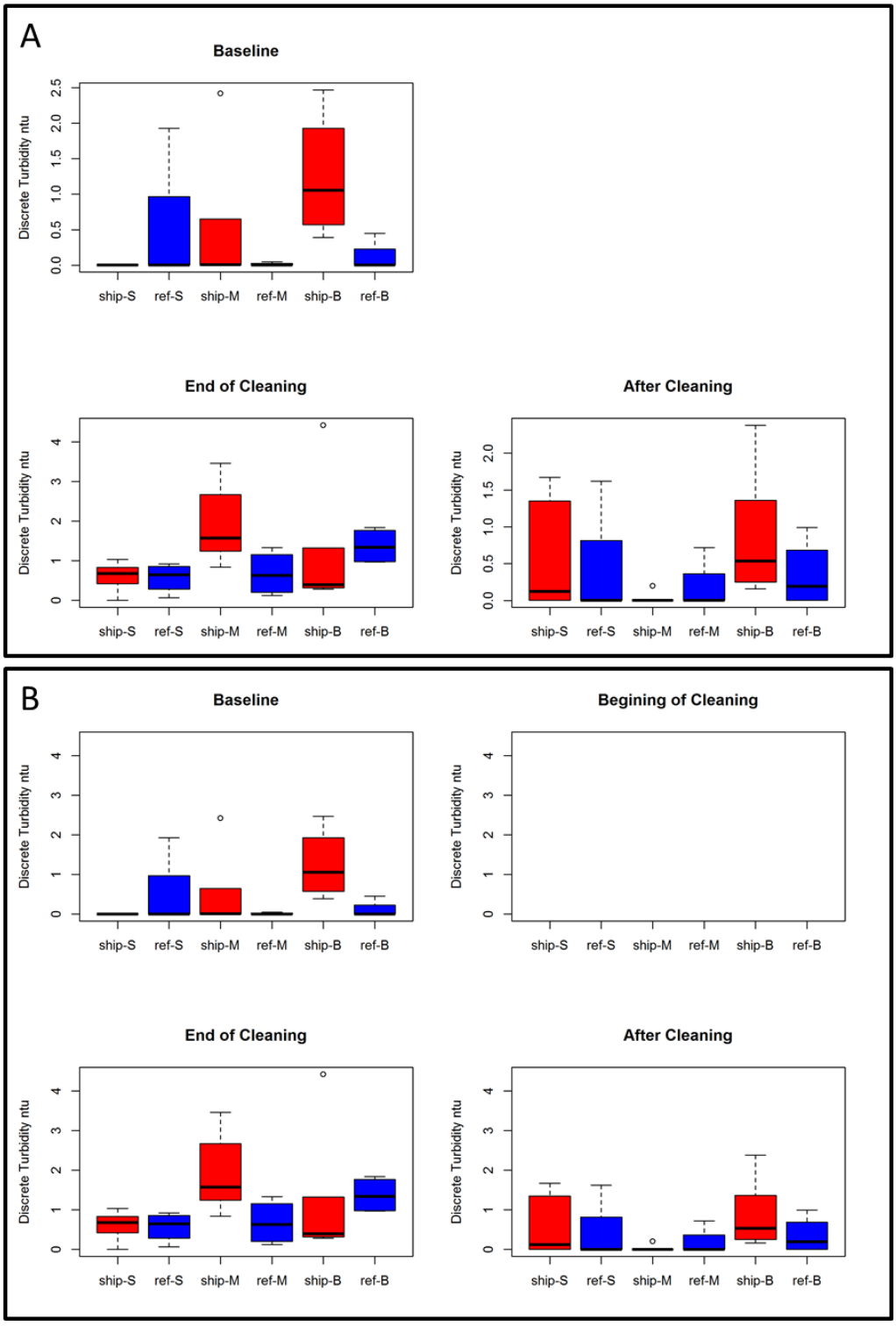

Figure C-12. Box and whisker plots for discrete turbidity (NTU) by sampling event, type and strata independently scaled by event in upper panel (A) and same scale for all events in lower panel (B). Note discrete turbidity was missing for event 2.

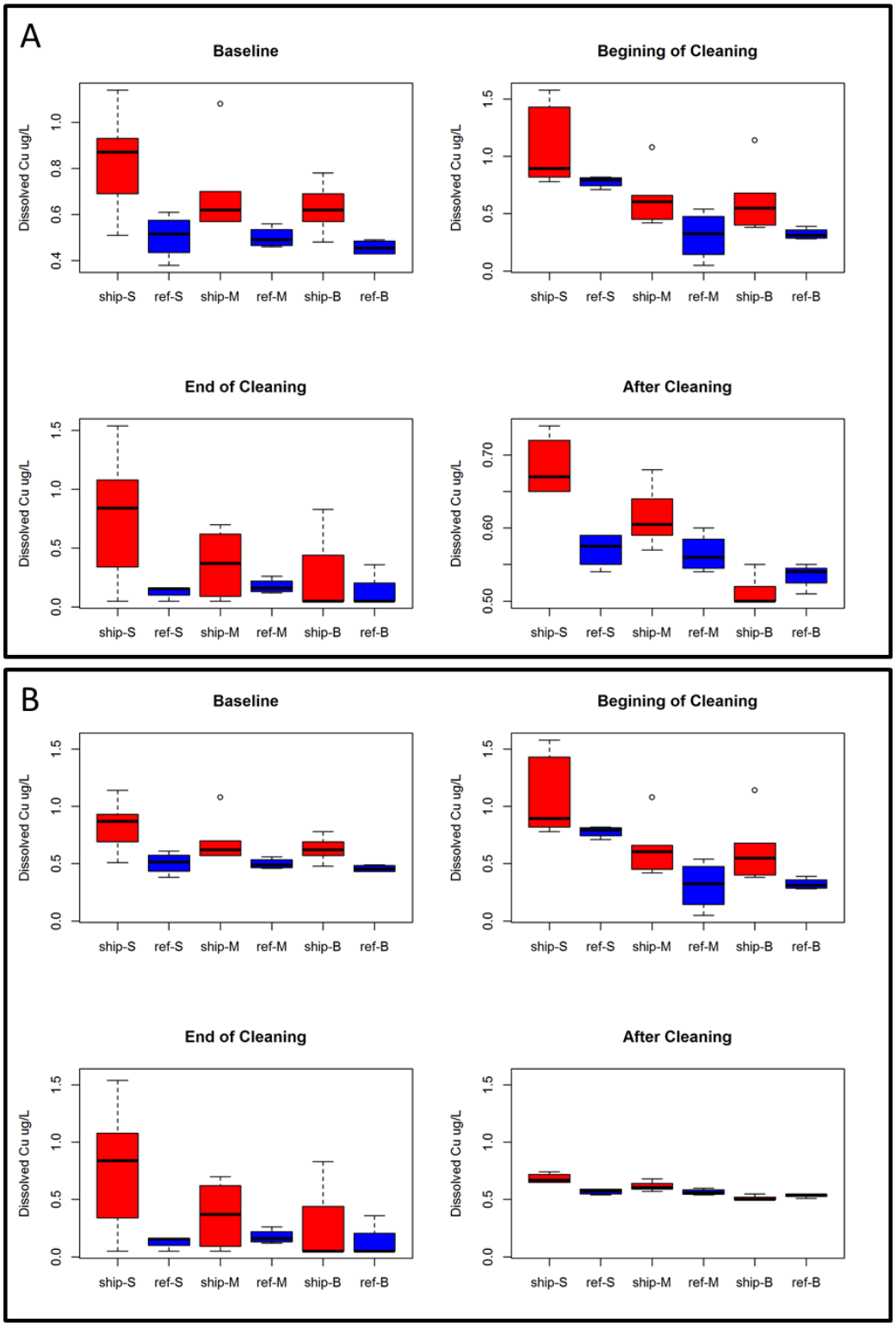

Figure C-13. Box and whisker plots for dissolved copper (µg/L) by sampling event, type and strata independently scaled by event in upper panel (A) and same scale for all events in lower panel (B).

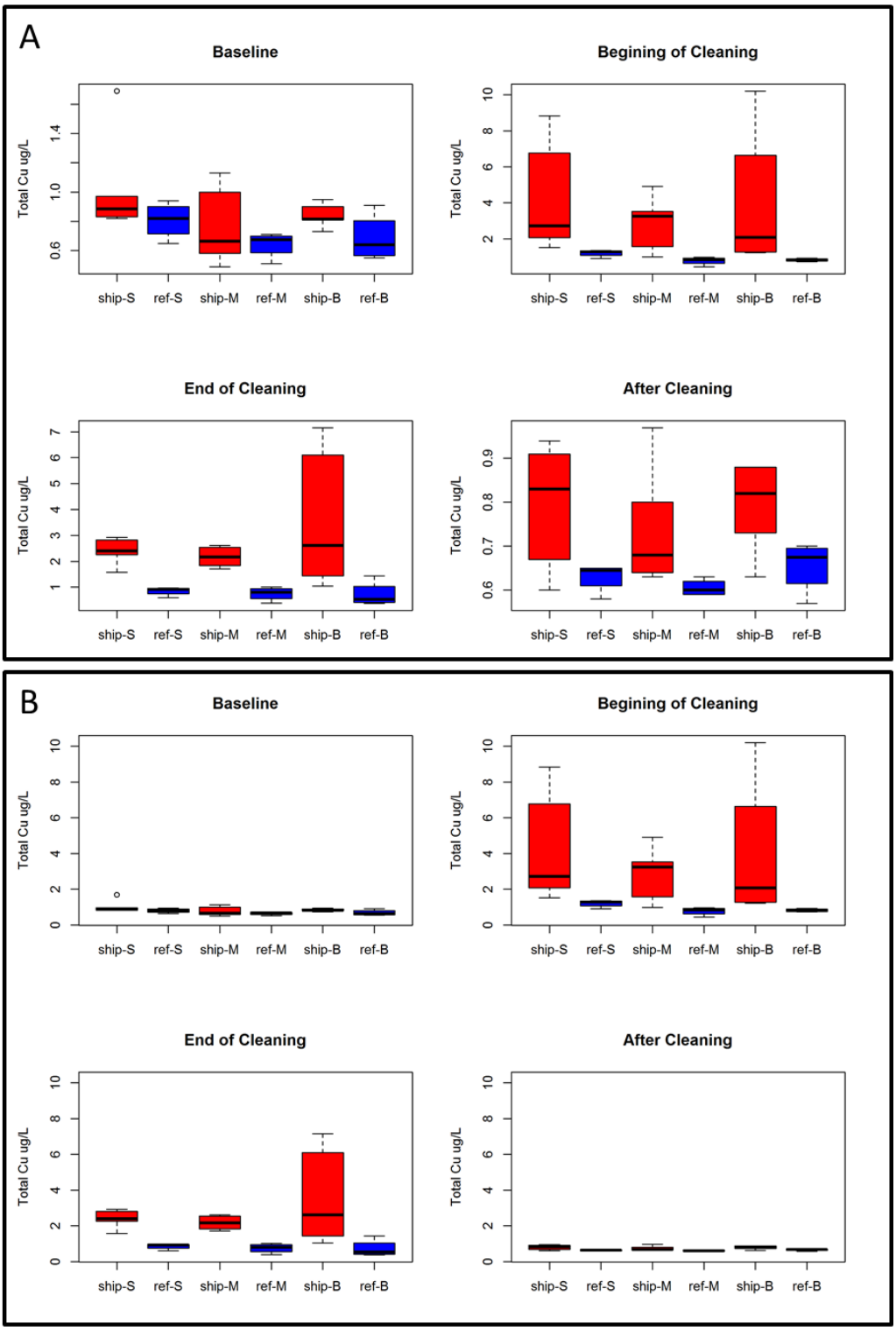

Figure C-14. Box and whisker plots for total copper (µg/L) by sampling event, type and strata independently scaled by event in upper panel (A) and same scale for all events in lower panel (B).

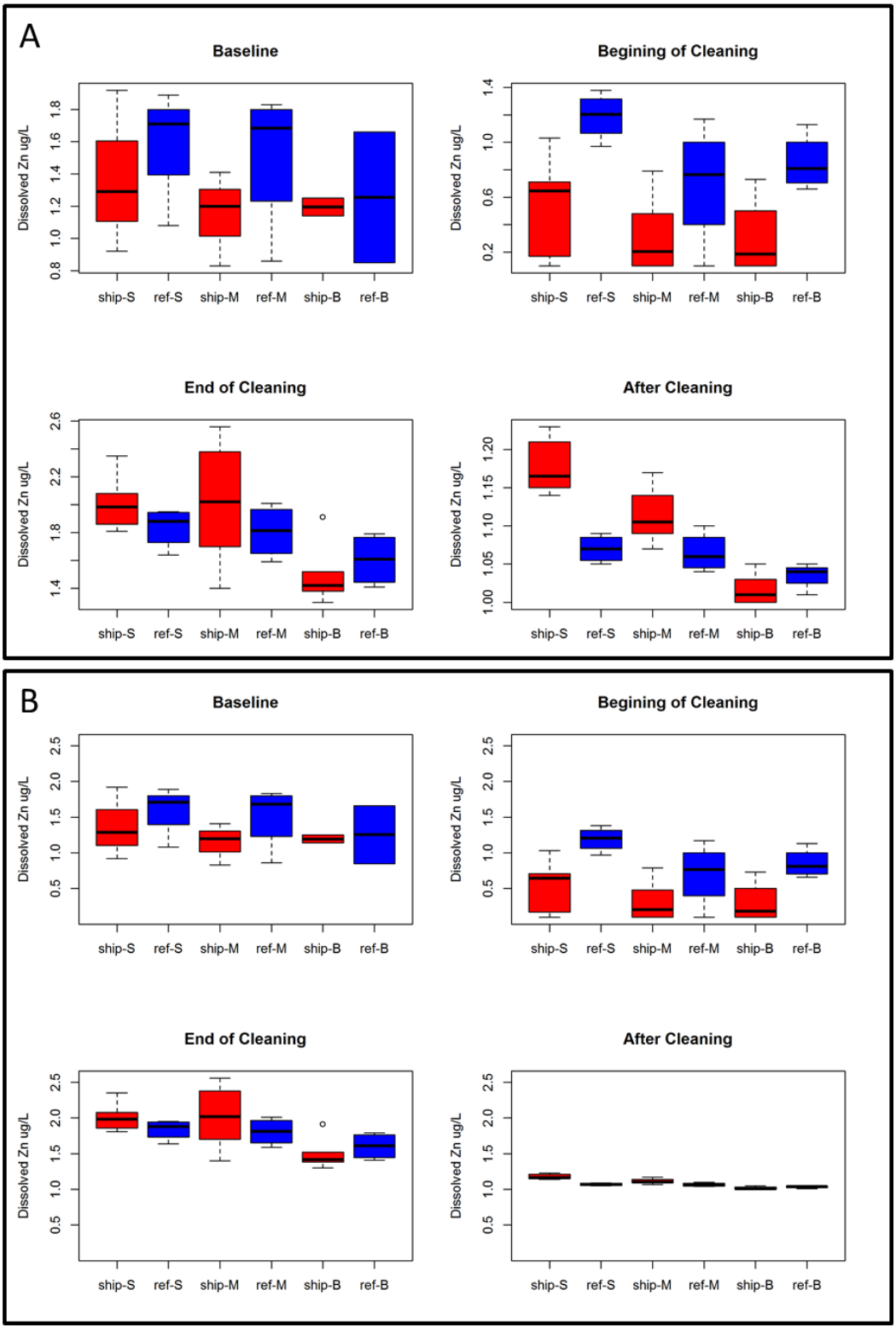

Figure C-15. Box and whisker plots for dissolved Zn (µg/L) by sampling event, type and strata independently scaled by event in upper panel (A) and same scale for all events in lower panel (B).

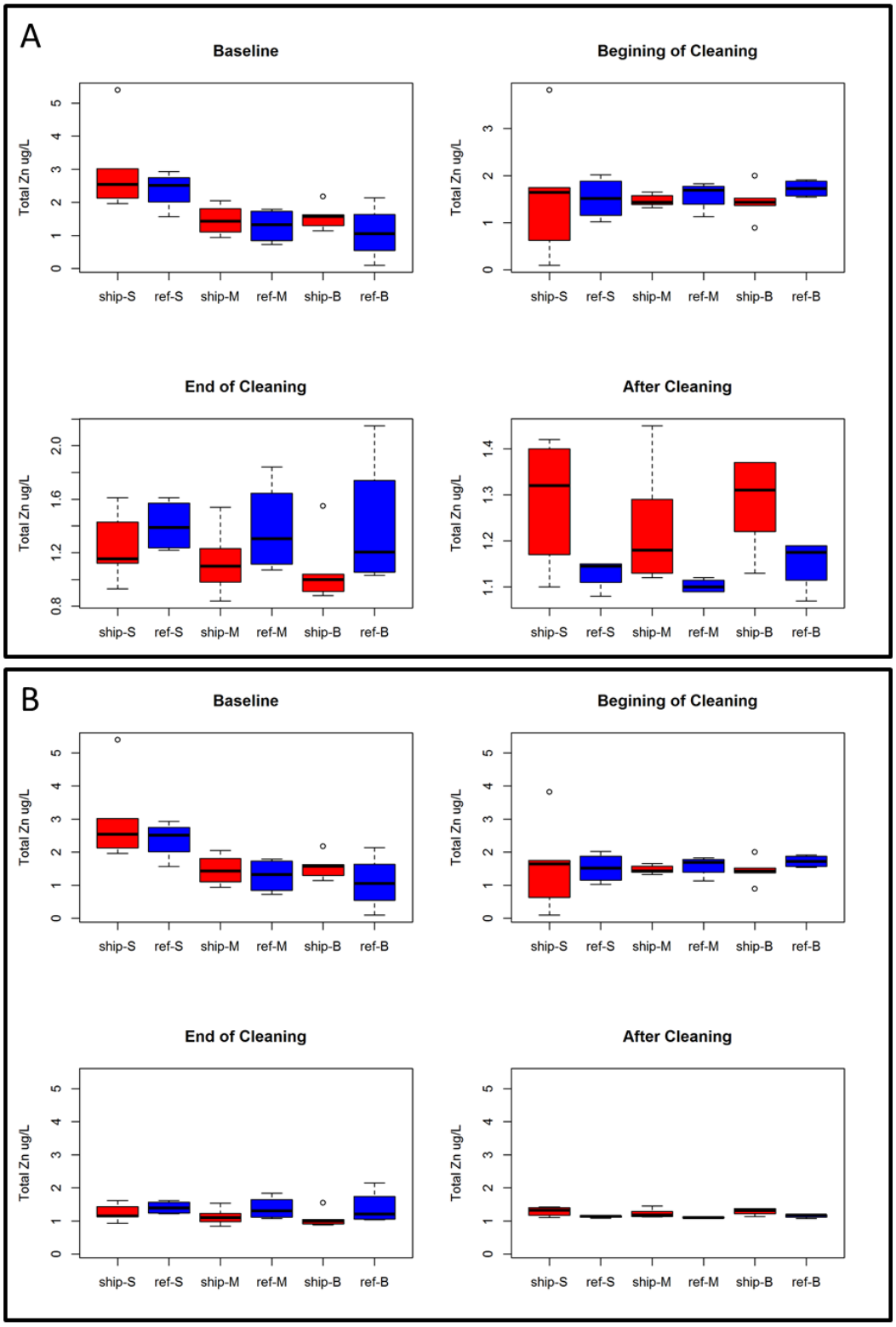

Figure C-16. Box and whisker plots for total zinc (µg/L) by sampling event, type and strata independently scaled by event in upper panel (A) and same scale for all events in lower panel (B).

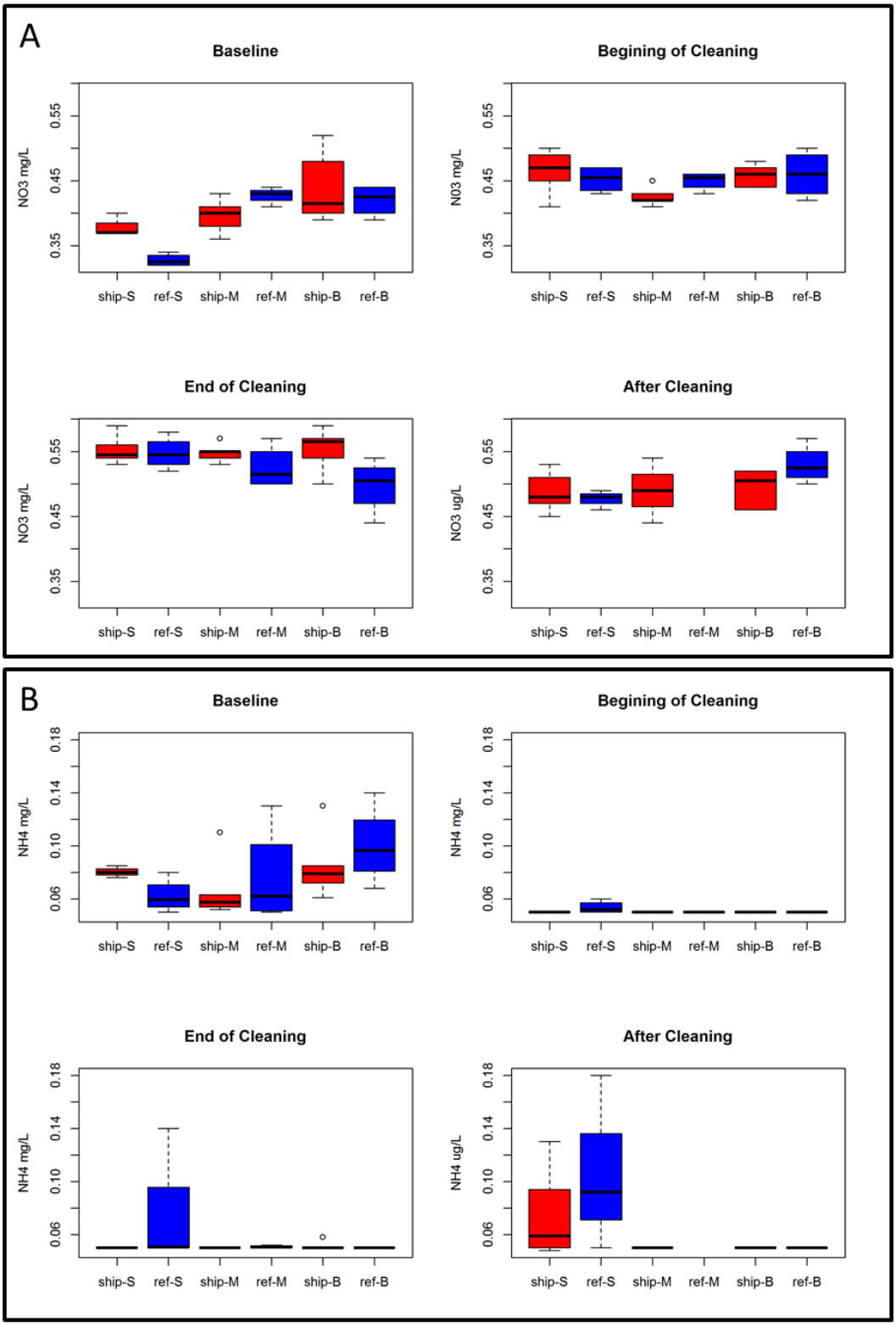

Figure C-17. Box and whisker plots for Nitrate-N (A) and Ammonia-N (B) expressed as nitrogen (mg/L) by sampling event, type and strata.

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14. ABSTRACT

This report contains details of Biofouling removal from the ex-INDEPENDENCE conducted on January 6 to 27, 2017, at Mooring G at Puget Sound Naval Shipyard (PSNS). This study was conducted to monitor and evaluate key water quality parameters at six sites located near the ship (area of influence) and four reference sites within western Sinclair Inlet. Details include results of four sampling events that were conducted, which included before removal (Event 1, Baseline, November 9 to 10, 2016), during removal (Event 2, During-removal, January 10, 2017), at the end of removal (Event 3, Week-post-removal, January 31, 2017), and 40 days after removal was completed (Event 4, Month-post-removal, March 7, 2017). Each sampling event consisted of collecting discrete water samples from the surface, mid-depth, and near bottom strata of the water column.

As part of the research, water samples were analyzed for dissolved and total Cu and Zn, nutrients, DOC, and BOD. In addition, in-situ sensors were utilized during the study to provide continuous monitoring of temperature, salinity, pH, DO, and turbidity within the water column at each sampling site. In summary, there was no evidence of any parameter exceeding regulatory thresholds and no evidence of a persistent water quality impacts from the ex-INDEPENDENCE biofouling removal operation, as water quality indicators returned to ambient conditions within 40 days after biofouling removal was completed.

15. SUBJECT TERMS

Hull Cleaning; CV-62; USS Independence; biofouling removal; Water Quality assessment; dissolved copper; Dissolved Zinc; turbidity; dissolved organic carbon; biological oxygen demand

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