SPAWAR Systems Center PACIFIC

TECHNICAL REPORT TR 3081
October 2017

Comprehensive Field Surveys and Evaluation of Biofouling and Coating System Conditions Associated with the Hull of the ex-USS INDEPENDENCE (CV 62)

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ADMINISTRATIVE INFORMATION

The work described in this report was performed for the Navy Sea Systems Command by the 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), Space and Naval Warfare Systems Center Pacific (SSC Pacific) San Diego, CA. Additional support was provided by the Naval Undersea Warfare Center (NUWC) and the McLaughlin Research Corporation.

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EXECUTIVE SUMMARY

OVERVIEW

Moored at Naval Base Kitsap Bremerton, WA, in Sinclair Inlet, Puget Sound since decommissioning in September 1998, the ex-INDEPENDENCE (CV 62) was scheduled to be towed in March 2017 to Brownsville, TX, for dismantling. Based on an informal consultation with the National Marine Fisheries Service (NMFS), the Navy was directed to clean the ship's hull prior to towing to mitigate the transfer of invasive species to other regions. The Navy prepared a Biological Evaluation (BE) that addressed the towing of the ex-INDEPENDENCE from Bremerton, WA, to Brownsville, TX, and the potential effects on species listed under the Endangered Species Act (ESA) and their designated critical habitat (Naval Undersea Warfare Center Division Newport, 2016). Biological surveys of the hull of the ex-INDEPENDENCE were conducted prior to hull cleaning to characterize the species and mass of biological material on the hull and, post hull cleaning, to assess the effectiveness of reducing the potential transport of invasive species. Additionally, sampling and analysis of the hull coating system condition was conducted to assess the release of metals (copper and zinc) associated with hull cleaning. Hull cleaning was conducted in January 2017 by Seaward Marine Inc., under contract to the Navy. This report documents the methods used by Marine Inc., and presents the results of the biological surveys and paint analysis.

Space and Naval Warfare Systems Center Pacific (SSC Pacific) Scientific Diving Services (SSC Pacific SDS) and Naval Undersea Warfare Center (NUWC) Division Newport conducted in-water hull surveys on the ex-INDEPENDENCE for the Naval Sea Systems Command (NAVSEA) Inactive Ships Office (ISO) SEA 21I prior to hull cleaning from 2016 12 08 through 2016 12 14 and 2017 01 31 to 2017 02 05 at Naval Base Kitsap in Bremerton, WA. The ex-INDEPENDENCE was towed from the Naval Inactive Ships Maintenance Office (INACTSHIPMAINTO), Bremerton, WA, on 2017 03 11 and arrived to Brownsville, TX, on 2017 06 01, for dismantling.

TEST OBJECTIVES

The primary objectives of the biological assessment were to: (1) determine the presence/absence of high-risk invasive species remaining on the hull of the ex-INDEPENDENCE post hull cleaning, (2) determine the amount of organic tissue (i.e., marine organisms) and the amount of inorganic material (i.e., shells) that were removed during the hull cleaning process, and (3) identify biofouling organisms to the lowest possible taxonomic level that was practical on the hull of the ex-INDEPENDENCE prior to hull cleaning.

The primary objective of the paint analysis was to assess the release of copper (Cu) and zinc (Zn) associated with the hull cleaning of the ex-INDEPENDENCE by (1) measuring the passive release of dissolved Cu and Zn from the hull using the SSC Pacific Dome technique pre and post hull cleaning, (2) measuring total (dissolved + particulate) Cu and Zn in samples collected from a simulated hull cleaning device prior to cleaning, and (3) measuring total and dissolved Cu and Zn in samples collected from the effluent of the Submerged Cleaning and Maintenance Platform (SCAMP®) during cleaning operations.

PRE-HULL CLEANING TAXONOMY AND BIOMASS ASSESSMENTS

Biological sample collection for biomass assessment and taxonomic identification was conducted pre-hull cleaning in December 2016. Biological samples for taxonomy and biomass were collected at randomly selected stations along transect belts on the hull, as well as on niche areas (other isolated areas of the hull where fouling is known to occur). A total of 19,092 organisms and 92 distinct taxa belonging to 11 phyla were detected in the pre-hull cleaning taxonomy survey samples along the hull of the ex-INDEPENDENCE. The majority of the taxa belonged to Phyla Arthropoda and Annelida, accounting for 54% and 27% of the total identified organisms, respectively. Seven species (*Jassa marmorata, Pinnixa sp., Mytilus galloprovincialis, Schizoporella unicornis, Watersipora subtorquata, Ciona intestinalis,* and *Botrylloides violaceus*) were determined to pose a potentially high risk to the western Gulf of Mexico if they remained on the hull post cleaning based on their environmental tolerances. Three crustacean (*Caprella mutica, Hemigrapsus oregonensis, and Ianiropsis serricaud*i) and one tunicate species (*Styela clava*) were considered to be a low risk as the environmental parameters in Gulf of Mexico are not suitable for colonization and/or survival of these species during the tow was unlikely.

The amount of biomass removed from the ex-INDEPENDENCE was estimated using the quantitative measurements of fouling biomass present at the randomly sampled locations on the hull and extrapolating the measured values to the extent of the wetted surface area of the hull. Based on the results of this study, the total wet weight of material removed from the hull was approximately 119,071 pounds (lbs; 54,000 kg). This corresponds to a dry weight of approximately 3,146 kg (7,000 lbs) of organic material (i.e., soft parts of marine organisms) and approximately 9,945 kg (22,000 lbs) of inorganic material (i.e., shells) that was removed from the hull of the ex-INDEPENDENCE during cleaning operations.

POST-HULL CLEANING BIOLOGICAL HULL SURVEY

Biological surveys to assess the invasive species remaining on the hull following hull cleaning were conducted in January to February 2017. These surveys utilized both qualitative and quantitative methods to estimate the percent coverage of biofouling organisms remaining on the hull and in niche areas following the cleaning. Qualitative inspection of niche areas revealed little to no fouling coverage, with isolated tubeworms, anemones, and hydroids surviving. Little to no biofouling organisms were observed during the quantitative biological hull survey (< 1%). The hull was predominately characterized by areas of exposed antifouling paint and small, random patches of bare hull, with remnants of dead barnacles and calcareous tubeworms. Given that the ex-INDEPENDENCE had an extensive hull cleaning resulting in minimal remaining growth and that no invasive species were observed on the hull post-cleaning, the risk of species transfer to the destination port is substantially reduced. Most, if not all, of the fouling community was effectively removed during hull cleaning of the ex-INDEPENDENCE and it is not likely that substantial biofouling growth would accumulate during the transit while the ship was moving.

PRE, DURING, AND POST-HULL CLEANING PAINT ASSESSMENT

Paint sampling was conducted between December 2016 and February 2017. Three sampling techniques were utilized to estimate metal (Cu and Zn) release from the hull of the ex-INDEPENDENCE during cleaning: (1) passive leaching rates of dissolved Cu and Zn from ship hull paint were measured pre and post hull cleaning utilizing the SSC Pacific Dome technique, (2) release of total (particulate and dissolved) Cu and Zn associated with hull cleaning were analyzed from simulated hull cleaning prior to actual hull cleaning, and (3) during hull cleaning, samples were collected from the SCAMP® effluent to evaluate the load of total and dissolved metals released during cleaning. Results of chemical analysis of the samples in all three cases indicated low passive metal leaching rates and low environmental loading of metals associated with hull cleaning.

Dome sample analysis showed a leaching rate of 1.1 ±1.1 µg Cu/cm^2 the day pre-hull cleaning and a nearly identical rate of 1.2 ±0.8 µg Cu/cm^2 the day post hull cleaning. Extrapolating to the wetted hull surface area of the ex-INDEPENDENCE (123,445 ft^2 [11,468 m^2]), these rates represented loading of 0.29 lbs of dissolved Cu per day to the environment. Zinc concentrations were very low or not detected indicating minimal or negligible release of Zn from the hull pre and post cleaning. Analysis of simulated hull cleaning samples indicated geometric mean release rates of 8.23 µg Cu/cm^2 and 1.53 µg Zn/cm^2 representing an estimated release of 2.08 lbs of Cu (total) and 0.39 lbs of Zn (total) during hull cleaning. SCAMP® effluent analysis indicated geometric mean release rates of 1.24 g Cu/m^2 (total), 0.39 g Cu/m^2 dissolved, 0.79 g Zn /m^2 (total), 0.25 dissolved g Zn/m^2 (dissolved). These SCAMP® rates represent an estimated release of 31.3 lbs of total Cu, 9.9 lbs of dissolved Cu, 12.7 lbs of total Zn, and 6.3 lbs of dissolved Zn during hull cleaning. Definitions of dissolved and total Cu and Zn and an assessment of potential water quality impacts to Sinclair Inlet associated with biofouling removal from the ex-INDEPENDENCE are discussed in a separate report focusing on water quality (SPAWAR and NUWC, 2017).

CONCLUSIONS

The survey methods employed pre, during, and post-hull cleaning on the ex-INDEPENDENCE succeeded in meeting the objectives set forth in the protective measures of the BE to reduce or avoid potential effects from invasive species to ESA-listed species and critical habitat. Three separate lines of evidence to estimate the metal loading to Sinclair Inlet associated with the cleaning of the ex-INDEPENDENCE's hull (analysis of dome samples, simulated hull cleaning, and SCAMP® effluent) indicate that the antifouling system of the inactive ex-INDEPENDENCE's hull was metal-depleted, and no longer releasing substantial amounts of Cu and Zn. Therefore, hull cleaning of the ex-INDEPENDENCE would likely have released relatively small amounts of Cu and Zn with minimal environmental loading compared to routine husbandry on active ships. Biofouling on the ex-INDEPENDENCE was removed by the hull cleaning operations and no appreciable marine growth, invasive or otherwise, remained on the hull post cleaning. Based on the results of this study, hull cleaning appears to be an effective management strategy to reduce the risk of transferring invasive species.

ACRONYMS AND ABBREVIATIONS

BE	Biological Evaluation
C	Celsius
CIA	Controlled Industrial Area
cm	centimeter (s)
cm^2	square centimeter (s)
CRM	Certified Reference Material
Cu	copper
CV	coefficient of variation
DO	dissolved oxygen
DoN	Department of the Navy
EEZ	Exclusive Economic Zone
EPA	Environmental Protection Agency
ESA	Endangered Species Act
ft	feet
ft^2	square feet
ft^3	cubic feet
FIAS	Flow Injection for Atomic Spectroscopy
g	gram (s)
HEPA	High Efficiency Particle Air
in	inch (es)
ICP-MS	Inductively coupled plasma – mass spectroscopy
IMF	Intermediate Maintenance Facility
INACTSHIPMAINTO	Inactive Ships Maintenance Office
ISO	Inactive Ships Office
kg	kilogram (s)
L	liter (s)
lbs	pound (s)
m	meter (s)
m^2	square meter (s)
MARAD	U.S. Maritime Administration
min	minute (s)
mL	milliliter (s)
µg	microgram (s)
µm	micron (s)

N	Normal
NAVSEA	Naval Sea Systems Command
NBK	Naval Base Kitsap
NMFS	National Marine Fisheries Service
NOD	Nature of Discharge
NUWC	Naval Undersea Warfare Center
NIST	National Institute of Standards and Technology
PEMES	Perkin-Elmer multi-element standard solution
ppb	parts per billion
PSNS&IMF	Puget Sound Naval Shipyard and Intermediate Maintenance Facility
PVC	polyvinyl chloride
PWP	Project Work Plan
QA	Quality Assurance
QC	Quality Control
$QNHO_3$	quartz still nitric acid
rpm	revolutions per minute
SCAMP	Submerged Cleaning and Maintenance Platform
SHCP	Ship Hull Characteristics Program
SSC Pacific	
SSDS	Surface-Supplied Diving Systems
SDS	Scientific Diving Services
sp(s)	species
SD	standard deviation
UNDS	Uniform National Discharge Standards
US	United States
Zn	zinc

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1. INTRODUCTION

During an informal consultation with National Marine Fisheries Service (NMFS), the Navy prepared a Biological Evaluation (BE) that addressed the towing of the ex-INDEPENDENCE (CV 62) from Bremerton, WA, to Brownsville, TX, and the potential effects on species listed under the Endangered Species Act (ESA) or their designated critical habitat (Naval Undersea Warfare Center Division, Newport, 2016). This BE evaluated protective measures including hull cleaning and hull surveys to reduce or avoid potential effects from invasive species to ESA-listed species and critical habitat. Invasive species are non-native species that have the ability to spread through an ecosystem and displace and outcompete native species. These protective measures were implemented and this report summarizes the results of these surveys.

Space and Naval Warfare Systems Center Pacific Scientific Diving Services (SSC Pacific SDS) and Naval Undersea Warfare Center (NUWC) Division Newport conducted in-water hull surveys on the ex-INDEPENDENCE (CV 62) for the Naval Sea Systems Command (NAVSEA) Inactive Ships Office (ISO) SEA21I from 8 December through 14 December 2016 and 31 January to 5 February 2017 at Naval Base Kitsap in Bremerton, WA. The ex-INDEPENDENCE was towed from Mooring G at the Naval Inactive Ships Maintenance Office (INACTSHIPMAINTO), Bremerton, WA, on 11 March 2017 and arrived to Brownsville, TX, on 1 June 2017, for dismantling.

The primary objectives of the surveys were to:

1. Determine the presence/absence of high-risk invasive species remaining on the hull of the ex-INDEPENDENCE post hull cleaning.
2. Determine the amount of organic tissue (i.e., marine organisms) and the amount of inorganic material (i.e., shells) that were removed during the hull cleaning process.
3. Identify biofouling organisms to the lowest possible taxonomic level that was practical on the hull of the ex-INDEPENDENCE prior to hull cleaning.
4. Assess the release of copper (Cu) and zinc (Zn) associated with the hull cleaning of the ex-INDEPENDENCE by measuring the passive release of dissolved Cu and Zn from the hull using the SSC Dome sampler pre and post hull cleaning, total (dissolved and particulate) Cu and Zn in samples collected from a simulated hull cleaning device prior to cleaning, and total and dissolved Cu and Zn in samples collected from the effluent of the SCAMP® during cleaning operations.

A project work plan (PWP) was prepared to document the sampling and analysis procedures that were followed for assessing potential impacts from biofouling removal from the ex-INDEPENDENCE moored in Sinclair Inlet, Puget Sound, WA (SPAWAR 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.

1.1 BACKGROUND

The ex-INDEPENDENCE, commissioned on 10 January 1959, is a *Forrestal*-class aircraft carrier. The vessel was decommissioned in 1998 after 39 years of active service and was transferred to INACTSHIPMAINTO. On 21 December 1999, she arrived at Mooring G, where she remained until towed on 11 March 2017 to Brownsville, TX, for dismantling. SEA 21I manages U.S. Navy ships that have reached the end of their lifecycles.

1.2 TOWING PROCESS

The ex-INDEPENDENCE is in an inactive status and therefore required towing from her berth to a dismantling facility in Brownsville, TX. The tow route began through the shipping channels of Puget Sound and into the open ocean and continued south around Cape Horn through the Strait of Magellan, because the vessel was too large for transit through the Panama Canal. The route north from Cape Horn continued through the South Atlantic Ocean and the Caribbean Sea, then into the Gulf of Mexico, arriving to Brownsville, TX, in the western Gulf of Mexico on 1 June 2017. The tow route occurred within international waters, the Exclusive Economic Zone (EEZ) and territorial seas of Chile and Argentina, and U.S. EEZ and territorial waters. During transit, the tug and tow normally traveled at speeds less than 10 knots in the open ocean.

1.3 PAINTING HISTORY

The last dry-dock event for the ex-INDEPENDENCE was on 26 April 1985 to 8 November 1986. The most recent painting report for ex-INDEPENDENCE, dated 17 December 1986, reported that the body of the ship was blasted 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 ex-INDEPENDENCE in 1985–1986 contained cuprous oxide as the active anti-fouling ingredient and consisted of coats of both International BRA 540® red and International® BRA 542 black (Department of the Navy, 1986). In the 30 years since then, the copper would be depleted from its original levels in the paint.

1.4 HULL CLEANING PROCESS

Due to the size of ex-INDEPENDENCE, there was only one dry dock on the west coast large enough to accommodate the ship, dry dock 6 at the Puget Sound Naval Shipyard and Intermediate Maintenance Facility (PSNS&IMF) in Bremerton, WA, which was fully booked meeting the repair and maintenance demand of active U.S. Navy ships. Therefore, the ex-INDEPENDENCE could only be cleaned using diver operated equipment. Due to the size of ex-INDEPENDENCE and the extent and type of biofouling present, the cleaning operation was estimated to take approximately 1 month to accomplish. Hull cleaning commenced on 6 January 2017 and was completed by 27 January 2017. Organisms and/or biofouling communities attached to the hull were removed using underwater hull cleaning methods and equipment as specified in the “Naval Ships” Technical Manual Chapter 081 Waterborne Underwater Hull Cleaning of Navy Ships”(Naval Sea Systems Command, 2006). Naval Ships’ Technical Manual 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 multiple brushes or single brushes fitted with different brush types depending on the type of machine and fouling conditions present. The Multiple-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 multiple-brush unit cannot access. For areas that are more difficult to reach, divers employ high-pressure water jets.

2. METHODS

2.1 STUDY SITE

In-water surveys on the ex-INDEPENDENCE were conducted off G Pier, Bremerton, WA, where the ex-INDEPENDENCE had been docked since 1999 (Figure 1). The surveys were designed using the docking plans of the ex-INDEPENDENCE (Department of the Navy, 1962) . These docking plans were reviewed to determine in-water sampling strategies and locations on the hull that could potentially support biofouling. Terminology used here to refer to parts of a ship conforms to that used by the U.S. Navy underwater ship husbandry manual (Department of the Navy 2008).

Figure 1. Ex-INDEPENDENCE at the Puget Sound Naval Shipyard and Intermediate Maintenance Facility, Bremerton, WA.

The ex-INDEPENDENCE has an overall length of 1,070 feet (ft; 326.1 meters [m]), a waterline length of 990 ft (301.8 m), a waterline beam of 130 ft (39.6 m), a maximum beam of 263 ft (80.1 m), and a draft of 37 ft (11.3 m). Her light displacement is 57,734 long tons (NSWC Carderock, 2016). All propellers, rudders, and rudder posts have been removed and all openings, including sea chests and overboard discharges have been sealed (NSWC Carderock, 2016). The mean depth of water where the ex-INDEPENDENCE is docked is 42 ft (12.8 m). At low tide, the clearance from the bottom of the vessel to the seafloor was approximately 10 to 15 ft (3.0 to 4.6 m) (Bryson, 2017).

2.2 SURVEY METHODS AND SAMPLE DESIGN

In consultation with NMFS, the Navy identified protective measures to reduce or avoid potential effects to ESA-listed species and critical habitat, including appropriate hull cleaning, pre and post-cleaning inspections, and a biological survey. Pre-cleaning inspections are briefly described to provide background for the biological survey, post-cleaning inspection, and coating system evaluation reported here.

Pre-cleaning hull inspections were conducted prior to in-water cleaning to determine if cleaning was necessary and what specific equipment and procedures would be employed. On 5 November

2016, Seaward Marine Services, Inc. was contracted by NAVSEA to assess and document the amount of biofouling. This inspection determined the average biofouling growth to be approximately 2 inches, with scattered tubeworms extending 3 ft (0.9 m). This comports with the findings from Global Diving & Salvage, Inc. which reported approximately two to three feet of heavy tubeworm growth throughout the vessel during a February 2016 inspection (Global Diving & Salvage, 2016). However, it is important to note that Seaward Marine was only able to perform an inspection on the bow of the vessel to 300 ft (91.4 m) aft due to pier load limits, which restricted access to the rest of the hull during the time of their inspection.

Three separate survey efforts were performed to address the overall goals and included (1) pre-hull cleaning biological sample collection for biomass assessment and taxonomic identification, (2) post-hull cleaning biological hull surveys for an assessment of invasive species remaining on the hull following hull cleaning that may pose a risk to ESA-listed species, and (3) pre, during, and post-hull cleaning paint analysis to estimate the Cu and Zn concentrations from the ablative copper coating on the hull of the ex-INDEPENDENCE released during hull cleaning and the potential load of these metals to Sinclair Inlet. The sample design for each of these surveys is described separately in the sections below. The scientific team involved in the pre-hull cleaning biomass and taxonomy surveys, as well as the paint assessment and post-hull cleaning surveys included scientists from SSC Pacific and NUWC Division Newport.

2.2.1 Personnel and Diving Apparatus

The underwater surveys of the ex-INDEPENDENCE were performed from 8 December through 14 December 2016 and 31 January to 5 February 2017. Dive support was provided by PSNS&IMF Dive Locker. All diving operations were conducted in accordance with the U.S. Navy Diving Manual (Department of the Navy, 2016). Diving was conducted using surface-supplied diving systems (SSDS) with umbilicals that allowed for communications with scientists on the surface and real-time video. MK-20 full face masks and KM-37 diving helmets were utilized.

2.2.2 Pre-Hull Cleaning Taxonomy and Biomass Assessment

A 0.25 square meter (m^2) (0.25 by 0.25 m) Plexiglas sampling box was constructed with an open end that was placed against the hull for collection of biological samples used for both biomass and taxonomy assessments. The opposite end of the box was covered with neoprene with a slit allowing access to the inside of the box. On the bottom of the box was a tube facilitating collection of the sample down into a tightly woven white cotton cloth sample bag that was attached on the outside of the tube (Figure 2). The sample bag had a mesh of 200 micron (μm) and was 14 inches (in) 35.6 centimeter (cm) long with a 4-in (10.2 cm) opening. A diver placed the open end of the sampling box firmly against the hull of the ship to prevent loss of any biota sampled. A second diver inserted a scraper into the neoprene slit and scraped biota from the hull, ensuring all of the specimens were collected in the cloth sampling bag. Motile fauna were also captured if they were located within the quadrat. The bag was then closed off and secured with a zip-tie to prevent loss of the biota sample. For several sampling stations, additional sample bags were used if necessary. The fouling density required the scientific diver to remove some of the biota prior to placement of the sampling box to ensure it was flush against the hull. Biota removed pre placement of the box was also collected in bags. Each bag was numbered prior to commencement of diving operations and those numbers were communicated to topside scientists for accurate recordkeeping. After biota were collected and secured in sample bags, the bags were stored in a large tool bag and returned to the surface. During the survey, in some instances, video was recorded to gather more information. Upon retrieval at the surface, all bags were immediately transferred and samples were prepared for laboratory analysis.

Figure 2. Biological specimen sample device.

2.2.3 Sampling Design for Taxonomy and Biomass Surveys

Biological sampling locations were established at six stations along five transect belts oriented perpendicular to the vessel centerline at approximately every 200 ft (61 m) from the bow, with the exception of Transect 5 which was moved forward 100 ft to allow for adequate keel depth (Figure 3).

The position of the transects were adjusted to ensure that two of the sampling transects were positioned so as to be shaded by the flight deck, and two were positioned where they were not shaded by the flight deck. Permanent vessel features (i.e., welded cleats, bollards, and ship waterline marks) were noted prior to sampling to allow divers the ability to return to the same location post-hull cleaning for the post-hull cleaning biological hull survey. A stratified random sample design by depth was employed from the waterline to a maximum depth of 28 ft (8.5 m) (Table 1). Sample stations were distributed along both sides of the hull from the waterline to the bottom of hull in three depth strata:

1. *Surface strata (S)*: approximately 2–5 ft below water line
2. *Mid-depth strata (M)*: approximately 10–15 ft below water line
3. *Deep (near bottom) strata (D)*: approximately 20–28 ft below water line (below bilge keel)

Table 1. Random Taxonomy Sample Survey Design Stratified by Depth (ft).

Vessel Side	Depth Strata	Transect Belt				
		1	2	3	4	5
Starboard	Surface	4	2	3	3*	2
	Middle	10	11	10	16*	15
	Deep	19	24	21	24*	24
Port	Surface	2	2	5	3	4
	Middle	12	10	15	11	14
	Deep	20	24	26	23	25

*Not sampled due to time constraints.

Note: Yellow shaded cells indicate that these stations were also sampled for biomass.

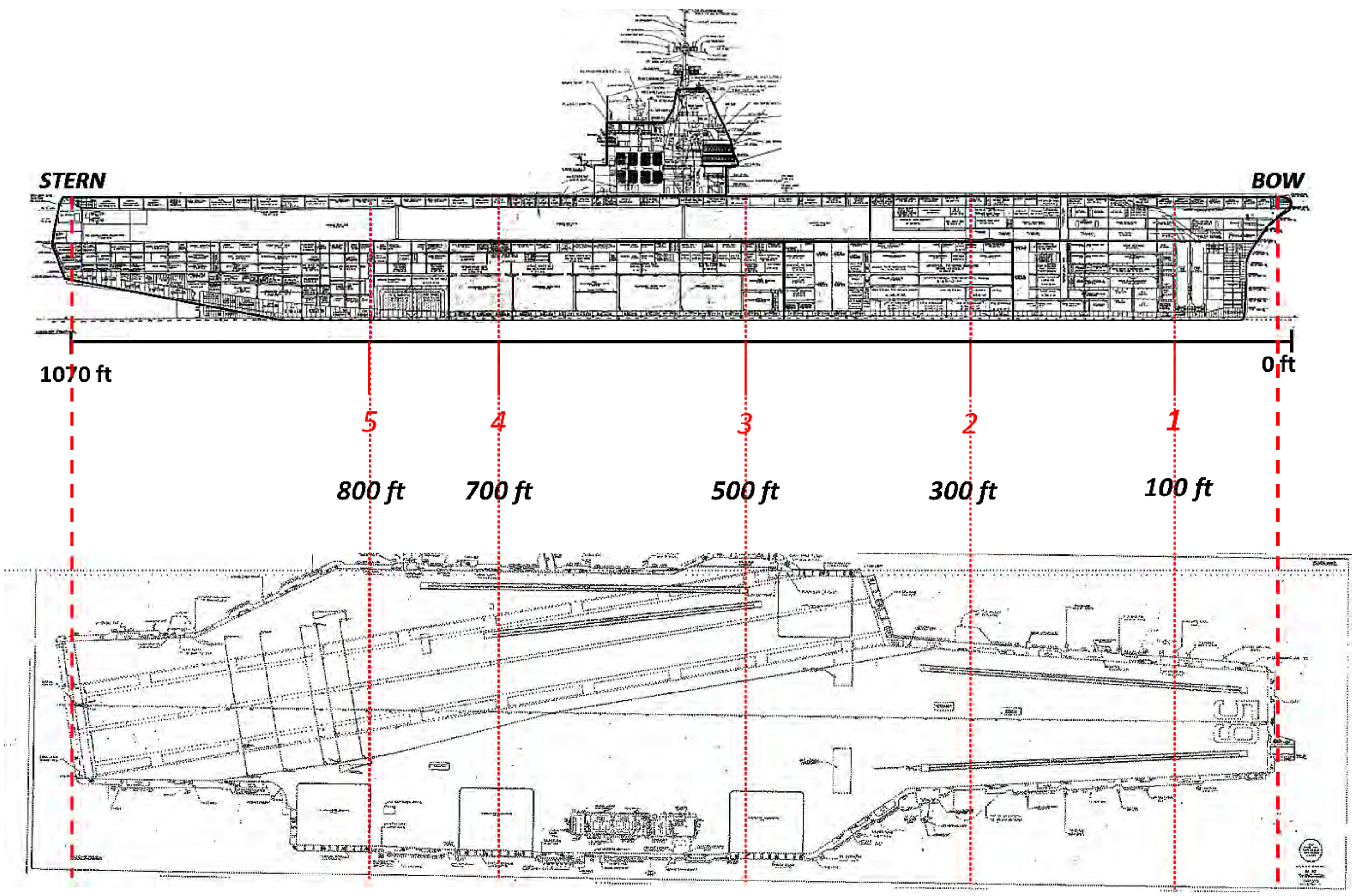


Figure 3. Approximate locations of sample transects for biological samples.

Ten additional sample stations were distributed randomly along stern niche area features including bilge keel, fairwaters, stern tubes, and struts (Table 2).

Table 2. Niche Area Sample Stations and Corresponding Vessel Feature on the ex-INDEPENDENCE.

Sample Station	Corresponding Niche Area
Stern Site 1	Stern tube
Stern Site 2	Strut
Stern Site 3	Strut
Stern Site 4	Inboard port shaft
Stern Site 5	Inboard shaft
Stern Site 6	Outboard stern tube
Stern Site 7[1]	Inboard strut
Stern Site 8	Aft strut
Stern Site 9	Inboard starboard stern tube
Stern Site 10	Aft starboard inboard strut

[1] Stern Site 7 sample originally collected for taxonomy was processed instead as biomass for Stern Site 8.

Note: Yellow shaded cells indicate that these stations were also sampled for biomass.

2.2.3.1 Taxonomy Survey and Analysis Procedure

A total of 36 taxonomy samples were collected and processed from stations distributed along the hull (27 samples) and niche areas (9 samples). All hull stations described in the sampling design were sampled and processed for taxonomy identification, with the exception of three stations on the starboard side of transect 4 which could not be accessed due to time constraints, and one niche area (Stern Site 7).

Samples were collected in bags and brought topside where they were transferred to containers, labeled, and fixed with formaldehyde and delivered to the laboratory. Taxonomy samples were fixed by adding equal parts of 10 percent (%) formalin solution (37% formaldehyde buffered with Borax™) and filtered seawater. This resulted in a 5% formalin solution within the sample container.

In the laboratory, samples were prepped for removal of macroinvertebrates by emptying the matrix into a mesh sieve and rinsing to remove preservative and fine sediment while retaining all macroinvertebrates. All material remaining after the rinsing and separation process was sorted under a dissecting microscope (minimum magnification = 10X). Benthic macroinvertebrates removed from this remaining matrix were counted as they were placed into vials containing 70% ethanol. To further ensure every macroinvertebrate sample met a standard minimum sorting efficiency, 10 percent of the samples were re-sorted to ensure that at least 90 percent of the organisms had been removed from those samples. In this project, all original sorts passed the 90% efficacy test and no additional sorting of samples was required.

All macroinvertebrates were then identified by highly-qualified, experienced and professionally certified taxonomists that specialize in each sample component: crustacea, polychaetes, and generals (i.e., all other organisms). The target taxonomic resolution for crustacea and polychaetes was to the family-level unless little effort was required to identify the organisms to genus or species. The target-level for general organisms was "lowest practical", which is generally considered to be genus/species. The coarser target resolution for crustacea and polychaetes was determined to be prudent considering project time constraints and the large numbers of organisms in some samples. To further expedite sample identification, some very abundant taxa of crustacea in six samples were selectively subsampled only to determine the proportions of different taxa in the sample. In such cases, subsampling was only applied to the abundant groups, thereby retaining accurate non-subsampled counts of less abundant taxa. Further, the resulting proportion of taxa identified in the subsampled groups was applied to the *actual* count of all individuals within those subsampled groups, which ensured that the actual total count of organisms in every sample was retained once added to the counts of non-subsampled groups in each sample. A matrix of species identified in the samples and their current status in Washington waters (i.e., native or non-indigenous species) was constructed based on taxonomy data. The presence or absence of any high risk invasive species was also noted.

Species diversity was calculated using the Simpsons reciprocal diversity index (1/D), see Equation (1),

where

$$\boldsymbol{D} = \frac{\sum_{i=1}^{R} n_i(n_i - 1)}{N(N-1)}, \tag{1}$$

and

n = number observed of the i^{th} species out of R total species
N = total number of individuals (observed across all species) for each individual sample, and then for pooled samples by depth, side, and transect.

Simpson's reciprocal diversity index is a measure of community evenness, which ranges from 1, indicating a community entirely dominated by one species to R indicating a community exactly evenly distributed among R total species. This was calculated at the lowest level of taxonomic certainty available. The remainder of the analyses was pooled to the Phylum level, because this level enables consistent identification, and provides ample taxonomic distinction while limiting analysis to a reasonable number of groups.

The total number of organisms observed was compared across transect, ship side (port vs. starboard), and depth strata (surface, middle, bottom). Statistical comparison between groups was accomplished by Bonferroni corrected T-test, to adjust for multiple comparisons. Because the primary purpose of this analysis was to assess transfer risk, analyses were performed on count data rather than biomass. However, note that the size range of organisms observed on the hull was considerable, and thus, an equally sized patch of large tubeworms would produce a much lower abundance than a similarly sized patch of ampeliscid amphipods, for example. Even within species, an equivalently sized patch of juvenile mussels (*Mytilus* sp.) would result in much higher abundance than adults of the same species.

2.2.3.2 Biomass Survey and Analysis Procedure

Using the 0.25-m^2 sampling device, a total of 23 samples were collected for biomass analysis from stations distributed along both sides of the hull from the waterline to the bottom, as well as niche areas. Biological samples were collected at randomly selected stations along five transect belts on the hull for a total of 20 hull biomass samples (see Figure 3 and Table 1). Three more samples were collected in the stern niche area features at Stern Sites 1, 2, and 7 for biomass analysis (see Table 2).

Samples were collected in bags and brought topside where they were transferred to 1-gallon buckets, labeled, and then transferred to the laboratory at the end of each survey day. At the laboratory, individually numbered pie tins were placed in a drying oven (around 100 °C) for 1 hour and then allowed to cool in a sealed desiccator prior to obtaining tare weight. Subsamples for biomass analysis were blotted dry and transferred from the sample container into a pie tin and sample name and weigh boat were recorded on a datasheet. Samples were weighed and the wet weight was recorded as sampled wet biomass (b_W, gram [g]). The samples were then placed in a drying oven for at least 24 hours until biological material was dry. Larger samples took longer to fully dry, and very large samples (> 1 kilogram [kg] wet weight) were split into two weigh boats. After drying, samples were transferred and allowed to cool in a desiccator until weights remained constant prior to obtaining sampled dry biomass weights (b_D, g). After dry weights were obtained, the samples were transferred into a muffle furnace for two hours at 550 °C. This process burns off the organic matter; the material that remained was cooled in the desiccator, weighed, and recorded as the sample ash weight (b_{Cal}, g). The difference between the dry weight and the ash weight was the non-calcareous organic matter ($b_{OM} = b_D - b_{Cal}$) and the ash weight represents the calcareous (inorganic) biogenic material.

The amount of biomass removed from the ex-INDEPENDENCE was estimated using the quantitative measurements of fouling biomass present at the randomly sampled locations on the hull and extrapolating the measured values to the extent of the wetted surface area of the hull. The wetted surface area of the hull was estimated using Equation (2) (Johnson et al., 1999; Naval Sea Systems Command 1996b) and the biomass was determined by Equation (3):

$$S = 1.7\,(L)(d) + (V\sigma)/d \tag{2}$$

$$B = (S/10.6)(b)(16)(.001) \tag{3}$$

S = wetted hull surface area [square feet (ft^2)]
L = length of vessel [ft]
d = molded mean draft at displacement [ft]
V = molded volume of displacement [tons]
σ = density of seawater [cubic feet (ft^3)/ton]
B = total biomass [kg] (either wet weight B_W, dry weight B_D, or calcareous weight B_{Cal})
b = sampled biomass [g] (either wet weight b_W, dry weight b_D, or calcareous weight b_{Cal})
10.6 = conversion factor ft^2/m^2
16 = conversion factor 0.0625 m^2 to m^2
0.001 = conversion factor g to kg

Equation 2 and the results from the random biomass quadrat samples (0.25 m x 0.25 m) analyzed for wet weight, dry weight, and ash weight after combustion were used to estimate the wet biomass (B_W, g/m^2), dry weight biomass (B_D, g/m^2), and calcareous biomass (B_{Cal}, g/m^2) present on the hull, respectively. An initial estimate of the vessel's wetted surface area was obtained using published values for depth and length at full displacement of the ex-INDEPENDENCE (Wikipedia, 2016) and the final estimate was updated using actual length and draft measurements obtained in October 2016 and displacement calculated with the Ship Hull Characteristics Program (SHCP), Version 4.40.02 (NSWC Carderock, 2016). The parameters and values used to calculate the initial and final wetted surface area are shown in Table 3. The accuracy of the calculation is within 5% (Naval Sea Systems Command, 1996a).

Biomass (wet weight) was compared across transect, ship side (port vs. starboard), and depth strata (surface, middle, bottom). Statistical comparison between groups was accomplished by Bonferroni corrected T-test, to adjust for multiple comparisons.

Table 3. Summary of parameters and calculations used to determine the wetted surface area of the hull[1].

(Variable) Units	Initial	Source	Final	Source
(*L*) length ft	1,070	Wikipedia 2016	1,045.83	NSWC Carderock 2016
(*d*) depth ft	37	Wikipedia 2016	27.61	NSWC Carderock 2016
(*w*) width f	130	Wikipedia 2016	129.22	NSWC Carderock 2016
m^3/ft^3	0.028316847		0.028316847	
lb/kg	2.20462262		2.20462262	
lb/ton	2,000		2,240	Naval Sea Systems Command 1996a
(σ) density Kg/m^3			1,023	Density of seawater at $30^{o}/_{oo}$ and 10℃ (Nayar et al., 2016)
ft^3/kg			36,126.90	
ft^3/lb			79,646.19	
(σ) ft^3/ton	35	Johnson et al., 1999	35.55633	Calculated for measured salinity ($30^{o}/_{oo}$) and temperature (10℃)
(*V*) displacement tons	80,643	Wikipedia 2016	57,739.44	NSWC Carderock 2016
(*S*) Wetted Hull Surface ft^2	143,586		123,445.34	Surface area of ship underwater
ft^2/m^2	10.76		10.76	
(S) Wetted Hull Surface m^2	13,340		11,468	Surface area of ship underwater

[1] Calculations are summarized for the initial and final wetted surface areas.

2.2.4 Post-Hull Cleaning Biological Survey

To conduct the post-hull cleaning biological survey, two main sampling methods were utilized to answer questions regarding the presence of invasive species and representative biofouling communities that may remain on the vessel following hull cleaning. The first method was used to document the benthic organisms at hull locations and structures where biofouling is typically found. The second method relied on random sampling to acquire representative estimates of percent cover on large surfaces of the vessel including the bottom. Areas typically supporting biofouling on a carrier include the shafts, struts, stern tubes, fairwaters, rudder posts, bilge keels, the flat bottom of the vessel (particularly at suction openings and locations of dry docking blocks), and waterline (Figure 4). To document the biofouling, the following was conducted:

1. Qualitative descriptions of the bilge keels and submerged aft section of the ship's hull and appendages, from the stern tubes to the end of the shafts, including fairwaters, secondary and main struts.
2. Quantitative sampling of the hull (port and starboard), bilge keel (port and starboard), and bottom of the ship using randomly-placed quadrats.

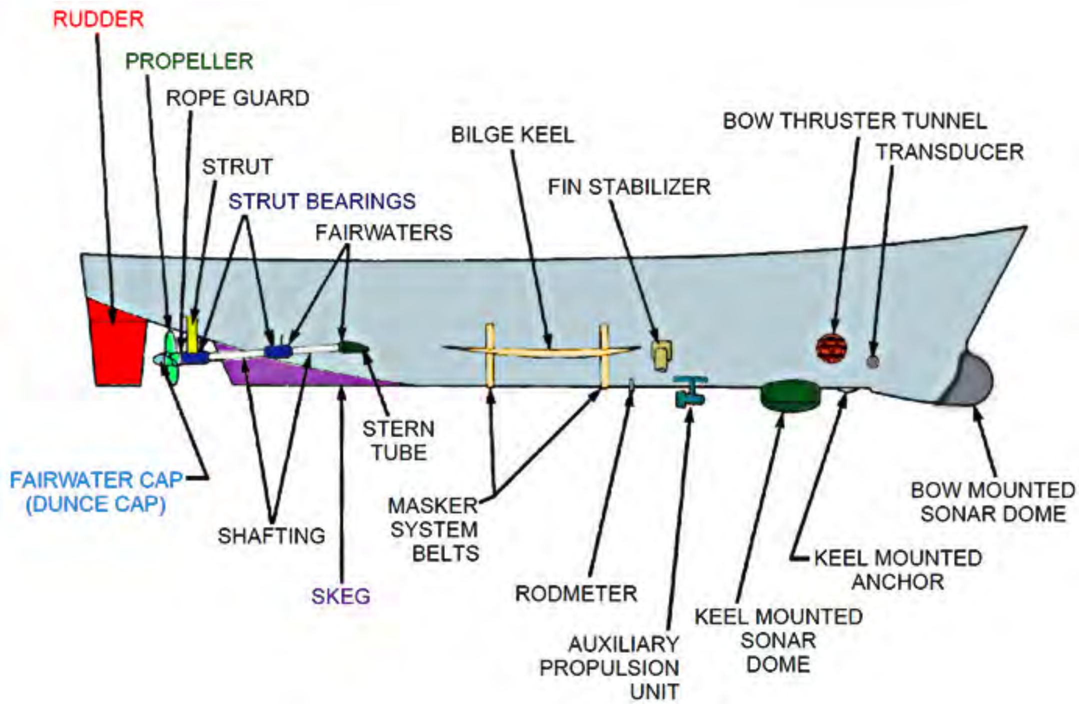


Figure 4. Areas typically supporting marine growth on a representative vessel (Department of the Navy, 2011). Note that not all of these features are present on the ex-INDEPENDENCE and additional features including discharges and sea chests may be present on the ex-INDEPENDENCE.

The sampling quadrat measured 0.5 m^2 (0.5 by 0.5 m) and was made of ¾-in polyvinyl chloride (PVC). It was subdivided into 100 squares, each representing 1% cover (Figure 5). Percent cover of each taxonomic group was estimated by counting the number of squares in which they occurred. The sessile benthic organisms found within the quadrat were identified to the lowest possible taxonomic level.

The following areas were identified in the Survey Plan, inspected, and documented for both fouling and general condition during the post-cleaning survey:

1. Each shaft, strut, stern tube, and fairwater
2. Appendages (e.g., bilge keels)
3. Interface between hull surface with struts, stern tubes, and other appendages
4. Sea chests, openings, and discharges all blanked for inactivation (opportunistic observations only)

Figure 5. Sampling quadrat with floats to ensure positive buoyancy (left). Sampling quadrat placed on the hull of the Ex-Saratoga surveyed in 2010 (right).

The qualitative and quantitative underwater sampling was monitored and recorded from the surface using real-time video (Outland Technology Inc., Underwater Video Systems, Color Camera UWS-3410/D utilizing the UWC-325/P fixed camera and LED light; Figure 6) and audio feeds (communication with divers via the MK-20, Mod-I full face mask and KM-37 diving helmet). The video footage and audio feed provided a permanent record of quadrats and observations. All video records were preserved on DVD disks. An SSC Pacific marine scientist and an enlisted Navy diver conducted the in-water sampling, while at topside, NUWC Division Newport and SSC Pacific marine scientists viewed the sampling on a monitor and communicated with the divers. This allowed for a collaborative sampling and taxonomic identification effort, and enabled real-time data validation and verification.

Figure 6. Underwater video system used to record the hull survey.

2.2.4.1 Qualitative Observations

2.2.4.1.1 Aft Section

Qualitative observations were made of the aft section of the ex-INDEPENDENCE and focused on tight, crevice areas specifically on the shafts, main and secondary struts, fairwaters, stern tubes and bilge keel. The composition of the biofouling community was identified on each structure.

2.2.4.1.2 Seafloor

Though not in the original scope of the project, during the post-hull cleaning biological survey, divers opportunistically surveyed the seafloor underneath the hull of the ex-INDEPENDENCE to qualitatively assess the biofouling that was removed from the hull during the cleaning process and settled to the underlying seafloor. Divers started at frame number 201 and swam forward relaying observations to topside scientists. Video footage of the tract divers swam was captured. On 30 March 2017, approximately 3 weeks after the departure of ex-INDEPENDENCE from Sinclair Inlet, PSNS&IMF Divers also video surveyed the bottom adjacent to Mooring G to document seafloor conditions after completion of the project. In this survey, divers opportunistically videoed the bottom at the locations where the post-cleaning sediment samples were obtained including CV 62-1, CV 62-2, CV 62-6, and a transect between CV 63-3 and CV 63-5 (SPAWAR and NUWC 2016).

2.2.4.2 Quantitative Assessments

Prior to the start of the survey, specific areas along the hull were marked with spray paint to denote certain frame numbers and distances from the bow that would direct divers to sampling locations along the port side of the ship. Frames are typically numbered from the bow aft in most Navy ships. Frame spacing and numbering are important to underwater ship husbandry personnel because they provide an easy reference for locating the diver along the length of the ship. By knowing the framing system and the plating arrangement, divers can precisely report the location of underwater damage or abnormal conditions and for the purposes of this survey, locations of survey transects and quadrats. Permanent vessel features (i.e., welded cleats, bollards, and ship waterline marks) were also noted prior to sampling to allow divers the ability to return to the same location if required. Quantitative measurements were also intended for the aft end niche areas, however, the irregular shapes of the hull features prevented use of quadrats to obtain data. In these cases, only qualitative observations were recorded. The quadrat was made positively buoyant using plastic floats attached to the corners of the quadrat (Figure 5). This ensured that the quadrat would lie flush against the bottom of the vessel. For each quadrat location, video and still images were obtained, and divers estimated the percentage of biofouling cover, then relayed the data for topside scientists to record. Due to technical difficulties with the underwater still camera housing on 31 January, still images were obtained from the video system's "snapshot" function for the remainder of the survey.

2.2.4.2.1 Longitudinal Belt Transects

A total of nine transects oriented port-to-starboard and perpendicular to the vessel centerline approximately 100 ft (30.5 m) apart were sampled and included six quadrats per transect for a total of 54 quadrats (Table 4). Random quadrat numbers were preassigned and the initial five transect locations used in the December biomass/taxonomy surveys were revisited as part of the nine transects (Figure 3).

Table 4. Longitudinal belt transect locations.

Transect #	Approx. Distance from Bow (ft)	Frame #	Quadrat #	Depth (ft)
1	92	23	1-P-S	2
			2-P-M	11
			3-P-D	22
			1-S-S	3
			2-S-M	15
			3-S-D	21
1.5	192	48	1-P-S	4
			2-P-M	12
			3-P-D	20
			1-S-S	4
			2-S-M	15
			3-S-D	25
2	272	68	1-P-S	2
			2-P-M	13
			3-P-D	24
			1-S-S	5
			2-S-M	13
			3-S-D	22
2.5	392	98	1-P-S	5
			2-P-M	14
			3-P-D	24
			1-S-S	4
			2-S-M	15
			3-S-D	25

P = port; S= starboard

S = shallow; M = mid; D = deep

[1]No transect 4.5 due to proximity to transects 4 and 5

[2] Depth adjusted due to limited keel depth at aft end of hull

Table 4. Longitudinal belt transect locations. (Continued)

Transect #	Approx. Distance from Bow (ft)	Frame #	Quadrat #	Depth (ft)
3	496	124	1-P-S	4
			2-P-M	14
			3-P-D	26
			1-S-S	5
			2-S-M	11
			3-S-D	25
3.5	592	148	1-P-S	5
			2-P-M	12
			3-P-D	26
			1-S-S	1
			2-S-M	14
			3-S-D	22
4[1]	680	170	1-P-S	2
			2-P-M	11
			3-P-D	22
			1-S-S	5
			2-S-M	12
			3-S-D	24
5	768	192	1-P-S	4
			2-P-M	14
			3-P-D	27
			1-S-S	4
			2-S-M	13
			3-S-D	21
5.5	920	230	1-P-S	2
			2-P-M	11
			3-P-D	16[2]
			1-S-S	2
			2-S-M	12
			3-S-D	16[2]

P = port; S= starboard

S = shallow; M = mid; D = deep

[1]No transect 4.5 due to proximity to transects 4 and 5

[2] Depth adjusted due to limited keel depth at aft end of hull

Quadrats were distributed along the transects in three depth strata:

1. *Surface strata (S)*: approximately 1-5 ft below water line
2. *Mid-depth strata (M)*: approximately 10-15 ft below water line
3. *Deep (near bottom) strata (D)*: approximately 20-28 ft below water line (below bilge keel). Bottom strata extended from 20 ft to approximately 2 ft shallower than the maximum draft of the hull at that location which ranged from 24-30 ft. As divers neared the aft end of the hull, keel depth decreased and the bottom quadrat was positioned as close to keel depth as practical.

Divers positioned themselves at the starting ship frame number that corresponded to the transect number and then were directed to travel to the initial depth strata location (i.e., near surface depth).

2.2.4.2.2 Bilge Keel Random Quadrats

The bilge keels were located between ship frame numbers 90 and 167 (approximate length of the bilge keel was 308 ft [93.9 m] long). Fifteen quadrat locations were randomly generated along the starboard and port sides using random frame numbers ranging from 90 to 167 (Table 5). To position the random quadrats, the divers positioned themselves at the starting frame number and they were given a random distance to travel either aft or forward from that starting point where they set the quadrat. Quadrats were always placed underneath the bilge keel.

Table 5. Bilge keel random quadrat locations.

Quadrat	Starboard		Port	
	Frame #	Distance from Bow (ft)	Frame #	Distance from Bow (ft)
1	91	367	94	372
2	107	428	97	387
3	109	436	107	428
4	110	440	111	442
5	111	444	117	469
6	113	450	121	484
7	118	470	122	488
8	119	475	129	516
9	---	---	130	520
10	137	547	132	529
11	138	553	133	533
12	145	580	137	548
13	146	585	151	604
14	147	589	158	632
15	158	632	163	652

Dashes indicate quadrat not sampled as it was positioned in the same location as another quadrat.

2.2.4.2.3 Horizontal Hull Random Quadrats

A total of 25 quadrats were randomly selected along the entire length of each side of the ship (Table 6). The quadrats were positioned above the bilge keel in the shallow-mid depth region (approximately 10 ft). To position the random quadrats, the divers positioned themselves at the starting frame number and they were given a random distance to travel from that starting point either aft or forward, where they set the quadrat.

Table 6. Horizontal hull random quadrat locations.

Quadrat	Starboard		Port	
	Frame #	Distance from Bow (ft)	Frame #	Distance from Bow (ft)
1	16	65	14	56
2	47	190	27	108
3	48	192	29	114
4	52	209	35	141
5	54	216	44	176
6	55	220	79	316
7	65	261	87	346
8	71	283	112	451
9	72	288	113	453
10	117	467	121	485
11	133	531	123	490
12	135	540	154	615
13	137	546	160	693
14	181	724	175	698
15	192	767	180	718
16	194	776	186	743
17	199	795	188	750
18	216	865	195	780
19	226	907	199	795
20	227	908	220	880
21	231	922	222	889
22	232	928	224	895
23	238	950	229	914
24	239	957	229	915
25	243	971	234	936

2.2.4.3 Data Analysis

Percent coverage analysis of biofouling was performed real-time during the field effort. Biomass and abundance estimates from pre-cleaning surveys were compared to observations from post-cleaning surveys to estimate the percentage of fouling removed by the cleaning process. Because of the general absence of fouling remaining on the hull post-cleaning, statistical comparisons were not warranted. Species remaining post cleaning were assessed for risk of transfer based on an estimate of likelihood of surviving transit and ability to colonize at the destination location (based on a comparison of environmental conditions at the destination with known environmental tolerances of the species in question).

2.2.5 Pre, During, and Post-Hull Cleaning Paint Assessment

Dome deployments (n = 17), simulated hull cleaning samples (n = 16), and SCAMP® effluent samples (n = 7) were collected and analyzed adhering to strict sampling and laboratory processes. During the dome deployments and collection of simulated hull cleaning samples, clean sample handling procedures (U.S. Environmental Protection Agency 1996) were utilized to minimize contamination and increase confidence in the analytical chemistry results.

Passive leaching of dissolved Cu and Zn was measured utilizing the SSC Pacific Dome technique (Seligman and Neumeister 1983). Prior to the hull cleaning operation and before the hull scrubbing simulation was initiated, random sites (n = 5) were selected to deploy the dome system for evaluating dissolved Cu and Zn leaching rates over the existing fouling and hull surface. Dissolved metal is operationally defined as the fraction that passes through a 0.45 µm pore-size filter, and measured after acidification to pH ≤ 2. The aim of this measurement was to quantify the metal leaching rate of the fouled coating system, however, dome placement required scraping away some fouling to ensure that the dome gasket sealed against the hull. Excess fouling that did not fit under the dome was trimmed. After hull cleaning, dome measurements (n = 12) were collected on to quantify the leaching rate of Cu and Zn associated with the freshly cleaned hull surface. Definitions of dissolved and total Cu and Zn are also discussed in a separate report focusing on water quality (SPAWAR and NUWC 2017).

Prior to hull cleaning, a hull scrubbing simulation was conducted utilizing a device that simulated cleaning to capture seawater and associated particulate matter for chemical analysis at randomly located sample sites (n = 16). The in-water simulated hull cleaning sampling method was used to measure the environmental loading of total Cu and Zn (i.e., measured from samples with no filtration, only acidification to pH ≤ 2 and including both particulate and dissolved metal fractions) associated with cleaning activities (Chadwick et al., 2008).

During hull cleaning, in situ grabs of Submerged Cleaning and Maintenance Platform (SCAMP®) effluent was conducted adhering to methods described in the Uniform National Discharge Standards (UNDS) Nature of Discharge (NOD) evaluation for underwater ship husbandry (U.S. Environmental Protection Agency 1999). SCAMP® samples (n = 7) were analyzed for total and dissolved Cu and Zn.

2.2.5.1 Dome Measurements

Metal concentrations associated with leaching rates of dissolved Cu and Zn were measured pre and post hull cleaning with the in-situ dome system (Seligman and Neumeister 1983; Valkirs et al., 2003) (Figure 7 and Figure 8). The dome system and methods developed by the U.S. Navy (Seligman and Neumeister 1983) and described in detail by Seligman et al., (2001) and Valkirs et al., (2003) were used for evaluating passive metal leaching rates. This system isolates a volume of ambient water over the hull and recirculates the water in the system. The confined volume of water is exposed to any effects of leaching from the surface. During each dome deployment, aliquots of 60 milliliters (mL) were withdrawn from the dome at 0, 15, 30, 45, and 60 minutes (min). Approximately 30 mL of each sample were filtered through a 0.45 µm disc filter, representing the dissolved fraction. Three dome sampling events were completed. On 13 December 2016, there was a single deployment for conditions pre-hull cleaning. Four dome deployments on uncleaned surfaces were accomplished on 11 January 2017, one week post hull cleaning started. The final dome sampling event (n = 12) was conducted on the 1-2 February 2017, with conditions representative of a recently cleaned hull surface.

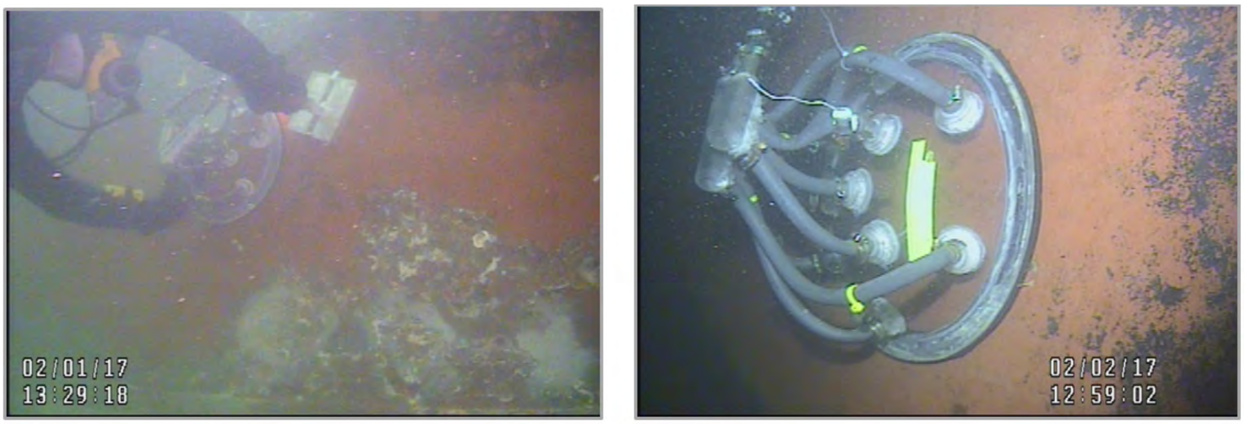


Figure 7. Dome placement on the hull of the ex-INDEPENDENCE post hull cleaning.

Figure 8. Topside sampling and pumping equipment setup for domes.

2.2.5.2 Hull Scrubbing Simulation

Prior to hull cleaning, a hull scrubbing simulation was conducted utilizing a device simulating a cleaning with a stiff nylon brush for fouling removal that best simulates brushes utilized for cleaning. The hull scrubbing device consisted of a clear polycarbonate cylinder, with an inside diameter of 11.4 cm, a sampling area of 101.6 cm^2, and a sample volume of 1,575 mL. The cylinder opening had an integrated double-edge gasket to seal against the test surface. On the opposite end of the cylinder, a shaft passed through an O-ring seal in a polycarbonate cap and attached to a spring-loaded brush inside the cylinder ensuring constant pressure during sampling activities (Figure 9). The device had an exterior handle on the shaft allowing the brush to be manually rotated for a set number of revolutions. For the cleaning simulation, the hull cleaning device was held in place with the gasket in full contact against the hull by divers to ensure seal integrity. The interior spring was released providing consistent pressure between the cleaning brush and the hull surface. The shaft was rotated 10 times at approximately 10–15 revolutions per minute (rpm). The spring was retracted and a polycarbonate sheet was slid between the hull and the cylinder gasket (ensuring that the sheet did not contact the hull surface) capturing the seawater and associated particulate matter. The hull cleaning device was taken out of the water, making sure the sheet stayed in place to avoid dilution of the sample with surrounding seawater. Once on board, a 60-mL aliquot was stored unfiltered representing the total metal concentration, associated with the cleaning simulation. A single hull scrub sampling event was conducted on 13 December 2016.

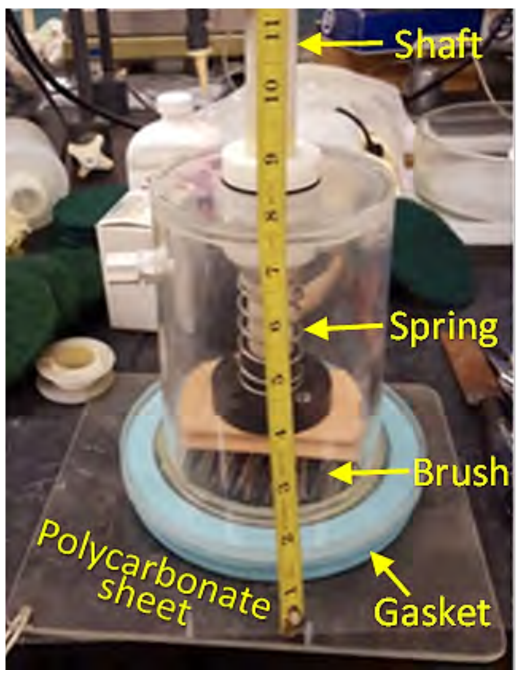


Figure 9. Hull scrubbing devices showing components.

The data from the hull scrubber samples were used to estimate the amount of total Cu and Zn released by hull cleaning using Equation (4) and Equation (5):

$$C_R = C_M(Samp_{vol})(Samp_{area}) \quad (4)$$

$$C_{MASS} = S(929.0304)(C_R)(0.000001) \quad (5)$$

C_R = chemical mass of metal released in scrubber sample per unit area [microgram (μg)/cm^2]
C_M = chemical concentration of metal measured in scrubber sample [μg/L]
$Samp_{vol}$ = volume of scrubber sample [1.575 L]
$Samp_{area}$ = area of scrubber sample [615.8 cm^2]
C_{MASS} = chemical mass released from scrubber sample [g] extrapolated to the wetted hull surface area of the ex-INDEPENDENCE
S = wetted hull surface area [123,445.34 ft^2]
929.0304 = conversion factor ft^2 to cm^2
0.000001 = conversion factor μg to g

2.2.5.3 SCAMP® Effluent Analysis

In-water hull cleaning was conducted using the SCAMP® multi-brush systems. These mechanical devices have three brushes and are held next to the hull from the pressure of a large impeller that pumps seawater at about 13,000 gallons per minute (U.S. Environmental Protection Agency, 1999). While the brushes rotate, the system moved forward at a maximum rate of 60 ft per minute, cleaning a 5-ft-wide swath. Seawater samples from the hull cleaning plume were collected in 125-mL polycarbonate plastic bottles by a diver from the effluent plume of the SCAMP® cleaning system used to clean the vessel hull. Half of each sample was filtered through a 0.4-µm disc filter, while the remaining were unfiltered, representing the dissolved and total metal fractions, respectively.

Total and dissolved metal mass loading resulting from hull cleaning were calculated based on measurements of the metal concentration in the effluent (g/L), observations of the flow rate, F (L/hr) from the SCAMP® impellers, and observations of the rate (R) of travel per unit time or area cleaned per unit time using the following formula, see Equation (6):

$$\text{Loading} = \text{metal concentration (g/L)}(F(L/hr)/\ R(ft^2/hr) \quad (6)$$

2.2.5.4 Analytical Chemistry

Prior to analysis, all samples were acidified to pH ≤ 2 with quartz still-grade nitric acid (Q-HNO_3) in a High Efficiency Particle Air (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) (U.S. Environmental Protection Agency, 1994). If deemed necessary, samples were diluted with 0.1 Normal (N) Q-HNO_3 made up in high-purity (18 MΩ cm^{-1}) water in order to minimize matrix-related interferences inherent to seawater. The samples were injected directly into the ICP-MS via a Perkin-Elmer Autosampler 100. Analytical standards were made with Perkin-Elmer multi-element standard solution (PEMES-3) diluted in 1N Q-HNO_3, which was matrix matched to the salinity of the test samples. Standards were analyzed at the beginning and end of the run, with acceptable calibration curves with $R^2 \geq 0.999$. Blanks made up of 18 MΩ cm^{-1} water acidified to pH ≤ 2 with Q-HNO_3 were analyzed every five samples, and had an average ± standard deviation of -0.035 ±0.13 μg/L, resulting in limit of detection (3 standard deviations [SD]) of 0.38 μg/L, and a limit of reporting (10 standards deviations) of 1.26 μg/L. Note that all quantified values are reported, and those used in the calculations are mentioned in the report, even though some values may be below either or both limits. The analysis also included measurement of sample duplicates and the Certified Reference Material (CRM) 1643e from the National Institute of Standards & Technology (NIST), a coefficient of variation (CV) of ≤15% for replicate measurements, as well as a recovery within 15% of SRM 1643e were required for acceptance of the quantifications. The actual recovery for SRM 1643e was 108 ±13%.

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. The FIAS 400 is coupled with the Autosampler 100 and set to inject the treated sample directly into the ICP-MS. A similar QA/QC control as the one described above is used in these analyzes, with the difference of using the CRM CASS 6, Nearshore Seawater Certified Reference Material for Trace Metals and other Constituents, instead of SRM 1643e, in order to match the salt matrix to the samples. The recovery for CASS 6 was 94 ±16%. Blanks made up of seawater from outside San Diego Bay that was 0.45 μm filtered and acidified to pH ≤ 2 with Q-HNO_3 were analyzed every five samples, with a mean value of 1.21 ±1.35 μg/L, a limit of detection of 4.04 μg/L, a limit of reporting of 13.46 μg/L.

3. RESULTS

3.1 PRE-HULL CLEANING TAXONOMY AND BIOMASS ASSESSMENT

3.1.1 Taxonomy

A total of 19,092 organisms were detected in the survey samples at stations along the hull of the ex-INDEPENDENCE. Given the volume and diversity of samples, taxonomic analysis was carried out to the lowest level practical. Some taxa were resolved to species, others to genus, family or order. Broad taxonomic summaries are given to phylum, whereas invasiveness is assessed at the species level by considering all taxa identified and researching their potential to include invasive species. Summaries of abundance, richness, diversity and potential invasive risk are presented below and in Table 7 and Table 8.

3.1.1.1 Abundance, Species Richness, and Diversity

3.1.1.1.1 Abundance

The highest abundance (number of individuals per sample) was detected at stations 4-P-S and 5-P-S, with a total of 2,978 and 1,396 individuals per quadrat, respectively (Table 7). These stations were located along transects near the aft end of the hull. Station 3-S-M (63 individuals) and Stern Site 10 (20 individuals), which corresponded to the aft inboard starboard strut, had the lowest abundance. Tests of significance across port, starboard, and stern areas showed significantly higher abundance (mean ± SD) on the port (698 ±123) than starboard (297 ±47) or stern niche area (415 ±72) ($p < 0.001$, $T = 6.8$, $df = 28$). Comparisons across depth strata showed that surface strata had the highest observed mean abundance (650 ±126) compared to mid-depth (368 ±61), deep (475 ±75), and stern niche areas (415 ±72). This difference was only statistically significant between surface and mid-depth strata ($p = 0.01$, $T = 4.5$, $df = 8$) and between surface and the stern niche areas ($p < 0.01$, $T = 4.6$, $df = 13$). Variation was also observed between transects bow-to-stern, with significantly higher abundances on transect 5, furthest aft (802 ±145) ($p = 0.02$, $T = 2.5$, $df = 48$) and significantly lower abundances on transect 3, mid-ship (133 ±19) ($p < 0.0001$, $T = 11$, $df = 48$). Transects on the bow of the ship (transects 1 and 2 [484 ±39]) had significantly lower abundance than transects at the aft end (transects 4 and 5[694 ±68]) ($p < 0.0001$, $T = 6.5$, $df = 18$).

3.1.1.1.2 Species Richness and Diversity

A total of 92 distinct taxa belonging to 11 phyla were identified during the survey. The majority of the taxa belonged to Phyla Arthropoda and Annelida, accounting for 54% and 27% of the total identified organisms, respectively (Table 8 and Figure 10). Diversity within individual quadrats was variable, ranging from a high index of 13.3 (Site 3-P-S) to a low of 2.3 (Site 5-S-D and Stern Site 9), indicating a patchy community. With respect to diversity, no patterns were evident between depth strata or ship side, with diversity levels generally between 4.5 and 5.5 for all cases, indicating a moderately diverse community. In general, transect 3 (mid-ship) had the highest diversity, followed by transect 2. These two transects also had the lowest abundances. However, the pattern of high diversity corresponding to low abundance does not hold in other cases. For example, abundance on surface quadrats was higher than deep quadrats, and stern transects showed higher abundance than bow transects, but there were no consistent patterns in diversity with depth or distance from the bow.

Table 7. Species abundance and diversity for ex-INDEPENDENCE taxonomy samples.

Sample Station	Total # Individuals	Total # Unique Species	Diversity Index
1-P-S	209	22	5.6
1-P-M	412	25	4.9
1-P-D	1,152	40	5.5
1-S-S	467	23	3.9
1-S-M	820	36	3.6
1-S-D	87	21	6.7
2-P-S	399	23	9.2
2-P-M	535	29	5.6
2-P-D	630	22	3.7
2-S-S	344	24	5.7
2-S-M	339	23	9.0
2-S-D	409	30	8.0
3-P-S	109	29	13.3
3-P-M	212	23	3.9
3-P-D	201	23	8.4
3-S-S	105	16	8.3
3-S-M	63	16	4.3
3-S-D	109	19	5.8
4-P-S	2,978	35	2.1
4-P-M	146	24	2.8
4-P-D	396	23	4.9
5-P-S	1,396	25	5.1
5-P-M	831	25	4.0
5-P-D	862	27	4.9
5-S-S	489	20	4.7
5-S-M	330	20	6.0
5-S-D	905	28	2.3
STERN SITE 1	544	29	2.7
STERN SITE 2	733	33	6.7
STERN SITE 3	764	33	3.3
STERN SITE 4	350	35	5.2
STERN SITE 5	234	24	5.1
STERN SITE 6	731	36	5.3
STERN SITE 8	151	29	9.5
STERN SITE 9	630	24	2.3
STERN SITE 10	20	10	6.7
TOTAL	19,092	92	

Note: P = port; S = starboard; S = surface strata; M = mid strata; D = deep strata

Table 8. Sum of phylum representatives.

Sample	Annelida	Arthropoda	Chordata	Cnidaria	Echino-dermata	Ectoprocta	Mollusca	Nemertea	Phoronida	Platy-helminthes	Porifera	Grand Total
1-S-D	44	15	8	6		3	10		1			87
1-S-M	277	476	10	19	1	3	28	3	2	1		820
1-S-S	162	263		15		3	22	2				467
1-P-D	304	493	74	84	1	3	175	13	3	2		1,152
1-P-M	110	265	10	4		4	18			1		412
1-P-S	60	110		20		3	14	2				209
2-S-D	115	125	31	42		1	94		1			409
2-S-M	176	45	23	67	3	2	23					339
2-S-S	156	102	2	59	1	3	21					344
2-P-D	142	330	20	90		2	44	2				630
2-P-M	186	202	19	58		3	22	2	43			535
2-P-S	123	231	4	27		3	11					399
3-P-D	119	20	2	2	1		52	4			1	201
3-P-M	45	124	9	7		3	24					212
3-P-S	32	26	4	9		4	33		1			109
3-S-D	17	63	6	11		3	8			1		109
3-S-M	16	31	6	4		2	4					63
3-S-S	60	19		11	1	2	12					105
4-P-D	221	122	8	18		2	18	7				396
4-P-M	30	92	4	14		3	2			1		146

Table 8. Sum of phylum representatives. (Continued)

Sample	Annelida	Arthropoda	Chordata	Cnidaria	Echino-dermata	Ectoprocta	Mollusca	Nemertea	Phoronida	Platy-helminthes	Porifera	Grand Total
4-P-S	347	2,138	20	364		5	89	2		13		2,978
5-P-D	260	539	8	42	1	1	8			3		862
5-P-M	276	414	9	107		2	22	1				831
5-P-S	315	885	17	129		3	45			2		1,396
5-S-D	199	594	24	46		1	37	2		1	1	905
5-S-M	49	135	21	113		4	7		1			330
5-S-S	253	182		17		1	36					489
Stern Site 1	98	376	17	17		1	30	3		1	1	544
Stern Site 2	285	283	4	56		2	93	10				733
Stern Site 3	285	435	1	18	1	2	15	3	1	2	1	764
Stern Site 4	64	194	8	13	3	1	65	2				350
Stern Site 5	102	102	4	16		1	6	2		1		234
Stern Site 6	182	434	13	26	2	2	68			4		731
Stern Site 8	40	40	12	13	1	3	42					151
Stern Site 9	66	442	12	48	1	1	59		1			630
Stern Site 10	12	5		1		2						20
Grand Total	5,228	10,352	410	1,593	17	84	1,257	60	54	33	4	19,092
% of Total	27.38	54.22	2.15	8.34	0.09	0.44	6.58	0.31	0.28	0.17	0.02	

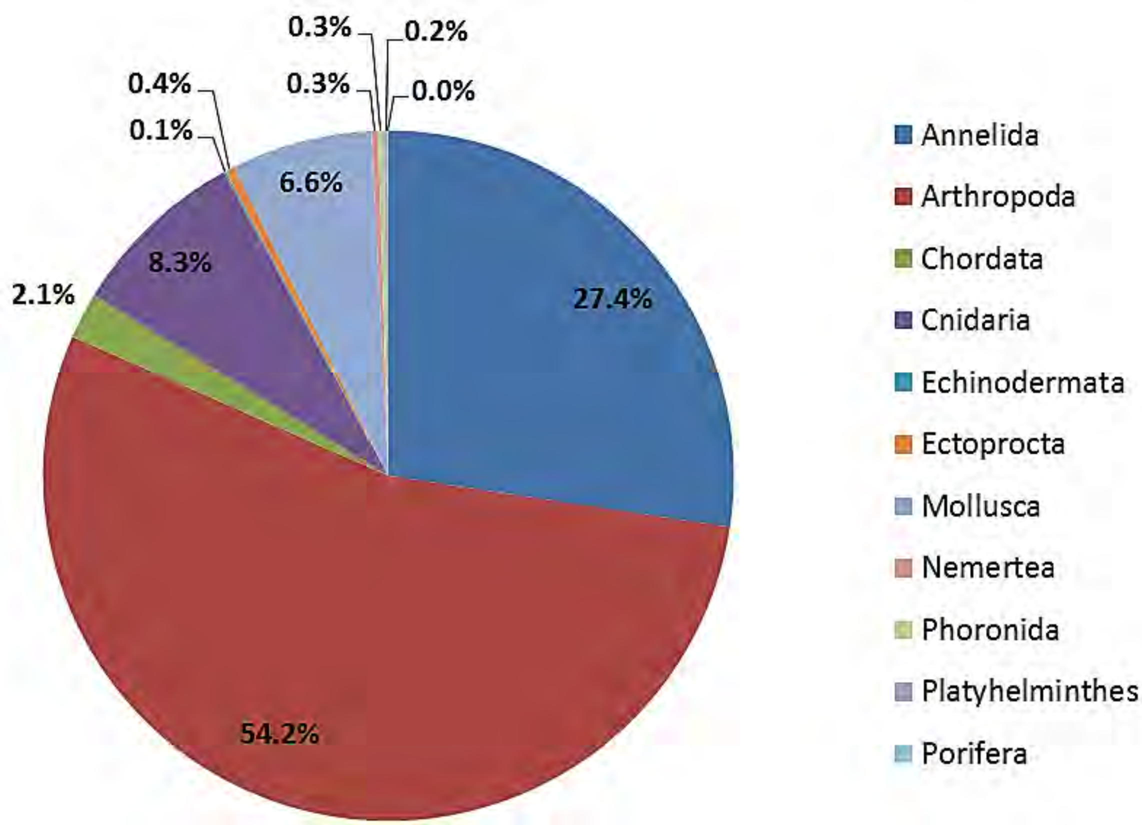


Figure 10. Observed phyla from the ex-INDEPENDENCE.

3.1.1.2 Non-Native and Invasive Species

Table 9 summarizes biofouling organisms observed on the hull of the ex-INDEPENDENCE by taxonomic group and ecological status. In this summary, species level assessments for potential presence of invasive species are evaluated for higher level groups. For example, taxa in the genus *Caprella* were identified in samples but not keyed to species. This genus contains nine species including both native and invasive species (e.g., *Caprella mutica*) in Puget Sound. Since it is possible that *C. mutica* is represented on the hull of the ex-INDEPENDENCE in the *Caprella* sp. sample, this is captured and reported in Table 9, with the status listed as N + I (native and invasive species represented in the genus).

Of the biofouling organisms observed on the hull, seven organisms which were identified to species (9%) were considered invasive in Puget Sound and elsewhere, including two amphipods (*Monocorophium acherusicum* and *M. insidiosum*), one gastropod (*Crepidula fornicata*), three bryozoans (*Amathia gracilis*, *Schizoporella unicornis*, and *Watersipora subtorquata*), and one tunicate (*Ciona intestinalis*). Additionally, eight taxa (10%), which were only identified to the genus level, contain native and at least one non-native/invasive species including three amphipod genera (*Caprella* sp., *Jassa* sp., and *Monocorophium* sp.), one decapod (*Hemigrapsus* sp.), one isopod (*Ianiropsos* sp.), one bivalve (*Mytilus* sp.), and one bryozoan (*Alcyonidium* sp.). Also present in samples was the pea crab genus *Pinnixia*, which includes several species native to Puget Sound, one of which (*Pinnixia occidentalis*) has been documented as invasive elsewhere. One cryptogenic (origin unknown) species was also observed, the bivalve *Hiatella arctica*, and may be either native or non-native/invasive. The ecological status of 12 families (primarily polychaetes) was not able to be determined as the level of taxonomy provided from the laboratory was too broad to assess whether an individual was native or invasive. Other taxa were considered to be native to Puget Sound (and not invasive elsewhere). A status summary is presented in Figure 11.**Error! Reference source not found.**

Only two invasive species were known at the port of Bremerton that are high risk for establishing in other regions. These are the red sheath tunicate (*Botrylloides violaceus*) and the transparent sea squirt (*Ciona savigny*i). Related taxa that are also likely high risk species were observed on the hull of the ex-INDEPENDENCE. One high risk invasive species, the sea vase tunicate (*Ciona intestinalis)*, was identified in the samples. Limited abundance of another high risk invasive species (*Botrylloides* sp.) was observed in-situ and in video during the pre-hull cleaning surveys, but was only identified to the family level within the samples (Figure 12).

All potential invasive species were further evaluated for their current status and invasion risk in the Gulf of Mexico (see Table 19 in Section 4.3).

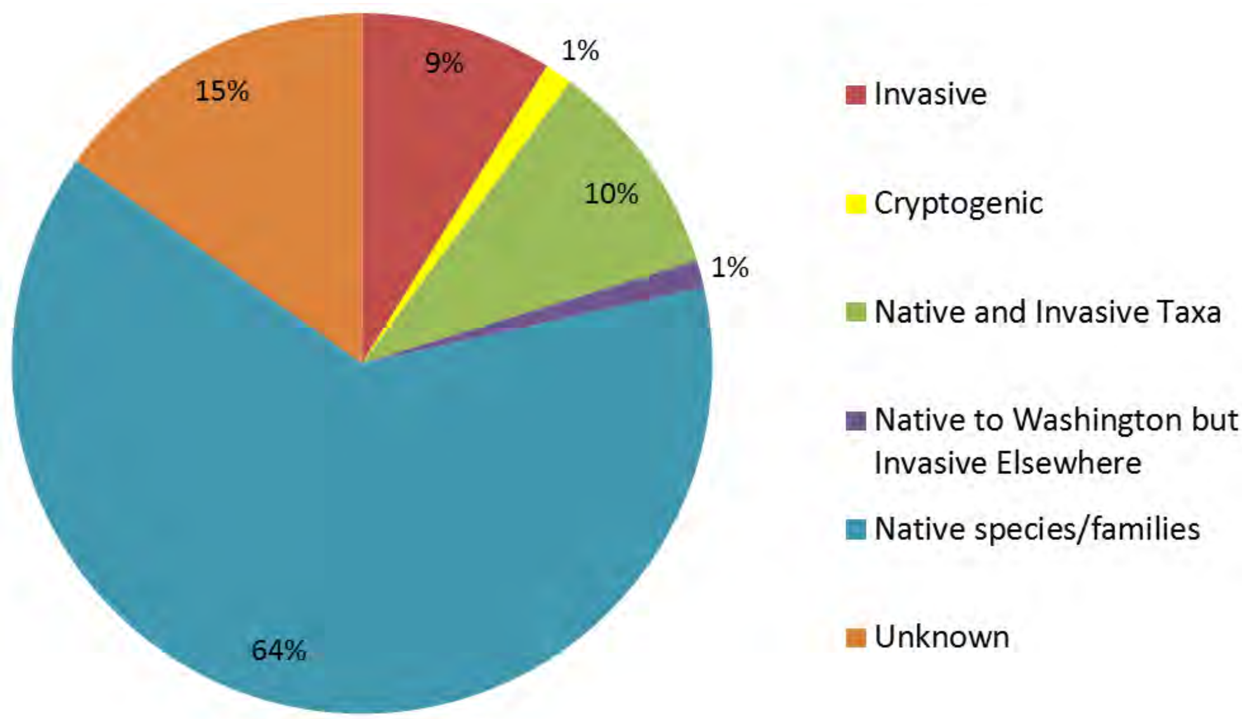


Figure 11. Breakdown of ecological status for the identified taxa. The “Native and Invasive Taxa” category includes genus or families that contain both native and invasive taxa. “Unknown” indicates that the level of taxonomy provided by the laboratory was too broad to assess whether an individual was native or invasive.

Table 9. Biofouling organisms observed on the hull of the ex-INDEPENDENCE and their ecological status.

Common Name	Scientific Name	Status	Notes
CRUSTACEANS			
Amphipods			
	Aoroides sp.	N	This genus is native to Puget Sound.
Caprellid amphipods	*Caprella* sp.	N+I	Very large genus of skeleton shrimp. Contains both native and invasive (*Caprella mutica*) species in Puget Sound.
Tube-dwelling amphipods	*Jassa* sp.	N+I	Amphipod genus which contains both known native and known invasive (*Jassa marmorata*) species in Puget Sound.
	Monocorophium acherusicum	I	
	Monocorophium insidiosum	I	
Tube-building amphipods	*Monocorophium* sp.	N+I	Two invasive (*M.acherusicum* and *M.insidiosum*) and at least one native (*M. carlottensis*) species
	Pleustidae	N	All known species are native
Decapods			
Shore crabs	*Hemigrapsus* sp.	N+I	Several possible species, some of which are invasive (e.g., *H. nudus, H. oregonensis*)
Sand crabs	*Heptacarpus* sp.	N	Coastal sand shrimp genus, likely native
Blackclaw crestleg crab	*Lophopanopeus bellus*	N	
Pea crabs	*Pinnixa* sp.	N*	Genus of pea crabs native to the Pacific Northwest, but with some species invasive elsewhere
	Epialtidae	N	Large family of globally distributed marine crabs (including kelp and spider crabs), few invasive species in this family
Porcelain crabs	Porcellanidae	N	No known invasive species present in this family
Other Crustaceans			
Isopods	*Ianiropsis sp.*	N+I	Four native and one invasive (*I. serricaudi*) species in Puget Sound
	Munnidae	N	Three species present, all native
Acorn barnacle	*Balanus crenatus*	N	Broadly distributed
Leptostracans	*Nebalia* sp.	N	Two species (one complex), both native
Mysids	Mysidae	N	Over a dozen native species, no noted invasive species

Note: blank status cells indicate that the level of taxonomy provided was too broad to assess whether an individual was native or invasive

I Invasive | N+I Includes Native & Invasive | N* Native to Pacific Northwest but Invasive Elsewhere | N Native

Table 9. Biofouling organisms observed on the hull of the ex-INDEPENDENCE and their ecological status.(Continued)

Common Name	Scientific Name	Status	Notes
MOLLUSCS			
Bivalves			
Spear scallop	*Chlamys hastata*	N	
Wrinkled rock-clam	*Entodesma navicular*	N	
Arctic hiatella	*Hiatella arctica*	C	Broadly distributed, cryptogenic
Sub-orbicular Kelly clam	*Kellia suborbicularis*	N	
Northern horse mussel	*Modiolus modiolus*	N	
	Mytilus sp.	N+I	Includes native (M. *edulis*) and invasive (*M. galloprovincialis*) mussels
Olympia oyster	*Ostrea lurida*	N	Taxonomic classification debated, but definitely native
Alaska jingle	*Pododesmus macrochisma*	N	
Gastropods			
Carinate dove shell	*Alia carinata*	N	
	Alia gouldi	N	
Dove snails	*Alia* sp.	N	
	Alvania compacta	N	
Common slipper shell	*Crepidula fornicata*	I	Invasive on Pacific coast
Pacific half-slipper snail	*Crepipatella lingulata*	N	
Shield limpet	*Lottia pelta*	N	
Minute slipper snails	*Odostomia* sp.	N	Very broad genus, several native and no known invasive members.
Other Molluscs			
Chitons	*Mopalia* sp.	N	
Other Arthropods			
Marine mites	Halacaridae		Very large family of marine mite species (1500+)
CNIDARIANS			
Anemones			
Frilled anemone	*Metridium senile*	N	

Note: blank status cells indicate that the level of taxonomy provided was too broad to assess whether an individual was native or invasive

I Invasive; C Cryptogenic; N+I Includes Native & Invasive; N* Native to Pacific Northwest but Invasive Elsewhere; N Native

Table 9. Biofouling organisms observed on the hull of the ex-INDEPENDENCE and their ecological status. (Continued)

Common Name	Scientific Name	Status	Notes
Hydroids			
	Obelia sp.	N	
	Orthopyxis sp.	N	Many species, difficult to identify, but likely native
BRYOZOANS			
Jelly bryozoans	*Alcyonidium* sp.	N+I	May be invasive, most known species not native
	Amathia gracilis	I	Possible cryptic taxonomy
	Celleporella hyalina	N	Probably native, or invasive from a very long time ago
Single horn bryozoan	*Schizoporella unicornis*	I	Native to Europe and invasive on both U.S. coasts
Red-rust bryozoan	*Watersipora subtorquata*	I	Highly invasive, local to British Isles
ECHINODERMS			
Orange sea cucumber	*Cucumaria miniata*	N	
Peppered sea cucumber	*Cucumaria piperata*	N	
White sea cucumber	*Eupentacta pseudoquinquesemita*	N	
	Pentamera lissoplaca	N	
ANNELID WORMS			
Polychaetes			
Vancouver feather duster	Sabellidae (*E. vancouveri*)	N	May not be exclusively this species
Threadworms	Capitellidae		Broadly distributed
	Chrysopetalidae		Globally distributed
	Cirratulidae		Family is globally distributed, poorly classified deposit feeders, probably native
	Dorvilleidae	N	Globally distributed, no broadly recognized invasive species
	Flabelligeridae		Marine Terebelid polychaetes. Large family (300+ species)
Bloodworms	Glyceridae		Globally distributed
Bristle Worms	Hesionidae	N	Many species native to the area. No well documented invasive species.

Note: blank status cells indicate that the level of taxonomy provided was too broad to assess whether an individual was native or invasive

I	Invasive	N+I	Includes Native & Invasive	N	Native

Table 9. Biofouling organisms observed on the hull of the ex-INDEPENDENCE and their ecological status. (Continued)

Common Name	Scientific Name	Status	Notes
Ragworms/clamworms	Nereididae		
	Orbiniidae		400+ species, dozens native to the Pacific Northwest region
Paddleworms	Phyllodocidae		800+ species
Scale Worms	Polynoidae	N	No known invasive species present in this family
Fanworms	Serpulidae	N	10 or so native species, no known invasive species
	Spionidae		Very broad family
	Syllidae		Very broad family
	Terebellidae		Very broad family
OTHER (NON-ANNELID) WORMS			
	Eurylepta aurantiaca	N	
	Notoplana longastyletta	N	
	Zygonemertes virescens	N	
Horseshoe worms	*Phoronis* sp.	N	Three species present, all native
Ribbonworms	*Tetrastemma* sp.	N	Very broad genus, but several native and no known invasive members
Orange ribbonworm	*Tubulanus* polymorphus	N	Broadly distributed
Flatworms	Euryleptidae		Relatively small family of flatworms. Several species native to study area.
SPONGES			
Purple scallop sponge	*Mycale adhaerens*	N	
TUNICATES			
Spiny-headed tunicate	*Boltenia villosa*	N	
Disc-top tunicate	*Chelyosoma productum*	N	
Sea vase tunicate	*Ciona intestinalis*	I	Globally distributed, highly invasive
Shiny red sea squirt	*Cnemidocarpa finmarkiensis*	N	Widely distributed
	Dendrodoa abbotti	N	

Note: blank status cells indicate that the level of taxonomy provided was too broad to assess whether an individual was native or invasive

I Invasive N Native

Table 9. Biofouling Organisms Observed on the Hull of the ex-INDEPENDENCE and their Ecological Status. (Continued)

Common Name	Scientific Name	Status	Notes
Mushroom ascidian	*Distaplia occidentalis*	N	
Sea peach	*Halocynthia aurantium*	N	
Peanut sea squirt	*Styela gibbsii*	N	
	Styelidae	N+I	Contains a few native species, but also major global invasive species (e.g. *Styela clava* and *Botrylloides violaceus*)

Note: blank status cells indicate that the level of taxonomy provided was too broad to assess whether an individual was native or invasive

N+I Includes Native & Invasive | N | Native

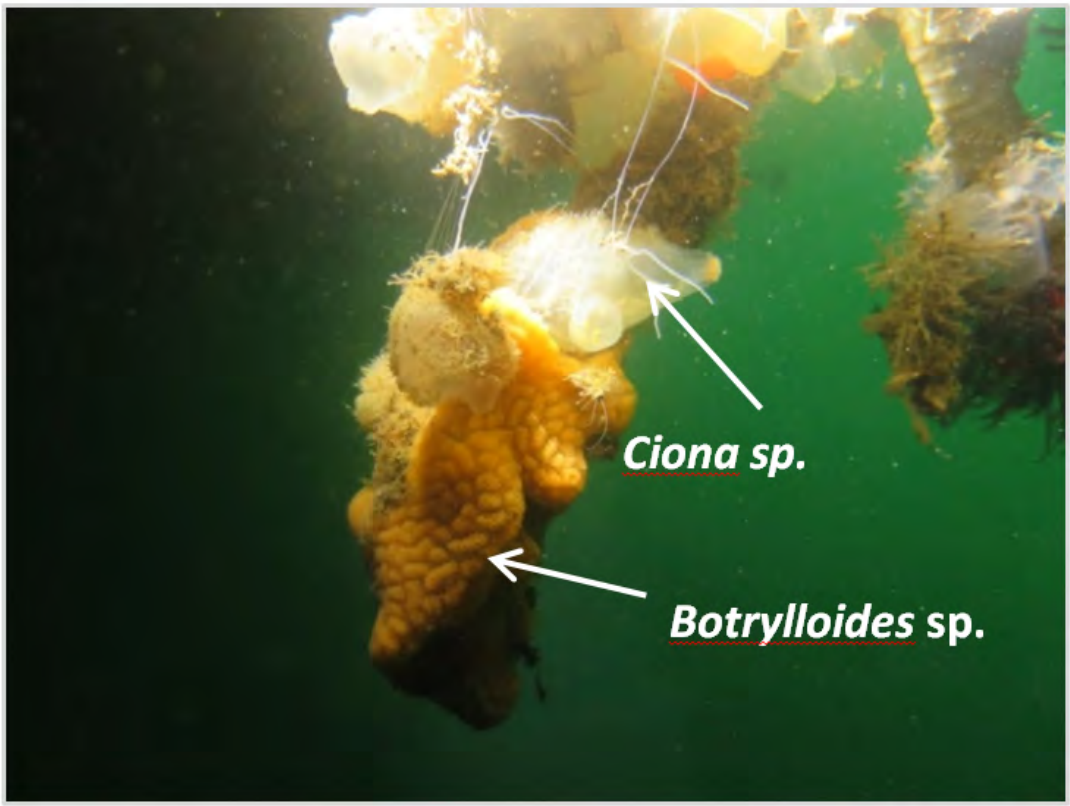


Figure 12. Potential high risk invasive species *Botrylloides* sp. and *Ciona* sp. observed on the hull of the ex-INDEPENDENCE during pre-hull cleaning inspections.

3.1.2 Biomass

Total wet weight biomass sampled in the quadrats ranged from 5.6 g at Stern Site 8 (aft strut) to 1,235.3 g at station 2-S-S, with an average of 472.2 g and a geometric mean (geomean) of 289.9 g (Table 10). On average, dry weight was approximately 25% of the material, which consisted of approximately 19% calcareous (inorganic shells) material and 6% organic matter (Table 10). The greatest dry weight biomass was also measured from transect 2 at station 2-P-S (328.9 g). The qualitative average degree of fouling prior to hull cleaning is depicted in the representative image in Figure 13.

The estimated total wet weight of material removed from the hull (see Equation (3)) was approximately 119,071 pounds (lbs; 54,000 kg) based on geomean calculations (Table 11). The estimate of total wet weight biomass removed by cleaning ranged from 1,043–230,177 kg, with an average of 88,099 kg and a geomean of 54,000 kg. Based on the geomean, this corresponds to a dry weight of approximately 3,146 kg (7,000 lbs) of organic material (i.e., soft material of marine organisms) and about 9,945 kg (22,000 lbs) of inorganic material (i.e. shell material) that was removed from the hull of the ex-INDEPENDENCE during cleaning operations (Table 11).

Biomass estimates were highly variable both within and between transects, with areas of a few large organisms (i.e., tubeworms) interspersed with areas of densely concentrated smaller organisms (i.e., amphipods) (Figure 14). The average biomass between transects was also highly variable, ranging from 5 to 16.7 kg/m^2. No discernable pattern in biomass was evident with depth, ship side, or distance from the bow, however, the greatest amount of biomass was encountered towards the mid to aft areas of the ship. Pattern analysis was somewhat limited because transect 4, near the stern of the ship, was not completely sampled due to logistical constraints.

Table 10. Weight of wet, dry, and calcareous biomass material sampled from the hull of the ex-INDEPENDENCE and the percent of dry, calcareous, and organic matter of each sample compared to total wet weight.

Sample ID	Sampled Weight			Sampled %		
	Wet b_W (g)	Dry b_D (g)	Calcareous b_{Cal} (g)	Dry %DW	Calcareous %Cal	Organic Matter %OM
1-P-D	97.9	31.1	26.7	31.8%	27.3%	4.5%
1-P-M	1,033.4	218.0	152.2	21.1%	14.7%	6.4%
1-S-D	105.5	32.3	27.2	30.6%	25.8%	4.8%
1-S-M	123.3	27.5	25.2	22.3%	20.4%	1.9%
1-S-S	211.3	75.8	67.8	35.9%	32.1%	3.8%
2-P-D	202.9	39.6	25.7	19.5%	12.7%	6.9%
2-P-M	185.4	49.0	43.3	26.4%	23.4%	3.1%
2-P-S	1,078.9	328.9	254.1	30.5%	23.6%	6.9%
2-S-D	600.8	115.9	87.9	19.3%	14.6%	4.7%
2-S-M	379.0	95.7	77.4	25.3%	20.4%	4.8%

Table 10. Weight of wet, dry, and calcareous biomass material sampled from the hull of the ex-INDEPENDENCE and the percent of dry, calcareous, and organic matter of each sample compared to total wet weight. (Continued)

Sample ID	Sampled Weight			Sampled %		
	Wet b_W *(g)*	*Dry* b_D *(g)*	*Calcareous* b_{Cal} *(g)*	*Dry* *%DW*	*Calcareous* *%Cal*	*Organic Matter* *%OM*
2-S-S	1,235.3	296.2	228.7	24.0%	18.5%	5.5%
3-P-D	91.0	27.9	23.4	30.7%	25.7%	4.9%
3-P-M	1,004.3	265.3	147.8	26.4%	14.7%	11.7%
3-P-S	568.6	156.8	123.4	27.6%	21.7%	5.9%
3-S-D	72.4	7.9	6.9	10.9%	9.5%	1.4%
3-S-M	471.3	113.0	87.0	24.0%	18.5%	5.5%
3-S-S	242.7	80.0	67.1	33.0%	27.6%	5.3%
4-P-D	1,044.1	221.5	133.8	21.2%	12.8%	8.4%
5-S-M	269.2	48.8	27.3	18.1%	10.1%	8.0%
5-S-S	381.1	109.9	87.8	28.8%	23.0%	5.8%
Stern Site 1	875.9	251.6	191.8	28.7%	21.9%	6.8%
Stern Site 2	594.6	139.6	87.0	23.5%	14.6%	8.8%
Stern Site 7	5.6	0.9	0.7	16.1%	12.5%	3.6%
n	23	23	23	23	23	23
AVERAGE	472.8	118.8	87.0	25%	19%	6%
STDEV	389.2	98.9	70.5	6%	6%	2%
CV	82.3%	83.2%	81.1%	24%	32%	41%
MIN	5.6	0.9	0.7	11%	10%	1%
MAX	1,235.3	328.9	254.1	36%	32%	12%
GEOMEAN	289.9	70.3	53.4	24%	18%	5%

Figure 13. Characteristic density of biofouling in some areas on the hull of the ex-INDEPENDENCE observed during pre-hull cleaning inspections. The high-risk species *Ciona sp.* is noted.

Table 11. Estimates of total biomass removed during hull cleaning for wet weight (B_W), dry weight (B_D), calcareous weight (B_{Cal}), and weight of organic matter (B_{OM}) over the entire wetted hull surface[1]

	Wet B_W (kg)	Dry B_D (kg)	Calcareous B_{Cal} (kg)	Organic Matter B_{OM} (kg)
Average	88,099	22,142.8	16,204.5	5,938.3
Min	1,043	167.7	130.4	37.3
Max	230,177	61,284.8	47,347.1	13,937.7
Geomean	54,010	13,091.4	9,945.2	3,146.3
	B_W (lbs)	B_D (lbs)	B_{Cal} (lbs)	B_{OM} (lbs)
Geomean	119,071	28,862	21,925	6,936

[1]Wetted Hull Surface = 11,468 m^2 (123,445 ft^2)

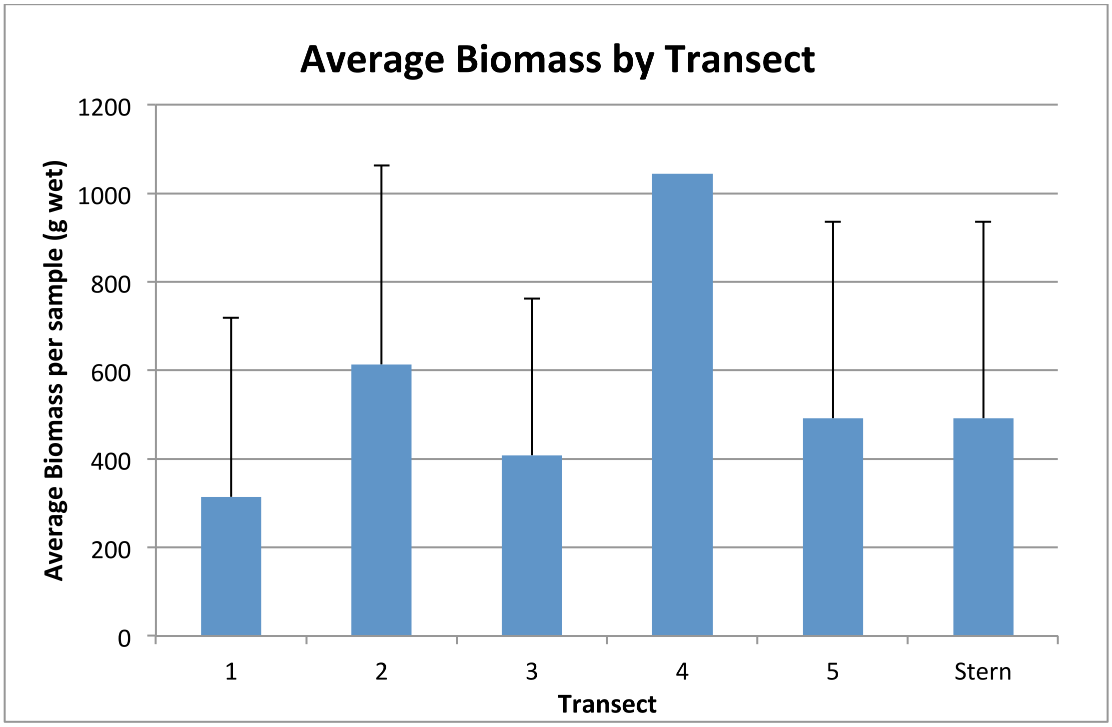


Figure 14. Average (error bars represent SD) wet weight biomass per sample by transect and stern sampling area.

3.2 POST-HULL CLEANING BIOLOGICAL HULL SURVEY

Qualitative and quantitative data were acquired to assess the presence/absence of invasive biofouling species and to evaluate their occurrence on the hull of the ex-INDEPENDENCE. The qualitative data included visual descriptions of the bilge keels and aft section of the vessel including niche areas. The quantitative data were collected to estimate biofouling cover on the hull, port and starboard bilge keel, and bottom of the vessel.

3.2.1 Qualitative Observations

At the aft section of the vessel the shafts, stern tubes (two inboard and two outboard), struts, and the bilge keels (starboard and port) were qualitatively examined. Because of the extent of their area, the bilge keels are described separately below. Observations from the opportunistic seafloor survey underneath the hull of the ex-INDEPENDENCE are summarized in Section 3.2.1.3.

3.2.1.1 Niche Areas

Table 12 summarizes the observations of the biofouling community and hull conditions at each of these hull features and provides representative underwater images. Niche areas were characterized by primarily paint and some areas of exposed hull (bare metal). Qualitative inspection of niche areas revealed little to no fouling coverage, with isolated tubeworms, anemones, and hydroids surviving. It is difficult to assess the percent cover in these niche areas; however, only 6 of the roughly 30 surveyed niche areas (20%) had any surviving biomass, and even these areas had only a few patchy remnants or new colonization of living organisms. One live feather duster tubeworm (*Eudistylia vancouveri*) was present in a fairwater port hole and on the port stern tube. Small numbers of live anemones (*Metridium* sp.) were observed on the port stern tube, aft end of the port bilge keel, and starboard bilge keel. Hydroids were detected on the end of the #4 shaft; these organisms could have colonized this area between the hull cleaning and the survey, or may have been established and missed during the cleaning (Figure 15). On this shaft, a layer of newly established biofilm and sedimentation had accumulated post hull cleaning (Figure 15). Lastly, an unidentified crab was observed inside the fairwater of the #1 port shaft, while an unidentified fish was present just outside of the #1 port stern tube (see Table 12).

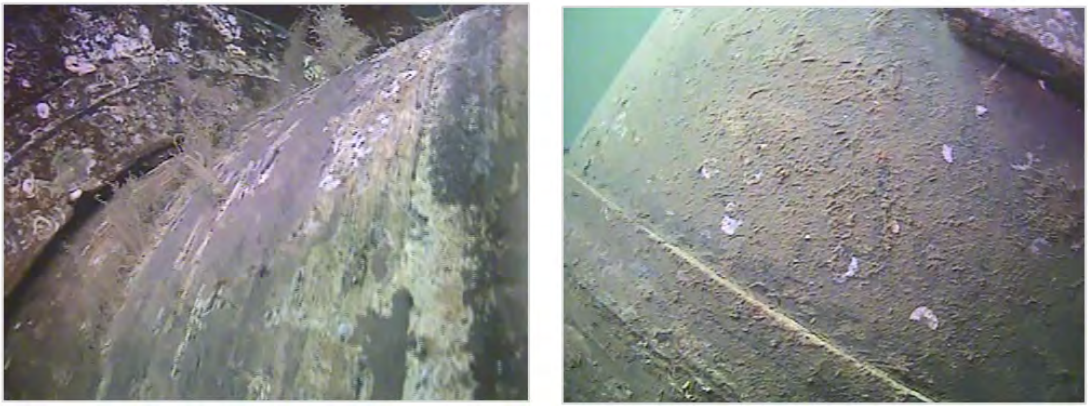


Figure 15. Possible hydroids (left) and a biofilm-sediment layer (right) on the end of the #4 shaft.

Table 12. Fouling summary on hull features of the ex-INDEPENDENCE.

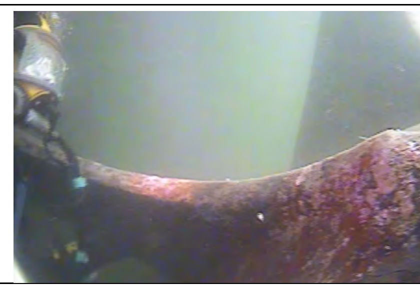
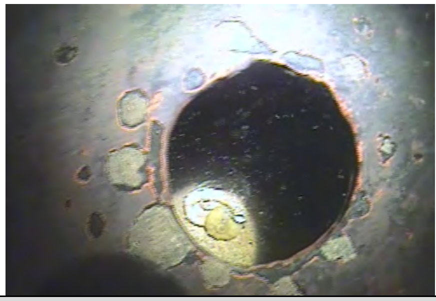
Vessel Feature	Description	Representative Image
Main Strut (Port #1)	No living fouling; paint and bare hull.	
Fairwater (Port #1 Main Strut)	No living fouling; paint.	
Shaft (Port #1)	No living fouling; tubeworm remnants; paint scrape marks.	

Table 12. Fouling summary on hull features of the ex-INDEPENDENCE. (Continued)

Vessel Feature	Description	Representative Image
Inboard Shaft (Port #2)	No living fouling; paint.	
Stern Tube (Port #2)	Live tubeworm; live anemone (*Metridium sp.*); dead barnacle.	
Stern Tube (Starboard #4)	No living fouling; paint and bare hull.	No photo available
Fairwater (Starboard Main Strut #4)	No living fouling; paint and bare hull.	No photo available
Shaft (#4)	No living fouling; tubeworm remnants.	

Table 12. Fouling summary on hull features of the ex-INDEPENDENCE. (Continued)

Vessel Feature	Description	Representative Image
Fairwater Hole (#1 Port Shaft)	Live tubeworm	
Fairwater (# Port Shaft)	No living fouling; tubeworm remnants; dead barnacle.	No photo available
Fairwater (#1 Port Shaft)	Live crab	
Stern Tube (Port #1)	Areas of no fouling, with a live tubeworm, mussel, and fish; dead tubeworm and barnacle.	

3.2.1.2 Bilge Keels

For the bilge keels, the divers began surveying at the aft ends at approximately frame 167 and moved forward relaying observations to topside scientists.

3.2.1.2.1 Port Bilge Keel

The aft end of the port bilge keel was characterized by predominately paint and bare hull with no living fouling (Figure 16). Three dead feather duster tubeworms (*Eudistylia vancouveri*) and one live anemone (*Metridium* sp.) were observed at the aft and mid sections of the bilge keel (Figure 17). On top of the bilge keel, divers noted one small live patch of green algae (Figure 18). Calcareous tubeworm remnants were also visible on painted surfaces of the bilge keel. Circular patches were present showing the underlying antifouling paint layer or bare hull through the top coat in some areas of the forward bilge keel and could be attributed to the removal of barnacles or tubeworms during the cleaning process (Figure 18 and Figure 19).

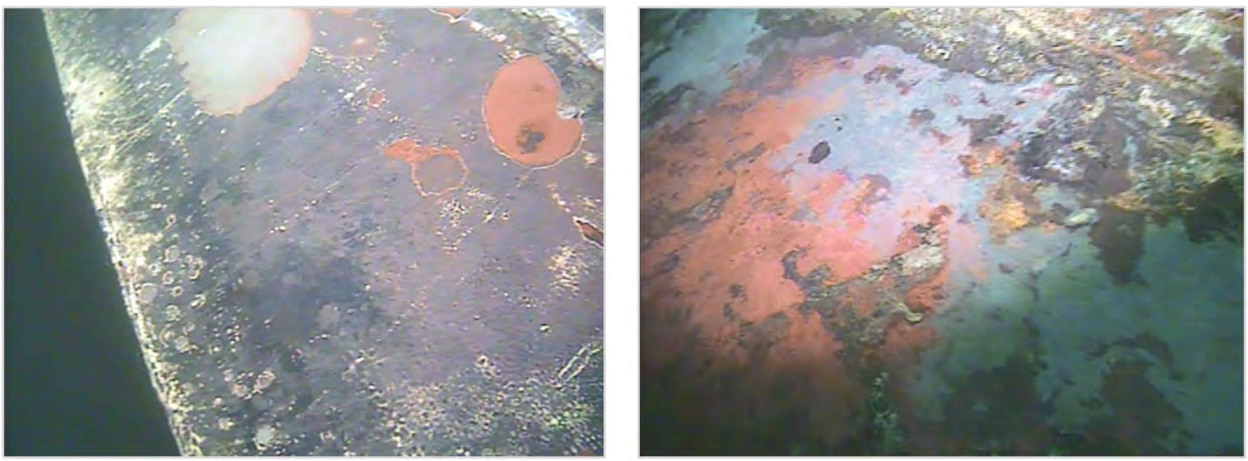


Figure 16. Top edge of port bilge keel showing paint (left) and top of port bilge keel showing paint and areas of bare hull (right).

Figure 17. Retracted anemone (*Metridium* sp.) on the port bilge keel.

Figure 18. Patch of green algae on top of the port bilge keel (left) and a remnant of a calcareous tubeworm (right). Circular patches from removed barnacles or tubeworms are also visible (right).

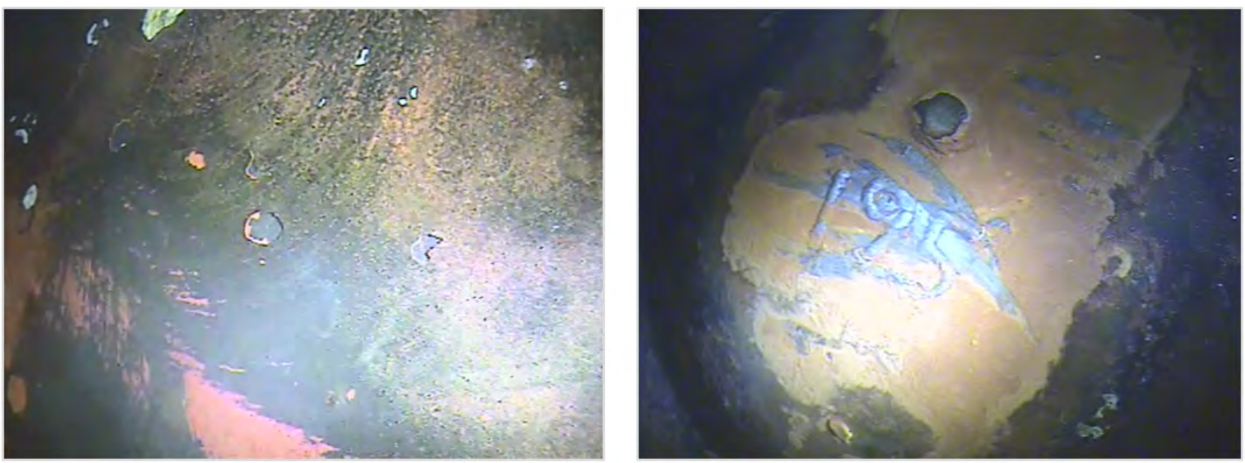


Figure 19. Brush marks on patches of underlying antifouling paint and bare hull on the port bilge keel (left) and bare hull exposed on a dome patch on the port bilge keel (right).

3.2.1.2.2 Starboard Bilge Keel

The starboard bilge keel had predominately antifouling paint, with only a few small patches of bare hull. Underlying antifouling paint layers were visible from the removal of marine growth (Figure 20). A small patch of live green algae was observed at the aft end of the bilge keel along with four live anemones (*Metridium* sp.) and some dead barnacles.

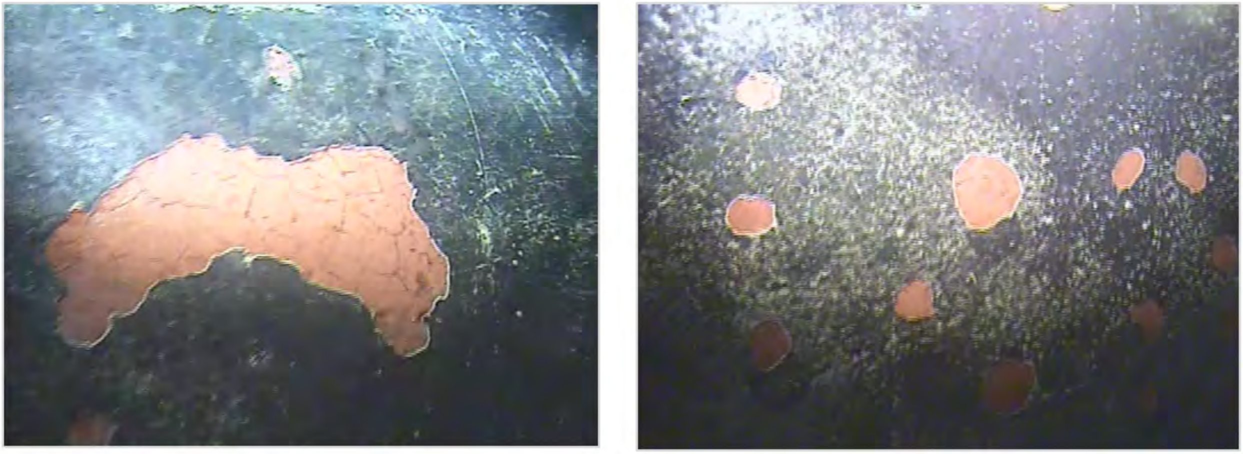


Figure 20. Antifouling paint layers (red and black) on the starboard bilge keel.

3.2.1.3 Seafloor Survey

For the post-hull cleaning opportunistic seafloor survey, divers started at frame number 201 and swam forward relaying observations to topside scientists. The opportunistic survey of the seafloor underneath the hull of the ex-INDEPENDENCE revealed an abundance of living and dead fouling organisms presumed to have been removed from the hull during the cleaning process (i.e., tubeworms and anemones), as well as a marine organisms foraging on the fouling debris (i.e., crabs, sea stars, and sea cucumbers) (Figure 21). A large amount of shell hash was also visible over the silt bottom. Outboard of the dive platform away from the hull, divers noted a primarily silt bottom, with a few scattered biofouling remnants (Figure 22).

The seafloor survey following the departure of the ex-INDEPENDENCE in March 2017 consisted of observations at sediment sampling stations including a transect on the bottom from about where transect 4 was located that contained some of the highest concentrations of fouling organisms. The video showed the organisms removed from the hull were still alive and were surviving on the bottom.

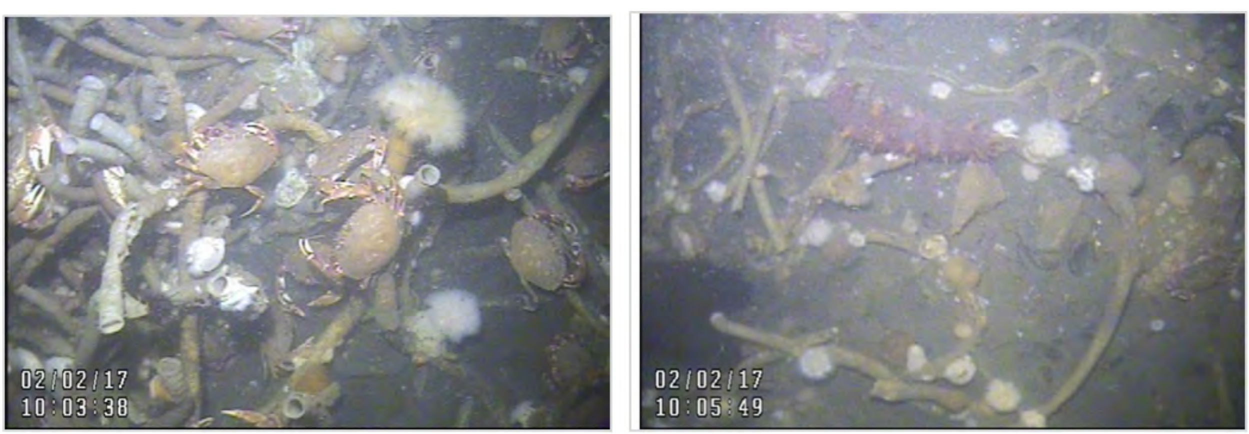


Figure 21. Seafloor under the hull of the ex-INDEPENDENCE post-hull cleaning, with crabs, tubeworms, anemones, sea cucumbers, shell hash, and silt visible.

Figure 22. Seafloor outboard of the ex-INDEPENDENCE showing primarily silt and tubeworm remnants.

3.2.2 Quantitative Assessment

The quantitative assessment involved an examination of the following hull features of the ex-INDEPENDENCE: the bottom, bilge keels (port and starboard), and hull (port and starboard) using the quadrats described above. Little to no biofouling organisms were observed during the quantitative biological hull survey (< 1%). The hull was predominately characterized by areas of antifouling paint and small, random patches of bare hull, with remnants of barnacles and calcareous tubeworms. Individual $0.25m^2$ quadrats ranged from 20 to 100% paint and 0 to 80% bare metal (exposed hull), however, on average, antifouling paint was largely intact. Individual transects ranged from 2 to 11% bare metal. The overall weighted average of all quantitatively surveyed quadrats was 95.5% paint, 4.5% bare hull, and 0.03% living organisms.

3.2.2.1 Longitudinal Belt Transects

Little to no fouling was detected on any of the five belt transects. The only biofouling present consisted of a small patch of green algae on transect belt 1.5 which may have been new growth since the hull cleaning. Percent cover of paint ranged from 55 to100%, with bare hull ranging from 0 to 45% (Figure 23 and Figure 24). Primer, the white coating observed in Figure 23, was also exposed at numerous stations. Remnants of barnacles (calcareous base plates), calcareous tubeworms, and unidentified mollusks were visible at a number of sample stations (Figure 25). Circular patches from the removal of barnacles and tubeworms could be seen at two sample stations (Figure 26).

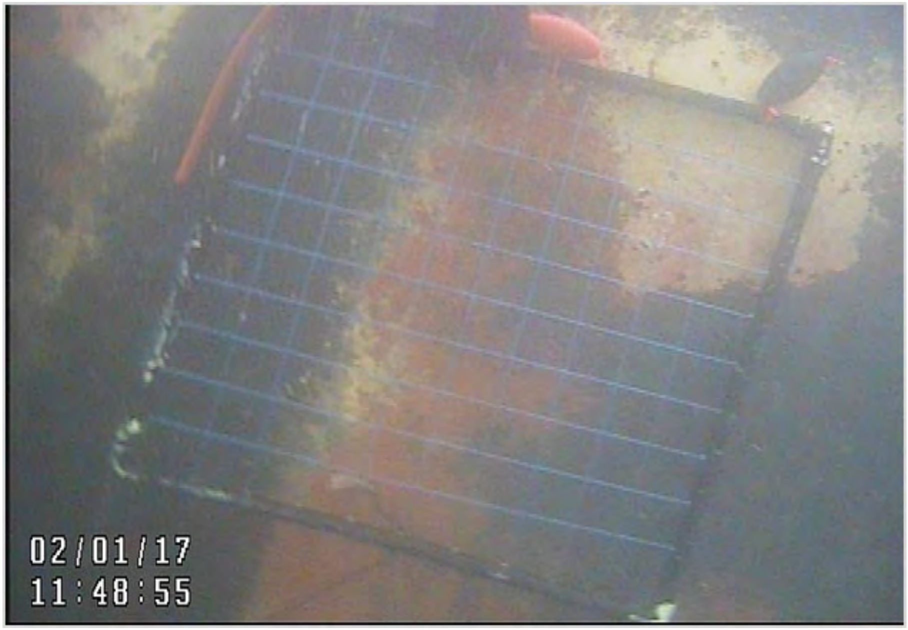


Figure 23. Transect 1.5 Quadrat 1-P-S (frame 48) showing various antifouling (black and red) and primer (white) paint layers (99% paint/1% bare hull).

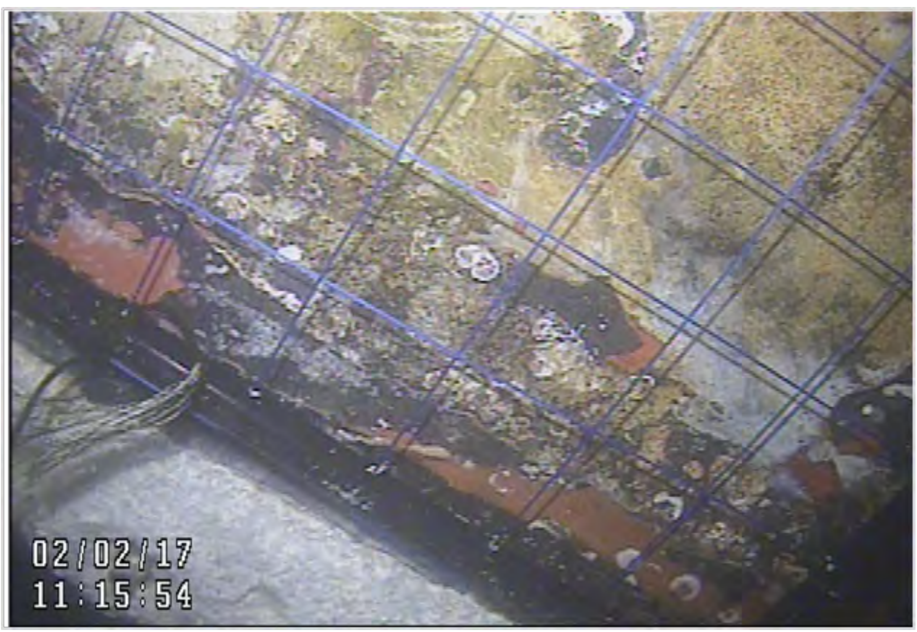


Figure 24. Transect 5.5 Quadrat 2-P-M (frame 230) showing areas of bare hull along with antifouling paint (55% paint/45% bare hull). Remnants of barnacles (calcareous base plates) are also visible.

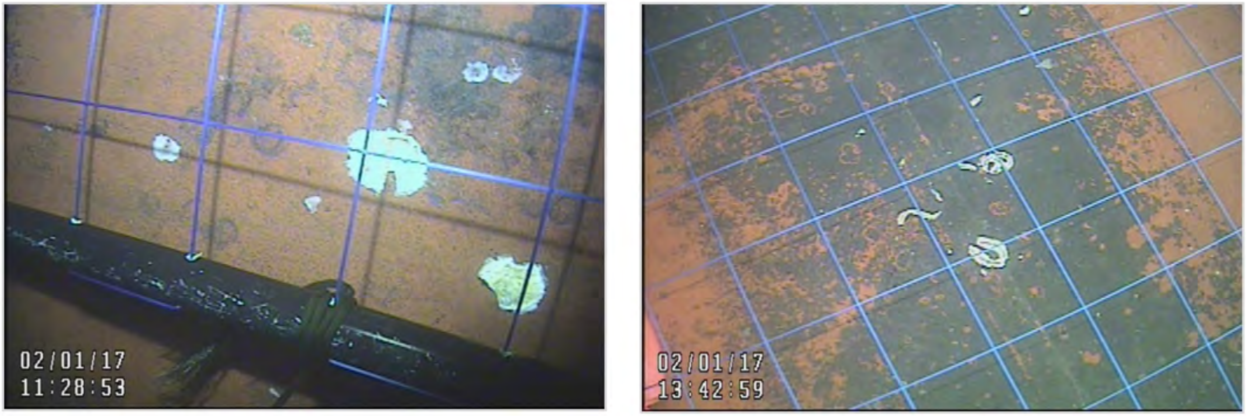


Figure 25. Remnant barnacles and mollusks at Transect 1 Quadrat 3-P-D at frame 23 (left) and remnant tubeworms at Transect 4 Quadrat 2-P-M at frame 170 (right). Both sample stations had 100% coverage of antifouling paint.

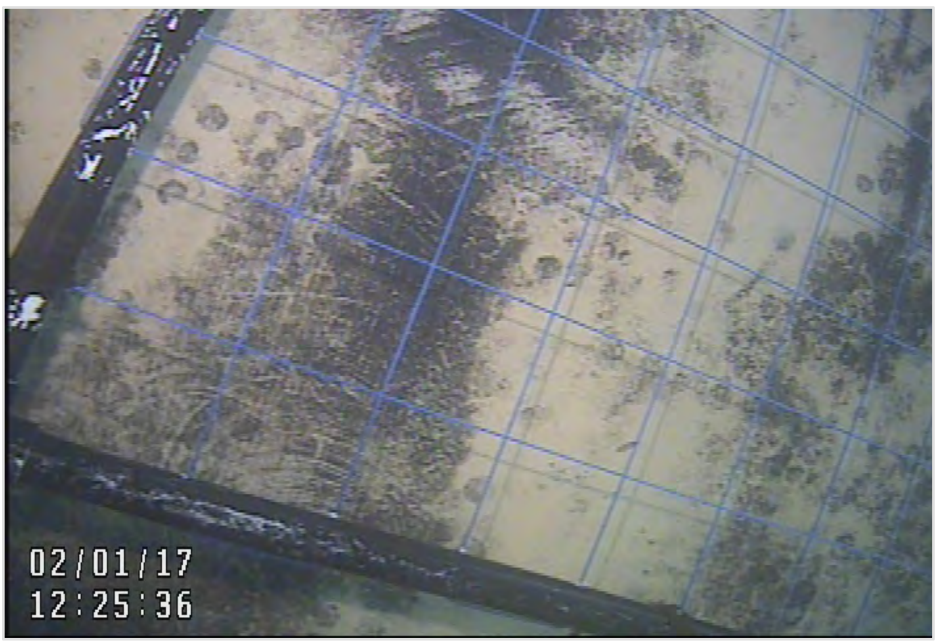


Figure 26. Transect 2.5 Quadrat 1-P-S (frame 98) showing brush marks from the hull cleaning and circular patches from the removal of barnacles and tubeworms (99% paint/1% bare hull).

3.2.2.2 Bilge Keel

No living biofouling was present on the portions of the port and starboard bilge keels surveyed during the quantitative assessment. Percent cover of antifouling paint ranged from 20 to 100%, with bare hull ranging from 0 to 80% (Figure 27). The port bilge keel had a higher percentage of bare hull than the starboard bilge keel, which was predominately antifouling paint. Circular patches showing underlying paint layers were visible through the paint in some areas of the forward bilge keel and are attributed to the removal of barnacles or tubeworms during the cleaning process (Figure 28). Remnants of calcareous tubeworms are shown in Figure 28.

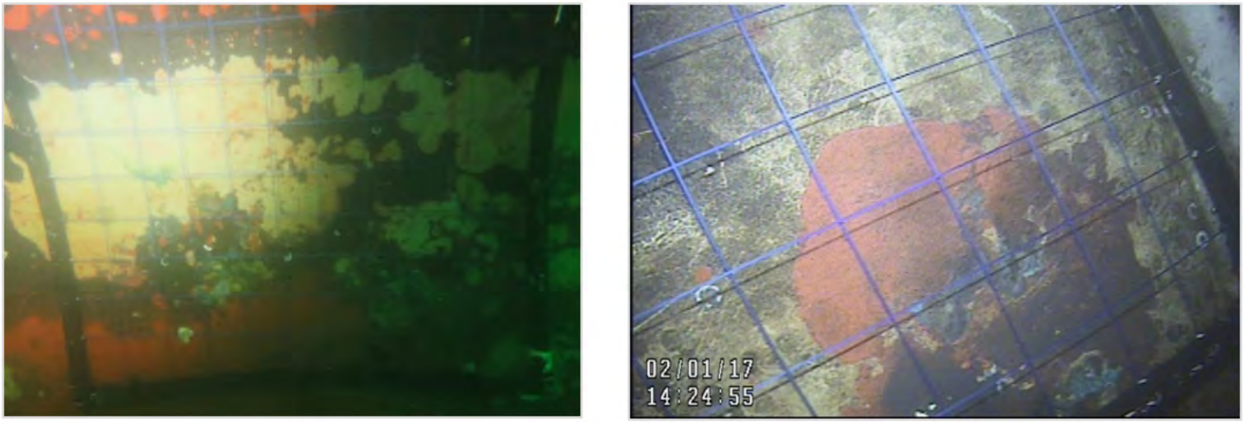


Figure 27. Quadrat 20 (frame 163) on the port bilge keel showing 45% paint/55% bare hull (left) and primarily paint with some bare hull and primer observed at Quadrat 14 (frame 147) on the starboard bilge keel (right).

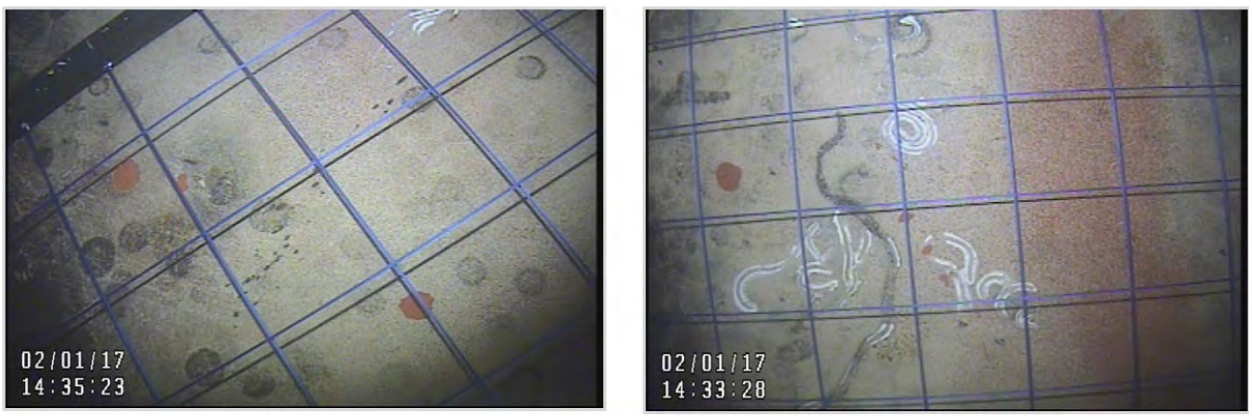


Figure 28. Circular patches from the removal of barnacles and tubeworms at Quadrat 3 (left) and Quadrat 4 (right) during the starboard bilge keel survey. Calcareous tubeworm remnants are also visible in Quadrat 4 (right).

3.2.2.3 Horizontal Hull Random Quadrats

No living biofouling was present on the portions of the hull surveyed during this quantitative assessment. Remnants of calcareous tubeworms and mollusks were evident at one quadrat (Figure 29). Percent cover of antifouling paint ranged from 20 to 100%, with bare hull ranging from 0 to 80% (Figure 30). Representative areas where bare hull was exposed are shown in Figure 31.

Figure 29. Calcareous tubeworm and mollusk remnants from Quadrat 5 on the starboard hull.

Figure 30. Antifouling paint (100%) and brush marks on the port hull at Quadrat 12 (left) and antifouling paint (20%) and bare hull (80%) at Quadrat 16 on the port hull (right).

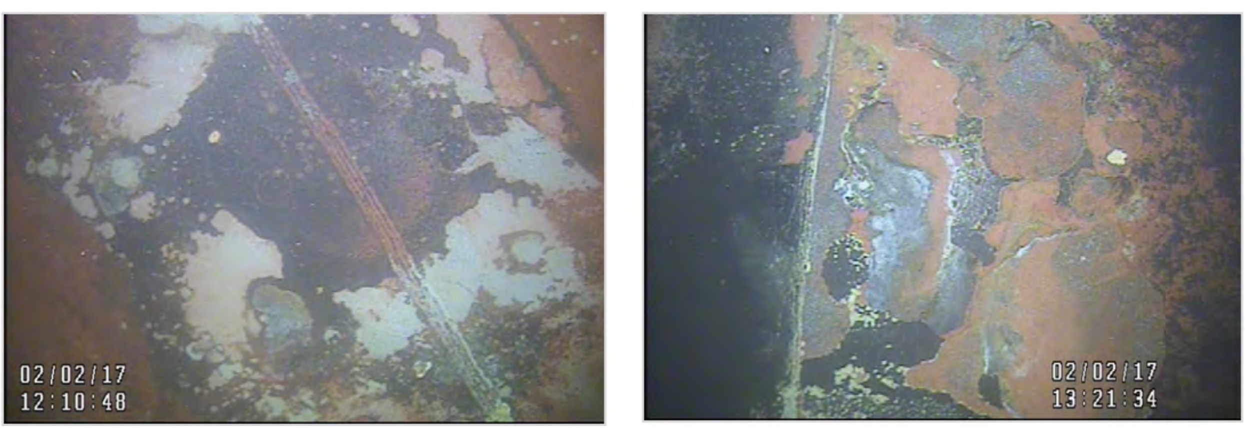


Figure 31. Examples of bare hull detected along hull seams within Quadrat 15 on the starboard hull (left) and outside Quadrat 7 on the port hull (right).

3.3 PRE, DURING, AND POST-HULL CLEANING PAINT ASSESSMENT

3.3.1 *Paint Analysis Prior to Cleaning*

3.3.1.1 *Dome Measurement*

Five dome deployments prior to cleaning, over fouled surfaces of the hull (Table 13) indicated an average leaching rate of 1.1 ±1.1 µg dissolved Cu/cm^2 day. Factoring the wetted hull surface area of the ex-INDEPENDENCE (123,445 ft^2 [11,468 m^2]), the daily loading was estimated to contribute 0.29 lbs of dissolved Cu per day to the environment. Zinc analysis was inconclusive showing steady low concentrations, with no significant correlation coefficient (R^2), limited by the capability of the field method. These results attest to a minimal, if not negligible, release of Zn from the hull pre cleaning.

Table 13. Dissolved Cu Leaching Rates (µg/cm^2 d) from dome deployments pre-hull cleaning.

Sample ID	R^2	Dissolved Copper Leaching Rate (µg/cm^2 d)
Pre Cleaning		
Dome Deploy 1	0.996	0.88
Dome Deploy 3	-0.610*	-0.6*
Dome Deploy 4	0.994	0.3
Dome Deploy 5	0.958	0.6
Dome Deploy 6	0.955	2.7
Average ± STD DEV		1.1 ±1.1

* Data that did not fulfill the a priori requirement of having an R^2 of ≥ 0.700. This data was considered an outlier and was not used for the average leaching rate calculations. The discontinuity in the numbering of dome deployments was due to labeling issues.

3.3.1.2 *Simulated Hull Cleaning*

A summary of the Cu and Zn concentrations measured from hull scrubber samples and extrapolated to the wetted hull surface area is presented in Table 14. Results show a geomean concentration of 8.23 µg Cu/cm^2, with a range of 0.23—45.89 µg Cu/cm^2 (total Cu discharged during simulated hull cleaning). Total Zn released per unit area during simulated hull cleaning showed a geomean of 1.53 µg/cm^2, with a range from 0.09—8.73 µg/cm^2.

Extrapolating sample data to the entire hull of the ex-INDEPENDENCE produces estimates of the total amount (chemical mass) of Cu and Zn released by hull cleaning (given by Equation 5) shown in Table 15. The estimated amount of Cu released ranged from 26—5,263 g, with an average of 1,985 g and a geomean of 944 g (2.08 lbs) based on simulated hull cleaning. The estimated amount of Zn released ranged from 11 to 1,002 g, with an average of 289 g and a geomean of 176 g (0.39 lbs) based on simulated hull cleaning.

Table 14. Summary of total Cu and Zn measured in hull scrubber samples from various locations and depths on the hull (C_M) extrapolated to the hull surface area (C_R)

Sample ID	Depth (ft)	Weight per Volume Measured in Hull Scrubber Sample (C_M)		Weight per Unit Area Released by Simulated Cleaning (C_R)	
		Total Cu (µg/L)	Total Zn (µg/L)	Total Cu (µg/cm^2)	Total Zn (µg/cm^2)
Hull Scrub 1	6	823	165	2.00	0.40
Hull Scrub 2	21	992	79	2.42	0.19
Hull Scrub 3	27	226	1,266	0.55	3.08
Hull Scrub 4	15	7,155	2,083	17.43	5.07
Hull Scrub 5	27	2,768	1,209	6.74	2.95
Hull Scrub 6	6	12,420	1,080	30.25	2.63
Hull Scrub 7	25	17,777	1,505	43.30	3.67
Hull Scrub 8	6	3,111	372	7.58	0.91
Hull Scrub 9	20	94	38	0.23	0.09
Hull Scrub 10	7	13,463	944	32.79	2.30
Hull Scrub 11	13	18,840	1,303	45.89	3.17
Hull Scrub 12	25	7,790	585	18.98	1.43
Hull Scrub 13	20	2,396	223	5.84	0.54
Hull Scrub 14	20	17,538	3,585	42.72	8.73
Hull Scrub 15	20	5,780	1,318	14.08	3.21
Hull Scrub 16	20	2,539	809	6.18	1.97
n				16	16
Average				17.31	2.52
STDEV				16.43	2.19
CV				94.9%	86.7%
Min				0.23	0.09
Max				45.89	8.73
Median				10.83	2.46
Geomean				8.23	1.53

Table 15. Estimated amount of total Cu and Zn released based on simulated hull cleaning, extrapolated to the wetted hull surface[1] of the ex-INDEPENDENCE (C_{MASS})

	C_{MASS}	
	Total Cu (g)	Total Zn (g)
Average	1,985	289
Min	26	11
Max	5,263	1,002
Geomean	944	176
	Total Cu (lbs)	Total Zn (lbs)
Geomean	2.08	0.39

[1]Wetted Hull Surface = 123,445 ft^2

3.3.2 Paint Analysis During Cleaning

3.3.2.1 SCAMP® Effluent

Effluent was collected in the plume created by the cleaning operation on 11 January 2017, and thus was representative of the most concentrated effluent during hull cleaning with respect to total and dissolved copper and zinc. Measured concentrations on the seven samples are shown in Table 16. Total and dissolved Cu in the discharge effluent averaged 39.76 ±22.67 µg /L (parts per billion [ppb]) and 12.45 ±4.43 µg /L, respectively. Total and dissolved Zn in the discharge effluent averaged 16.08 ±7.36 µg /L and 8.08 ±6.17 µg/L, respectively.

Table 16. Cu and Zn concentrations of effluent from in-water hull cleaning of the ex-INDEPENDENCE with the SCAMP®[1].

Sample ID	Dissolved Metal Concentration in Effluent (µg/L)		Total Metal Concentration in Effluent (µg/L)	
	Cu	Zn	Cu	Zn
SCAMP® 1	6.64	17.09	26.47 ±1.31	8.49 ±0.16
SCAMP® 2	11.59	3.40	39.50	12.70
SCAMP® 3	12.00	2.26	16.87	10.88
SCAMP® 4	9.56 ±2.20	0.59 ±0.06	15.29	9.66
SCAMP® 5	12.76	12.85	76.81	20.22
SCAMP® 6	13.57	8.89	59.62	26.05
SCAMP® 7	21.01	11.51	43.75	24.54
Mean	12.45	8.08	39.76	16.08
STD DEV	4.43	6.17	22.67	7.36
Geomean	11.81	5.20	34.14	14.68

[1]The first four samples were collected during the morning shift, and the last three samples in the afternoon shift.

Note: Value after ± is one standard deviation for samples analyzed in duplicate.

Using the following effluent measurements and assumptions for Equation (6):

Total Cu concentration = 0.00003976 g/L
Dissolved Cu concentration = 0.00001245 g/L
Total Zn concentration = 0.00001608 g/L
Dissolved Zn concentration = 0.00000808 g/L
F = 13,000 gpm (49,205 L/min)
R = 17.0 ft2/min (1.58 m2/min) (based on the logged hours of SCAMP® active use over the cleaning event (121 hours)

We obtain the following results:

- **Total Cu** = (0.00003976 g/L) (49,205 L/min)/1.58 m^2/min = **1.24 g Cu/m^2** surface cleaned, or 1.96 g /min of brush time.
- **Dissolved Cu** = (0.00001245 g/L) (49,205 L/min)/1.58 m^2/min = **0.39 g Cu/m^2** surface cleaned, or 0.61 g/min of brush time.
- **Total Zn** = (0.00001608 g/L) (49,205 L/min)/1.58 m^2/min = **0.50 g Zn/m^2** surface cleaned, or 0.79 g/min of brush time.
- **Dissolved Zn** = (0.00000808 g/L) (49,205 L/min)/1.58 m^2/min = **0.25 g Zn/m^2** surface cleaned, or 0.40 g/min of brush time.

Utilizing these data and assuming a wetted hull surface area of 123,445 ft^2 (11,468 m^2) for the ex-INDEPENDENCE, SCAMP® estimates of mass loading of total and dissolved metals were:

- **Total Cu** = (1.24 g Cu/m^2)(11,468 m^2) (1/1000 kg/g) = **14.22 kg (31.3 lbs)**
- **Dissolved Cu** = (0.39 g Cu/m^2)(11,468 m^2)(1/1000 kg/g) = **4.47 kg (9.9 lbs)**
- **Total Zn** = (0.50 g Zn/m^2)(11,468 m^2)(1/1000 kg/g) = **5.7 kg (12.7 lbs)**
- **Dissolved Zn** = (0.25 g Zn/m^2)(11,468m^2)(1/1000 kg/g) = **2.87 kg (6.3 lbs)**

SCAMP® values were higher than metal loading estimates from the hull scrubber and dome system analysis, however SCAMP® data support the summary findings in this report of low metal concentrations associated with the antifouling paint of the ex-INDEPENDENCE.

3.3.2.2 Dome Measurement

During the post-cleaning assessment, the SSC Pacific sampling dome was deployed in twelve locations at various depths along randomly distributed biological assessment transects (Table 17). Measurements pre-hull cleaning were done on areas covered by fouling. For measurements post hull cleaning, care was taken to select deployment areas where bare metal was not present so that any measurements were not skewed by the lack of paint.

Table 17 contains the data from the dome deployments post cleaning and shows an average leaching rate of 1.2 ±0.8 µg Cu/cm^2 day, which was almost identical to the leaching rate pre-cleaning of 1.1 ±1.1 µg/cm^2 d. The overall average leaching rate was 1.2 ±0.8 µg Cu/ cm^2 day. Factoring the wetted hull surface area of the ex-INDEPENDENCE (123,445 ft^2 [11,468 m^2]), the daily loading was estimated to contribute 0.29 lbs of dissolved Cu per day to the environment post cleaning. This is the same estimate obtained from dome sampling pre-hull cleaning. Zinc analysis was inconclusive due to low concentrations limited by the capability of the field method.

Table 17. Dissolved Cu leach rates (µg/cm^2 d) from dome deployments post hull cleaning

Sample ID	R^2	Copper Leaching Rate (µg/cm^2 d)
Dome Deploy 2	0.240*	0.6*
Dome Deploy 7	0.956	0.9
Dome Deploy 8	0.902	1.2
Dome Deploy 9	0.984	2.3
Dome Deploy 10	0.977	0.5
Dome Deploy 11	0.980	0.7
Dome Deploy 12	0.945	1.1
Dome Deploy 13	0.944	1.1
Dome Deploy 14	0.993	2.7
Dome Deploy 15	0.735	0.6
Dome Deploy 16	0.562*	1.1*
Dome Deploy 17	0.986	0.5
Average Post-Cleaning ± STDEV		1.2 ±0.8
Average Pre-Cleaning ± STDEV		1.1 ±1.1
Average Pre + Post Cleaning ± STDEV		1.2 ±0.8
Geomean Pre + Post Cleaning		0.9

*Data that did not fulfill the requirement of having an R^2 of ≥0.700. These data are considered outliers and are not used for the average or geomean leaching rate calculations.

4. DISCUSSSION

4.1 THE BIOFOULING COMMUNITY OF THE EX-INDEPENDENCE

4.1.1 Pre-Cleaning

During pre-cleaning taxonomy and biomass surveys, substantial biofouling was observed on all parts of the vessel, despite antifouling coatings used to prevent biofouling. Due to the age of the antifouling coatings, depletion in the Cu had occurred, which had reduced its effectiveness to prevent fouling over time. Heterogeneous "niche" areas such as shafts, struts, stern tubes, and bilge keels are extremely susceptible to fouling accumulation (Coutts and Taylor, 2004a). Long residence time at G Pier in Bremerton, WA, combined with no hull husbandry since the ship was last dry-docked in 1986, allowed for the development of high-density biofouling assemblages.

4.1.1.1 Taxonomy

The detailed taxonomic analysis provided valuable information regarding the biofouling community on the hull of the ex-INDEPENDENCE prior to hull cleaning and further emphasized the importance of conducting this type of survey as in situ and video observations alone do not allow for comprehensive species identification. Larger morphology of certain species (e.g., tubeworms and anemones) obscured the underlying biofouling community, composed of a high density of smaller species, not visible to divers. Furthermore, many invasive species look visually similar to native species and taxonomic analysis is the only method to definitively identify species and their ecological status.

The abundance and diversity of biofouling organisms were inversely correlated within individual quadrats, but did not share similar spatial patterns with respect to depth or distance from the vessel bow. This indicated that high abundances within sample quadrats and transects tended to be driven by the presence or absence of dense monocultures of one or two highly abundant species (e.g., the amphipod *Aoroides sp.*, which reached densities exceeding 2,000 individuals in a single quadrat), rather than by overall increases and decreases in the density of the fouling community.

Higher abundances of biofouling organisms were observed on the port side of the vessel than on the starboard side, where the vessel experienced some shading from a barge and the adjacent ex-Kitty Hawk. Sun exposure influences the amount of soft fouling (notably algae) and abundances are typically lower on areas of a vessel hull receiving little light as a result of shading effects from certain hull features (Coutts and Taylor, 2004b). The vessel flight deck also provided some shading for areas of the hull corresponding to transect 3, which exhibited the lowest overall abundance compared to other transects sampled.

Detailed taxonomic identification to species level was not possible for all individuals colonizing the hull of the ex-INDEPENDENCE prior to hull cleaning due to logistical constraints. Of the positively identified species found on the hull of ex-INDEPENDENCE prior to hull cleaning, seven species were considered invasive to the Pacific Northwest where the vessel was berthed (*Monocorophium acherusicum, M. insidiosum, Crepidula fornicata, Amathia gracilis, Schizoporella unicornis*, *Watersipora subtorquata*, *Ciona intestinalis*, and *Pinnixa* sp.).

The tube-building amphipods *Monocorophium acherusicum* and *M. insidiosum* are globally distributed with unknown native ranges (Fofonoff et al., 2003b). These species have likely spread through hitchhiking on the hulls of commercial ships or with oyster transplants. Though found burrowing in soft substrates, they can also attach to hard substrates such as rocks, shells, docks, woody debris, and ship hulls. They have been known to be a pest species that fouls maritime structures, though their large-scale impacts have not been quantified (Fofonoff et al., 2003b).

The common Atlantic slippersnail (*Crepidula fornicata)* is a limpet-like marine snail, native to the Northwest Atlantic from Newfoundland to the Gulf of Mexico (Fofonoff et al., 2003a). This species has been introduced to the Northeast Pacific where it can grow on a variety of hard substrates, including rock, wood, shells, docks, and ship hulls (Fofonoff et al., 2003a). This species has been known to affect the survival and growth of commercially important shellfish, as it can grow on the shells of other mollusks.

Bryozoan species found on the hull that are non-indigenous to the Pacific Northwest were *Schizoporella unicornis*, *Watersipora subtorquata*, and *Amathia gracilis*. *S. unicornis* and *W. subtorquata* are encrusting byrozoans. *W. subtorquata* is widely distributed around the globe, though its native range is poorly understood. It is found on hard substrates including rocks, oyster shells, pilings, floats, oil platforms, and ship hulls. Its calcareous crusts and curled edges create secondary habitat for the settlement of other marine invertebrates. Introduced populations in the U.S. have been recorded on the west coast and Hawaii. This species grew tolerant of copper and mercury antifouling paints, enabling them to outcompete congeneric species within their introduced range (Fofonoff et al., 2003d). They are often found to be the only species able to have settled on surfaces with antifouling paints. (Fofonoff et al., 2003d). *S. unicornis* forms extensive layered sheets over rocks, docks, seaweed holdfasts, and shells (Fofonoff et al., 2003c). This species is a freshwater species with occurrences in tidal fresh or brackish waters. The bryozoan *A. gracilis,* a relatively recent invader on U.S. west coast, is native to Europe and is possibly cosmopolitan or cryptogenic on the east coast of the U.S. East coast and Canadian Maritime (Waeschenbach et al., 2016). This species grows on hard substrate including overgrowing mussel beds, dock lines, and pilings (Marić et al., 2016; Temereva and Kosevich, 2016).

The sea vase tunicate *Ciona intestinalis,* a non-native species to the Pacific Northwest, is known as a fouling organism of vessels and docks throughout the world (Fofonoff et al., 2003e). Its most serious economic impacts have been on shellfish aquaculture in Nova Scotia, Canada (Fofonoff et al., 2003e; Ramsay et al., 2008) and South Africa (Robinson et al., 2005) where they overgrow mussel crop, ropes and equipment. Fouling of cultured shellfish has also been reported in Spain, Chile, Japan, and New Zealand (Fofonoff et al., 2003e). The sea vase is a formidable competitor due to its quick growth rate and its ability to displace other species in a fouling community (Lambert, and Lambert 2003). Studies in San Francisco Bay, CA indicate that the sea vase competes with other native and introduced fouling organisms (Blum et al., 2007). Diversity within fouling communities was negatively correlated with *C. intestinalis* abundance in that study, and experimental removal of *C. intestinalis* resulted in increased diversity (Blum et al., 2007).

The pea crab (*Pinnixia* sp.) is considered to be native to the Pacific Northwest, however some species in this genus (e.g., *Pinnixia occidentalis*) are invasive elsewhere outside this region. Pea crabs are often found inside other host organisms such as oysters. The pea crabs of the Pacific Northwest utilize a number of hosts including bivalves, tubes of certain tubiculous polychaetes, burrows of ghost shrimp and worms (Burgess and Eagleston, 2016; Fretwell and Starzomski 2015). These types of relationship are thought to be symbiotic for some cases and parasitic in others (Burgess and Eagleston, 2016).

A number of native and invasive taxa were only able to be identified to genus or families in the taxonomic analysis. Of particular interest, Family Styelidae included both native and invasive species including the high-risk invasive species *Botrylloides violaceus* (red sheath tunicate), which was also observed in pre-cleaning underwater video efforts. Although identification was not able to be confirmed from video analysis, *B. violaceous* is the most likely species identification from these observations. Samples of material were also identified to family level in the taxonomy samples. *B.violaceus* colonizes man-made structures including dock floats, pilings, piers, aquaculture structures, and boat hulls (Carman et al., 2010; Simkanin et al., 2012). Red sheath tunicates frequently displace other fouling organisms, including native and introduced tunicates, bryozoans, barnacles, and mussels through competition for space and food. Evidence of this was found during experiments with fouling plates in New England waters (Agius, 2007; Altman and Whitlatch, 2007; Bullard et al., 2004; Dijkstra and Harris, 2009; Myers, 1990; Osman and Whitlatch, 1995, 2000, 2007; Rajbanshi and Peterson, 2007; Stachowicz et al., 2002a; Stachowicz et al., 2002b; Stachowicz et al., 1999). On the U.S. west coast, red sheath tunicates are a prevalent invader of San Diego Bay, California. At two locations in California it has formed extensive areas of 100% cover, indicating a strong competitive ability (Lambert and Lambert, 2003). From 2003 through 2006, introduced tunicates, including red sheath tunicates, replaced the mussel (*Mytilus edulis*) as the dominant species in fouling communities in Portsmouth Harbor, NH leading to a major functional habitat change (Dijkstra and Harris, 2009). This species has been shown to be a competitor for space with mussels (Rajbanshi and Pederson, 2007).

4.1.1.2 Biomass

No discernable pattern in biomass was evident with depth, ship side, or distance from the bow, although this analysis was hampered by the inability to complete transect 4 due to logistical constraints. Visual observations indicated more biomass toward the aft and starboard side of the vessel than was near the bow, so there is uncertainty in the upper end of the biomass removed estimated from the quantitative data.

One important aspect of the biofouling assessment was to estimate the composition and the amount of biofouling present on the hull that would be released by cleaning. The total mass of material was needed to estimate the amount of Cu and Zn released by the cleaning and bound the estimate of how much material would be released into the inlet for degradation, decomposition, and recycling by the marine food web. The organic matter from the biomass removed was the material available for biodegradation and digenesis, which could consume dissolved oxygen (DO) in the bottom water near the site. The amount of biomass removed provided an estimate of the source term for the material released for degradation. Whether there was any short-term reduction in DO that could be attributed to the biofouling removal was evaluated by the water quality (SPAWAR, 2017).

4.1.2 Post-Cleaning

4.1.2.1 Biofouling Assessment

The ex-INDEPENDENCE primarily exhibited areas of antifouling paint and bare hull. The circular patches that were prevalent on the hull were either from the removal of barnacles or tubeworms and the circles were left when the paint failed and attached to the organisms upon removal by cleaning, revealing bare hull or lower paint layers. The minimal growth that was observed during the post-hull cleaning surveys could have been either existing growth missed during the cleaning or newly settled growth between the time following the cleaning and the time of the biological survey. The majority of the living fouling observed was soft fouling (algae and hydroids). The live tubeworm observed in the fairwater, however was most likely part of the original fouling community and was missed by hull cleaning tools as the inside of features such as these are difficult to access. The live anemones detected on various niche areas could have been dislodged during the cleaning process but reattached to a surface thereafter. Anemones are primarily sedentary marine animals colonizing hard substrates such as floats, docks, pilings, rocks, and ship hulls (Fretwell and Starzomski, 2015); however, anemones can move by sliding along surfaces or flexing their bodies when disturbed, resulting in limited swimming through the water column.

The feather duster tubeworm (*Eudistylia vancouveri*) and the frilled anemones (*Metridium* spp.) are both considered to be native species, and are both prevalent in the Pacific Northwest. Thus, the tubeworm species observed post-cleaning are unlikely to pose a colonization risk in the destination port, as most species are temperate/boreal with a range from Alaska to Central California and would likely not survive in the Gulf of Mexico (Fretwell and Starzomski, 2014). Observed surviving anemone species (e.g., *Metridium senile*), while globally distributed, are not commonly found below approximately 40^{o} N latitude and would not likely be able to colonize in the Gulf of Mexico (Encyclopedia of Puget Sound, 2015).

The quantitative effectiveness of in-water hull cleaning could not be fully evaluated as percent cover data from the ex-INDEPENDENCE (prior to hull cleaning) were not available; nevertheless, observations showed that the fouling had been effectively removed from the vessel. Due to the difficulty of cleaning tools accessing niche areas (e.g., inside fairwaters, rope guards, stern tubes, fairing, and around appendages), it is unreasonable to assume that hull cleaning would result in a 100% elimination of fouling cover. Based on the results of the post-cleaning biological survey, it can be concluded with confidence that hull cleaning was extremely effective in removing the fouling community on the hull of the ex-INDEPENDENCE, reducing the risk of invasive species transfer. Video analysis of the hull prior to cleaning showed between 80 to 100% cover of live fouling, with extremely dense cover in some places, and occasional small patches of exposed hull surface (mostly paint, very occasionally bare metal). Conservatively assuming 90% live cover pre cleaning, and based on previous calculations, it is estimated that the cleaning process removed approximately 99.9% of live fouling from the hull of the vessel.

It is extremely difficult to calculate the exact percent reductions in fouling, particularly for niche areas; however, generating a rough estimate is practical with the available information. Niche areas were not quantitatively surveyed; however, they likely had the highest remnant levels of fouling, since they are more difficult to clean than open smooth hull surfaces. Approximately one in five (20%, see Table 12) of the surveyed niche areas had fouling present. The remaining 80% had no live fouling observed, and most of these areas where fouling was present had only a single individual or a small patch of fouling. Because these areas were not smooth, it was not possible to quantify the percentage cover in those cases with the sampling quadrat. Using a "worst case" assumption of 25% live fouling in those cases where fouling was observed, a conservative estimate of 5% live fouling coverage on niche surfaces overall (20% of niche surfaces with 25% fouling coverage) can be obtained. Since niche areas constituted a very small portion of the hull, approximately 1% or less of the overall hull area of the vessel, this "worst case" estimate can be used above for niche areas. When this estimate is combined with the quantified fouling rate observed on the surveyed portions of the hull from the post-cleaning survey, the result is an estimate of the overall percent cover of live fouling on the vessel. This technique yields an estimate that overall fouling rate on the ex-INDEPENDENCE was no higher than roughly 0.08%.

4.1.2.2 Seafloor Observations

The area of seafloor and man-made structures (e.g., piers and pilings) surrounding the ex-INDEPENDENCE is colonized by species similar to those observed on the hull during pre-cleaning surveys. Sparse aggregations of tube-building polychaetes, anemones, and hydroids (likely the same species observed on the ex-INDEPENDENCE) have been observed in nearby areas. Sediment sampling conducted at the site prior to hull cleaning showed a very limited presence of macro-invertebrates in the sediments under the hull (SPAWAR and NUWC, 2017). However, when a sea

bottom site survey was conducted on 30 March, 2017, many organisms removed from the hull were found to be living and contributing to the benthic community (SPAWAR and NUWC, 2017). This was approximately
3 weeks after the ex-INDEPENDENCE was towed from Mooring G and more than eight weeks after hull cleaning was completed. Additionally, the February 2017 (post cleaning survey) and the March 2017 survey videos show a high abundance of crabs and other bottom scavengers feeding on the biofouling material (SPAWAR and NUWC, 2017). These observations suggest that biofouling removal had an ecological benefit by adding diversity and structure to the seafloor community where the ex-INDEPENDENCE was previously berthed.

4.2 ENVIRONMENTAL LOADING

All of the ex-INDEPENDENCE data used to estimate metal loading to Sinclair Inlet indicated the antifouling system was metal-depleted and no longer performing as an antifouling agent. This conclusion is supported by the observed covering and volume of organisms living on the hull of the ex-INDEPENDENCE pre-cleaning. SSC Pacific dome measurements pre and post cleaning provided the same estimates of 0.29 lbs of dissolved Cu in both cases. Dissolved Cu leaching rates obtained from dome samples ranged from undetected to low rates (0.3 $\mu g/cm^2$ d to 2.7 $\mu g/cm^2$ d, see Table 13 and Table 17). Dome measurements also similarly indicated that the release of Zn from the hull was minimal or negligible as it was below the limit of detection of the method both pre and post hull cleaning. In contrast, estimates of Cu in the effluent from the SCAMP® during cleaning indicated somewhat higher loading with a total (dissolved and particulate) Cu load of 31.3 lbs, dissolved Cu load of 9.8 lbs, total Zn load of 12.7 lbs, and dissolved Zn load of 6.4 lbs. Metal loads estimated from the hull scrubber data provided values between the Dome and SCAMP® measurements, with 2.08 lbs of total Cu, and 0.39 lbs of total Zn.

Table 18. Summary of estimates of the load of Cu and Zn released from the ex-INDEPENDENCE hull cleaning obtained from dome deployments, simulated hull cleaning, and SCAMP® effluent .

Method	Dissolved Cu (lbs)	Total Cu (lbs)	Dissolved Zn (lbs)	Total Zn (lbs)
Domes (pre)	0.29		Non-detect	
Hull Scrubs (pre)		2.08		0.39
SCAMP® (during)	9.8	31.3	6.4	12.7
Domes (post)	0.29		Non-detect	

Overall, the three lines of evidence indicated that the metal load associated with the ex-INDEPENDENCE hull cleaning is low, representing a fraction of the load expected from ships with an effective antifouling systems in place. The differences between the measured and expected metal loadings are an indication that the antifouling system on the ex-INDEPENDENCE was depleted with respect to the mass of Cu and Zn in the hull coating system. Such depletion is expected to have mitigated the mass loading of Cu and Zn metals released to the surrounding seawater during the cleaning of ex-INDEPENDENCE in 2017.

The similarity in the low range of passive metal leaching rates measured pre and post cleaning of the ex-INDEPENDENCE also corresponds with a depleted and ineffective antifouling system.

Effective antifouling Cu-Zn systems are characterized by a relatively large leaching rate of 21 to 65 µg Cu/cm^2 d (Earley et al., 2014; Valkirs et al., 2003) when recently painted, and peak release rates of about 34 µg Cu/cm^2 d post cleaning (Earley et al., 2014). However, the metal release in effective antifouling coatings exposed to natural seawater tends to decrease asymptotically with time to a lower value between 3 to 4 µg Cu/cm^2 (Earley et al., 2014; Valkirs et al., 2003). These reported peak leaching rates for freshly painted and cleaned antifouling systems were not observed for the ex-INDEPENDENCE, and even the maximum leaching rate observed of 2.7 µg Cu/cm^2 is at the lower end of leaching rates previously reported for active vessels with an intact anti-fouling system. This information supports the observed ineffectiveness of the antifouling system on the ex-INDEPENDENCE (i.e., high level of biofouling present pre-cleaning), low metal loads measured from sampling in this study, and low metal loads expected from this coating system during hull cleaning. An assessment of potential water quality impacts to Sinclair Inlet associated with biofouling removal from the ex-INDEPENDENCE is discussed in a separate report focusing on water quality (SPAWAR and NUWC, 2017).

4.3 MOVING THE EX-INDEPENDENCE

The ex-INDEPENDENCE is being dismantled in Brownsville, TX, having been towed in late winter from Bremerton through the Strait of Magellan and through the South Atlantic Ocean, the Caribbean Sea, then into the Gulf of Mexico. Moving inactive ships with fouling communities is of concern because of the possibility of introducing new species to an area where habitat conditions are suitable for survival and establishment. The transfer and establishment of non-native species (species that live outside of their historical geographical range) is not necessarily harmful to native flora and fauna; however, non-native species that have the ability to spread, displace and outcompete native species can directly impact species, communities and ecosystems. Such invasive species are of environmental concern because they have been shown to have negative effects on ecosystem diversity (i.e., reduction or elimination of native species), local and regional economies (i.e., reduction or impairment of natural resources, cost associated with removal), industrial hazard (i.e., clogged intake pipes, nets, or other gear), and public health (i.e., spread of infectious diseases).

Until recently, studies of vessel-mediated exchange of aquatic species have focused on ballast water. National and international policies aimed at reducing the risk associated with ballast water introductions are now being implemented (e.g., United States National Invasive Species Act of 1996; International Convention for the Control and Management of Ships' Ballast Water and Sediment). Despite the fact that biofouling on ships' hulls is a centuries-old mechanism of species introductions and that biofouling represents an equal or greater risk for species introductions than ballast water (Davidson et al., 2009; Davidson et al., 2008; Drake and Lodge, 2007), there is no legislation in place regarding hull transfers of invasive species.

A study by Llansó and Sillett (2008) for the U.S. Maritime Administration (MARAD) assessed biofouling of obsolete vessels and evaluated the effectiveness of in-water hull cleaning as a vector management option. Results of this study suggested a relationship between the initial amount of biofouling and the number of species in surveys that occurred after-transit. The amount of fouling existing on the hull and the three-dimensional structure provided by this community at the origination port was thought to enhance species settlement and attachments while in transit (Llansó and Sillett, 2008). Hull cleaning of the ex-INDEPENDENCE appears to have been an effective management strategy to reduce the risk of species transfers and introductions at or near the destination port in the Gulf of Mexico.

The purpose of the underwater hull cleaning was to reduce the risk of transferring invasive species to sensitive areas along the tow route and in the destination port. Of the invasive species initially known to occur in Bremerton, two were identified as high risk for transfer to sensitive areas along the tow route: red sheath tunicate (*Botrylloides violaceus*) and transparent sea squirt (*Ciona savignyi*). An additional *Ciona* sp. was also identified in the taxonomy samples, *Ciona intestinalis*, and later determined to be a high risk invasive or potentially invasive species capable of surviving and establishing in Brownsville, TX, and the Gulf of Mexico as it meets the temperature and salinity requirements for this region.

Additional species were identified during the taxonomic analysis that were later determined to be either invasive, cryptogenic , the invasive members of a genus or family that contains both native and invasive taxa, or species that are native to the Pacific Northwest but would be invasive elsewhere. A risk analysis was then preformed to determine the potential risk of establishment in in the Gulf of Mexico following the tow (Table 19). To become established outside of a native region or a region to which it has already been introduced, nonindigenous aquatic species must first be successfully transported to the new region. Factors affecting the transport of nonindigenous aquatic species to a new region include vessel speed, voyage duration, source region, and similarity of environmental factors during the voyage and at the destination port compared to the source (salinity, temperature, and nutrients). Of the taxa analyzed, seven species were determined to pose a potentially high risk to the western Gulf of Mexico based on their environmental tolerances (see Table 19). Three crustacean (*Caprella mutica*, *Hemigrapsus oregonensis*, and *Ianiropsis serricaudi*) and one tunicate species (*Styela clava*) were considered to be a low risk as the environmental parameters in Brownsville are not suitable for colonization and/or survival of these species during the tow is unlikely. Species or taxa that pose no risk included those species that are already present in Brownsville.

The ex-INDEPENDENCE remained at the pier in Bremerton for approximately 7 weeks post-cleaning and before it left on transit to Brownsville, TX. In this period of time, it was possible for some biofouling species to recolonize the hull. Biofouling organisms are able to recruit to suitable hard substrates (uncoated or exhausted painted surfaces) within one week, and depending on geographic location, moderate to extensive biofouling communities can develop over a 3–4 week period (Floerl et al., 2010). Recruitment by biofouling organisms is more seasonal in temperate latitudes such as Bremerton, but while more limited, certain species can still recruit in colder months, particularly on to bare substrate with little or no competition for space. Tows occurring as close as possible to the completion of a hull cleaning would potentially prevent the recruitment of new biofouling organisms. Several aspects of the movement of both active and inactive suggest these vessels are unusually potent sources of potential species transfer when not cleaned at regular intervals (Davidson et al., 2008; Drake and Lodge, 2007; Godwin et al., 2004). Inactive vessels are typically towed at slow speeds when moved from the origination port to the final destination, which allows for retention of the initial colonies during transit (Davidson et al., 2008). Upon arrival at the new location, vessels likely have long residence times as they remained in the water during the dismantling process, potentially increasing the likelihood of species transfer to the surrounding waters either due to reproduction, or removal from the hull. Given that the ex-INDEPENDENCE had an extensive hull cleaning resulting in minimal remaining growth and that no invasive species were observed on the hull post-cleaning, the risk of species transfer to the destination port was reduced.

Table 19. Potential risk of invasion to Brownsville by species known to be invasive.

Common Name	Scientific Name	Bremerton Status	Brownsville Status	Invasion Risk	Notes
CRUSTACEANS					
Amphipods					
Japanese skeleton shrimp	*Caprella mutica*	Invasive	Not Present	LOW	Upper temp limit 28° C, 20° C for reproduction, unlikely to survive in the Gulf of Mexico.
	Jassa marmorata	Invasive	Cryptogenic	HIGH	Easily misidentified. May or may not already be present in Gulf of Mexico. Habitat suitable for colonization.
	Monocorophium acherusicum	Invasive	Present	NONE	Already present in the Gulf of Mexico. Origin uncertain.
	Monocorophium insidiosum	Invasive	Native	NONE	Native to U.S. East coast
Decapods					
Shore crabs	*Hemigrapsus oregonensis*	Native	Not Present	LOW	Not identified to species, and not likely to survive transit through Antarctic waters. *H. oregonensis* is found in (Southern California) and could colonize in the Gulf of Mexico, and genus has known invaders (e.g. *H. sanguineus*).
Pea crabs	*Pinnixa* sp.	Native	Not Present	HIGH	Native to Pacific Northwest, and found as far south as Baja California. Known invasive in Africa
Other Crustaceans					
Isopods					
	Ianiropsis serricaudi	Invasive	Not Present	LOW	Not identified to species in survey, but *I. serricaudi* is a known invasive on both U.S. coasts. Temperature limit is 24° C, so unlikely to permanently establish in the Gulf of Mexico.
MOLLUSCS					
Bivalves					

Key	
High	Species is known to be invasive and environmental parameters suitable for colonization
Low	Environmental parameters not suitable for colonization and/or transport survival unlikely
None	Species already present in Brownsville

Table 19. Potential risk of invasion to Brownsville by species known to be invasive. (Continued)

Common Name	Scientific Name	Bremerton Status	Brownsville Status	Invasion Risk	Notes
Arctic hiatella	*Hiatella arctica*	Cryptogenic	Present	NONE	Cryptogenic, but already present in the Gulf of Mexico.
Mediterranean Mussel	*Mytilus galloprovincialis*	Invasive	Likely Not Present	HIGH	Requires genetic techniques to distinguish from native species *M. edulis*. Not known in Gulf of Mexico, but may be able to survive (upper thermal tolerance approximately 31° C).
Gastropods					
Common slipper shell	*Crepidula fornicata*	Invasive	Native	NONE	Native to Gulf of Mexico
BRYOZOANS					
	Amathia gracilis	Invasive	Present	NONE	Possible cryptic taxonomy, present and probably native in Gulf of Mexico
Single horn bryozoan	*Schizoporella unicornis*	Invasive	Not Present	HIGH	Possible cryptic taxonomy with *S. japonica*. Temperature range not well studied and not currently found in Texas, but recorded (invasive) in Florida.
Red -rust bryozoan	*Watersipora subtorquata*	Invasive	Cryptogenic	HIGH	Cryptic taxonomy. No specific records in Gulf of Mexico, but present (cryptogenic) in Florida and Caribbean, so likely also Brownsville.
TUNICATES					
Sea vase tunicate[1]	*Ciona intestinalis*	Invasive	Not Present	HIGH	Globally distributed, highly invasive, but not recorded in Gulf of Mexico. Similar species cannot tolerate temperature above 27° C.
Club tunicate[1]	*Styela clava*	Invasive	Not Present	LOW	Highly invasive, but may not be able to survive in Gulf of Mexico. Upper thermal limit approximately 27° C.
Red sheath tunicate[1]	*Botrylloides violaceus*	Invasive	Not Present	HIGH	Not conclusively identified to species in this survey. Not currently found on U.S. East Coast further south than Chesapeake Bay, but highly invasive and on Pacific coast, common as far south as southern Mexico.

[1] Species analyzed in the BE

Key	
High	Species is known to be invasive and environmental parameters suitable for colonization
Low	Environmental parameters not suitable for colonization and/or transport survival unlikely
None	Species already present in Brownsville

5. SUMMARY OF CONCLUSIONS

Biological surveys were conducted prior to hull cleaning to characterize the species and mass of biological material on the hull, and post hull cleaning, to assess the effectiveness of reducing the potential transport of invasive species. Before, during, and after-hull cleaning paint sampling was conducted to estimate the amount of Cu and Zn that could be released into the environment of Sinclair Inlet from biofouling removal. The survey methods employed on the ex-INDEPENDENCE successfully met the objectives set forth in the protective measures of the BE to reduce or avoid potential effects from invasive species to ESA-listed species and critical habitat. Hull cleaning of the ex-INDEPENDENCE served to reduce the risk of species transfer from Bremerton to sensitive areas along the tow route in the Gulf of Mexico.

5.1 BIOMASS AND TAXONOMY ASSESSMENT

- A total of 19,092 organisms and 92 distinct taxa belonging to 11 phyla were detected in the pre-hull cleaning survey samples at stations along the hull of the ex-INDEPENDENCE. The majority of the taxa belonged to Phyla Arthropoda and Annelida, accounting for 54% and 27% of the total identified organisms, respectively.
- Seven species (*Jassa marmorata*, *Pinnixa* sp., *Mytilus galloprovincialis*, *Schizoporella unicornis*, *Watersipora subtorquata*, *Ciona intestinalis*, and *Botrylloides violaceus*) were determined to pose a potentially high risk to the western Gulf of Mexico based on their environmental tolerances if they remained on the hull after cleaning. Three crustacean (*Caprella mutica*, *Hemigrapsus oregonensis*, and *Ianiropsis serricaudi*) and one tunicate species (*Styela clava*) were considered to be a low risk as the environmental parameters in the Gulf of Mexico are not suitable for colonization and/or survival of these species during the tow is unlikely.
- The estimated total wet weight of material removed from the hull was approximately 54,000 kg (119,071 lbs) based on geomean calculations. Based on the geomean, this corresponds to a dry weight of approximately 3,146 kg (7,000 lbs) of organic material and approximately 9,945 kg (22,000 lbs) of inorganic material that was removed from the hull of the ex-INDEPENDENCE during cleaning operations.

5.2 BIOLOGICAL HULL SURVEY

- Qualitative inspection of niche areas revealed little to no fouling coverage, with isolated tubeworms, anemones, and hydroids surviving.
- Quantitative assessments of biofouling remaining post hull cleaning showed little to no biofouling organisms (< 1%) remained on the hull. The hull was predominately characterized by areas of exposed antifouling paint and small, random patches of bare hull, with remnants of dead barnacles and calcareous tubeworms.
- The extensive hull cleaning resulted in minimal growth present post cleaning. Furthermore, invasive species were not observed anywhere on the hull post-cleaning. Therefore, the risk of species transfer during towing is negligible.
- Most, if not all, of the fouling community was effectively removed during hull cleaning of the ex-INDEPENDENCE and it is not likely that substantial biofouling growth would accumulate during the transit while the ship is moving. Hull cleaning of the ex-INDEPENDENCE appears to have been an effective management strategy to reduce the risk of species transfers to the Gulf of Mexico.

5.3 PAINT ASSESSMENT

- **Dome Measurements (dissolved Cu and Zn)**: Dome deployments post hull cleaning showed an average leaching rate of 1.2 ±0.8 μg Cu/ cm^2 day, which is almost identical to the leaching rate pre-cleaning of 1.1 ±1.1 μg/cm^2 d. The overall average leaching rate was 1.2 ±0.8 μg Cu/ cm^2 day. The estimated load of dissolved Cu was 0.29 lbs per day to the environment, both pre and post cleaning. The release of Zn from the hull was minimal or negligible as it was below the limit the detection method prior and post hull cleaning.
- **Simulated Hull Cleaning (total Cu and Zn):** The estimated amount of total (dissolved + particulate) Cu released by hull cleaning based on simulated hull cleaning samples extrapolated to the hull surface area of the ex-INDEPENDENCE ranged from 26–5,263 g, with an average of 1,985 g and a geomean of 944 g (2.08 lbs). The estimated amount of total Zn released based on simulated hull cleaning samples ranged from 11–1,002 g, with an average of 289 g and a geomean of 176 g (0.39 lbs).
- **SCAMP® Effluent (total and dissolved Cu and Zn)**: The mass loading of total (dissolved and particulate) Cu measured from SCAMP® effluent during cleaning and extrapolated to the hull surface area of the ex-INDEPENDENCE was estimated to be 14.2 kg (31.3 lbs), while the mass loading of total Zn was estimated to be 5.7 kg (12.7 lbs). The mass loading estimates of dissolved metals from SCAMP® measurements were 9.8 lbs of dissolved Cu and 6.4 lbs of dissolved Zn.
- **Overall:** The three sample methods indicated a low range of leaching rates pre and post hull cleaning and a low environmental loading associated with simulated and actual hull cleaning of the ex-INDEPENDENCE. These results indicate a metal-depleted and ineffective antifouling coating system on the hull of the ex-INDEPENDENCE, which is demonstrated by this study to have resulted in minimal metal loading to the environment during hull cleaning.

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October 2017	Final	

4. TITLE AND SUBTITLE	**5a. CONTRACT NUMBER**
Comprehensive Field Surveys and Evaluation of Biofouling and Coating System Conditions Associated with the Hull of the ex-USS INDEPENDENCE (CV 62)	
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER

6. AUTHORS			**5d. PROJECT NUMBER**
Patrick J Earley Leslie A Bolick Donald E. Marx Jr I. R. Duarte R.K. Johnston **SSC Pacific**	Natasha C. Dickenson **Naval Undersea Warfare Center**	Jason S. Krumholz **McLaughlin Research Corporation**	**5e. TASK NUMBER** **5f. WORK UNIT NUMBER**

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	**8. PERFORMING ORGANIZATION REPORT NUMBER**
SSC Pacific 53560 Hull Street San Diego, CA 92152–5001	TR 3081

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	**10. SPONSOR/MONITOR'S ACRONYM(S)**
Naval Sea Systems Command 1333 Isaac Hull Avenue, SE Washington Navy Yard, DC 20376	NAVSEA
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The primary objectives of the biological assessment were to: (1) determine the presence/absence of high-risk invasive species remaining on the hull of the ex-INDEPENDENCE after hull cleaning; (2) determine the amount of organic tissue (i.e., marine organisms) and the amount of inorganic material (i.e., shells) that were removed during the hull cleaning process; and (3) identify biofouling organisms to the lowest possible taxonomic level that was practical on the hull of the ex-INDEPENDENCE prior to hull cleaning. The primary objective of the paint analysis was to assess the release of copper (Cu) and zinc (Zn) associated with the hull cleaning of the ex-INDEPENDENCE by: (1) measuring the passive release of dissolved Cu and Zn from the hull using the SSC PAC Dome technique before and after hull cleaning, (2) measuring total (dissolved + particulate) Cu and Zn in samples collected from a simulated hull cleaning device prior to cleaning, and (3) measuring total and dissolved Cu and Zn in samples collected from the effluent of the Submerged Cleaning and Maintenance Platform (SCAMP®) during cleaning operations.

15. SUBJECT TERMS

biological evaluation; controlled industrial area; certified reference material; coefficient of variation; exclusive economic zone; EPA; Environmental Protection Agency; Endangered Species Act; flow injection for atomic spectroscopy; HEPA; high efficiency particle; ICP-MS; inductively coupled plasma – mass spectroscopy; IMF; intermediate maintenance facility; INACTSHIPMAINTO; Inactive Ships Maintenance

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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