TS-788-22
Ref. No. [UMCES] CBL 2023-017

Guidelines for Testing Ship Biofouling In-Water Cleaning Systems

26 October 2022

ALLIANCE FOR COASTAL TECHNOLOGIES

Maritime Environmental Resource Center

Guidelines for Testing
Ship Biofouling In-Water Cleaning Systems

These Testing Guidelines were developed as a collaborative effort of international subject matter experts, coordinated by the Alliance for Coastal Technologies (ACT) and Maritime Environmental Resource Center (MERC), and with support from US Department of Transportation, Maritime Administration (MARAD).

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Acknowledgements: We would like to thank the Working Group on Ballast and Other Ship Vectors (a joint working group of the International Council for the Exploration of the Sea [ICES], Intergovernmental Oceanographic Commission [IOC] of UNESCO, and International Maritime Organization [IMO]), and the Clean Hull Initiative (CHI), for their helpful peer-review of these Testing Guidelines.

Citation: ACT/MERC, 2022. Guidelines for Testing Ship Biofouling In-Water Cleaning Systems. TS-788-22, CBL/UMCES 2023-017.

Cover Art: Jean Mackay, jeanmackayart.com.

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1. Introduction

1.1. Ships and biofouling

Like all substrates placed in coastal waters, the wetted surfaces of ships are quickly colonized by a succession of diverse sessile or sedentary micro- and macro-organisms, collectively known as biofouling (Wahl, 1989; Flemming, 2002; Aldred and Clare, 2008). The adverse effects of biofouling on ships and their operations are well known and have been managed since ancient times (e.g., Phoenicians 1300 BCE; Woods Hole Oceanographic Institute, 1952).

Negative impacts of biofouling on the shipping industry include:

- reduced ship performance and fuel efficiency (Townsin et al., 1981; Townsin, 2003; Schultz, 2007; Schultz et al., 2011);
- corrosion and decreased durability (Jones and Little, 1990; Blackwood et al., 2017);
- increased greenhouse gas emissions (IMO, 2011; Faber et al., 2021);
- failure to meet associated legal/contractual requirements (BIMCO, 2013; 2019);
- increased underwater noise (Baudin et al., 2015); and
- unintended translocation of aquatic species (Hewitt and Campbell, 2010; Bailey et al., 2020).

1.2. Biofouling and aquatic non-indigenous species

In recent decades, the importance of ship biofouling as a pathway for aquatic non-indigenous species (NIS) translocations has become increasingly apparent (James and Hayden, 2000; Hewitt and Campbell, 2010; Ruiz et al., 2015; Davidson et al., 2018; Bailey et al., 2020). Entire biological communities can be moved around the world by oceangoing ships and substantial numbers of species, including pathogens, can be introduced as a result (Anderson et al., 2003; Minchin et al., 2006; Georgiades et al., 2021). While not all NIS have immediately noticeable or significant impacts, a subset of NIS have a broad range of effects on the aquatic environment and the communities reliant upon local ecosystem services (Ruiz et al., 1997; Grosholz, 2002; Hewitt et al., 2004). Guidelines and regulations to prevent NIS introductions via ship biofouling are beginning to emerge to protect environmental, economic, social, and cultural values (e.g., IMO, 2011; California Code of Regulations, 2017; Ministry for Primary Industries, 2018).

1.3. Coating systems

The International Maritime Organization (IMO) defines antifouling systems (AFS) as a coating, paint, surface treatment, surface, or device that is used on a ship to control or prevent attachment of unwanted organisms (IMO, 2001). The primary AFS are coatings, applied during dry-docking to surfaces below the maximum waterline of ships, that are designed to either prevent macrofouling attachment (using biocides) or reduce adhesion (foul-release) to wetted surfaces (Dafforn et al., 2011; Arndt et al., 2021). In some areas (e.g., Baltic Sea), non-ablative or non-polishing hard coatings are used in combination with regular cleaning as a fouling prevention strategy (Watermann, 2019).

The service life of modern coatings for commercial ships is typically five years (e.g., Arndt et al., 2021; Lagerström et al., 2022). Despite substantial improvements over the last 40 years, surface coatings do not consistently prevent biofouling accumulation on all ship surfaces over the course of their service lives (Dobretsov, 2010; Georgiades and Kluza, 2017). Accumulations tend to occur as coatings age (Georgiades et al., 2020; Chan et al., 2022) and when ships have extended stationary periods (BIMCO, 2013; 2019; Davidson et al., 2020; Ruiz et al., 2022).

Even when antifouling coatings are used, there are also substantial areas of ships' immersed surfaces that are more prone to biofouling (Coutts and Taylor, 2004; Coutts and Dodgshun, 2007; Davidson et al., 2009; Georgiades et al., 2018) because they:

- cannot be painted (e.g., anodes);
- are prone to damage (e.g., bulbous bow, tug and fender points, area below anchor chain);
- are challenging to coat (e.g., dry-dock blocking areas); or
- are sub-optimal for coating performance (e.g., gratings, rudders, propellers, and sea chests).

Given the existing limitations of coatings, especially during extended periods between dry-docking, in-water cleaning of ship biofouling (within coating service life) is often required or advantageous (e.g., IMO, 2011).

1.4. In-water cleaning systems

In-water cleaning (IWC) of biofouling – used to either maintain or reset ship immersed surfaces to a hydrodynamically smooth state – is a common approach to increase ship performance and fuel efficiency between dry-dockings (Naval Sea Systems Command, 2006; Schultz et al., 2011). IWC is also recognized as beneficial for reducing both green-house gas emissions (IMO, 2011; Pagoropoulos et al., 2018; Faber et al., 2021) and biosecurity risks (Scianni and Georgiades, 2019; Tamburri et al., 2021).

IWC systems typically involve the use of diver- or remotely- operated cleaning units (i.e., cleaning carts) that remove biofouling from hull surfaces (McClay et al., 2015; Morrisey and Woods, 2015). IWC is generally described as either Proactive or Reactive (Scianni and Georgiades, 2019). Proactive IWC is the periodic removal or reduction of biofilm growth (i.e., microfouling or slime layer) on ship surfaces. Proactive IWC also removes newly settled or attached microscopic stages of macrofouling organisms, to ultimately minimize macrofouling growth (Tribou and Swain, 2010; Scianni and Georgiades, 2019). Reactive IWC is used to remove already established macrofouling organisms (Scianni and Georgiades, 2019; Tamburri et al., 2020) and often includes debris capture, treatment, and disposal.

While IWC has the potential to provide significant ship operations and biosecurity benefits, there are two main IWC processes that may result in inadvertent environmental harm: (a) lack of, or incomplete, capture of dislodged debris by the cleaning unit; and (b) release of untreated, or incompletely treated, effluent from debris processing (Scianni and Georgiades, 2019; Tamburri et al., 2021). Potential environmental impacts from these two IWC processes include:

- increased discharge of coating biocides and microplastics to ambient waters (Morrisey et al., 2013; Tamburri et al., 2020; Jones and McClary, 2021; Soon et al., 2021);

- release of live biofouling organisms, their propagules, or pathogens, into local habitats (Tamburri et al., 2020; Georgiades et al., 2021; Jones and McClary, 2021); and
- diminished coating condition (e.g., dry film thickness [DFT] or scuffs and chips) that reduces antifouling performance and longevity (Naval Sea Systems Command, 2006; Lewis, 2013).

Given the potential for environmental harm, independent, transparent, and predictive testing of efficacy is needed to evaluate the performance of both Proactive and Reactive IWC systems. Such robust and standardized testing is critical for responsible use of IWC systems and the success of biofouling related policy and regulations (Tamburri et al., 2021).

1.5. Scope of IWC Testing Guidelines

The aim of these Testing Guidelines is to provide standardized, science-based test procedures that produce the data (and level of confidence) needed by permitting or regulatory authorities when assessing applications for IWC systems (Tamburri et al., 2021). These Guidelines describe how to produce data and reporting on the efficacy and safety of IWC systems for cleaning of ship surfaces and for the capture and disposal of cleaning debris. The data and reporting from test events should readily inform IWC service providers, ship operators, and jurisdictional agencies on the effects of IWC and can be used in permitting processes. The following procedures and methods could also serve as a resource for technology developers, environmental regulators, and other stakeholders interested in the safe and effective use of IWC systems.

More specifically, these Testing Guidelines provide detailed and rigorous procedures for the independent performance testing of all forms of IWC systems (i.e., both Proactive and Reactive systems) for external ship surfaces. This includes the various components or options of multicomponent systems for fouling removal (e.g., cleaning unit for flat hull surfaces and smaller handheld tools for more complex niche areas) or effluent treatment (e.g., physical separation for captured solid material and treatment for dissolved biocides and/or live organisms). These Guidelines were developed so specific IWC systems can be tested in a standardized way that is appropriate to their design, operational requirements/limits, and their individual service providers' claims.

The development of specific IWC performance criteria or standards are outside the scope of these Testing Guidelines. Similarly, systems designed to kill or prevent biofouling on external surfaces without removal, and systems that remove or treat biofouling on internal surfaces (e.g., sea chests, seawater intakes), are also outside the scope of this document.

These Testing Guidelines represent the consensus among international technical experts on the best currently available scientific approaches. However, it is also expected that some test methods will evolve or improve over time as our collective knowledge of this complex issue grows. Performance and safety of IWC systems is context-dependent with many sources of variation across ships, environments, and associated biota. As a result, use of these Testing Guidelines does not guarantee that a specific IWC system will always, or under circumstances other than those used in testing, operate at the levels reported.

1.6. Independent testing

These Testing Guidelines provide scientific guidance to a variety of stakeholders and end users, but have been specifically designed to inform the independent testing of IWC systems. To be acceptable as an independent testing organization (TO), a group should:

- have the recognized expertise and resources to completely, competently, and consistently test and evaluate IWC systems;
- be an independent, third-party – not owned, controlled, or influenced by any client, industrial organization, or any other person or institution with a financial interest in the product, system, or service being tested; and
- have a quality management system in place that:
  - is consistent with ISO/IEC 17025 (International Standards Organization, 2017) for testing; and
  - meets the requirements of ISO/IEC 17020 (International Standards Organization, 2012) as an inspection body (See also Sections 8 and 9 below for information on Data Management and Quality Assessments, respectively).

2. Fundamental Information Needed for Testing of IWC Systems

There are numerous factors which can impact IWC system performance and the means by which comprehensive, standardized testing is performed (Tamburri et al., 2020; 2021). These include, but are not limited to:

- ship (e.g., type, design, coating(s), ship and coating ages, operational profile and routes);
- biofouling (e.g., life history stage, type, coverage, location);
- environmental conditions (e.g., visibility, swell, current, ambient water quality);
- the IWC system (e.g., unique design features, operational requirements/limits, cleaning procedures); and
- IWC system operator training and experience.

Testing IWC systems is most appropriate and informative when performed under real-world conditions. However, the cost and complexity of full-scale operations on ships can prohibit extensive experimental replication, controls, and the isolation of single factors to measure their impact on overall performance and safety. Given the complexity of these variables, it is not feasible to examine all possible factors (singularly or in combination) that can impact IWC system performance and safety. Therefore, a list of fundamental parameters that should be either documented, characterized, or specifically tested for, as part of any independent evaluation, has been provided (Table 1). The listed parameters allow for linking test results to performance under specifically known (or measured) ship, biofouling, environmental, and IWC system characteristics. The experimental design and specific performance parameters for IWC system testing are described in Sections 3 – 7.

Table 1. Parameters that impact IWC system performance and evaluations. Reporting for these parameters falls into three general categories that serve to:

- *Document* - information that should be provided by the ship owner/operator and/or IWC system service provider, which appropriately describes test conditions;
- *Characterize* - information that should be collected (e.g., direct observations of cleaning mobilization/operations/demobilization and measures of test conditions) and reported by the independent TO conducting the testing; and
- *Test* - factors that should be directly targeted or manipulated as fundamental test variables (e.g., a direct test of IWC system claims).

Ship Parameters	**Document**	**Characterize**	**Test**
Ship type/function, age, size, and design drawings, with any relevant modifications (including complexities and niche areas)	X		
Ship recent routes/voyages and operational history over at least the past 12 months (including dry-docking, long idle periods, lay-up, and repairs)	X		
Ship availability/access for cleaning and/or testing (including dates, ports, time at dock or anchorage, any access restrictions)	X		
Ship coating(s) type, age, applied location, and history (including prior cleaning, damage, or repair)	X	X	X e.g., test to IWC system service provider claims on coating type (biocidal or fouling release), age or damage, which can influence environmental results
Ship fouling rating prior to testing (including type and percentage cover) and distribution on various surfaces		X	X e.g., test to IWC system service provider claims on fouling type/level/location and results from recent/relevant in-water biofouling inspections
Environmental Parameters	**Document**	**Characterize**	**Test**
Water visibility/clarity		X	
Tides, currents, wind, and waves		X	
Water quality at location of testing and during testing, at minimum: (a) salinity, (b) temperature, (c) total suspended solids, (d) particle size distribution, (e) dissolved organic carbon, and (f) particulate organic carbon		X	
Ambient levels of biocides during testing, if applicable (e.g., background levels of copper and zinc) and other contaminants of interest (e.g., microplastics) in water column at location of testing		X	

IWC System Parameters*	Document	Characterize	Test
IWC system design and function, and IWC mobilization, operations and demobilization	X		X
IWC system specifications, requirements, and limits	X		X e.g., test within specifications or to limits, including fouling (e.g., type, stage, and coverage), ship (e.g., size, materials, curvature, niche areas), coating type and appropriateness for cleaning, and environmental parameters (e.g., currents and visibility)
Mode of cleaning unit operations (e.g., diver-, remotely-, or autonomously-operated)	X	X	
Mode of cleaning unit attachment to, and movement on, ship surfaces	X	X	
Operator/diver skill and experience (as described by IWC service provider)	X	X	
Mode of biofouling (biofilms and/or macrofouling) removal (e.g., brushes, blades, or water jets, with details on type, amount, configuration, etc.)	X	X	
Rate and pattern of individual cleaning operations (e.g., speed of cleaning unit, number and overlap of passes, etc.)	X	X	X
If applicable, frequency of cleaning operations	X	X	X e.g., for Proactive IWC systems that prescribe a frequency of cleaning
If applicable, debris capture methods (e.g., cleaning unit shroud and suction)	X	X	X e.g., test within specifications or to efficacy limits
If applicable, flow rate of debris/wastewater capture	X	X	
If applicable, debris and wastewater transport and processing (e.g., particle settlement processes, type and level of filtration/separation, secondary treatment of biological waste [e.g., UV or chlorination], type media for removal of metals) and maximum load capacity	X	X	X e.g., test within specifications or to limits
If applicable, waste disposal processes (including volumes and mass)	X	X	
Various pre-set modes of operations and adjustments during cleaning, including contingency plans and response to unexpected conditions (e.g., presence of macrofouling during Proactive IWC) and system failures	X	X	

*Proprietary, or commercially sensitive, information on specific IWC technologies or approaches can be held confidential, provided enough basic information on system specifications, design, function, and operations are available to allow for an adequate understanding of performance and safety.

3. Test Experimental Design

The full IWC system is to be tested on a minimum of three distinct ships (i.e., $n \geq 3$). This level of replication is meant to provide fundamental information on system performance, environmental safety, and applicability across different conditions within the operational claims and parameters of the individual IWC system. A single test ship will not be able to provide all relevant challenge conditions for predictive IWC system testing.

The test ships and conditions chosen should capture as much relevant variability in key parameters listed in Table 1 as feasible. While the overall IWC system test unit of replication is the number of test ships (i.e., $n \geq 3$), additional sample replication within individual test trials of biofouling removal/prevention, changes to water quality, debris capture/processing, and ship coatings impacts, are described in Sections 4 – 7 (Figure 1).

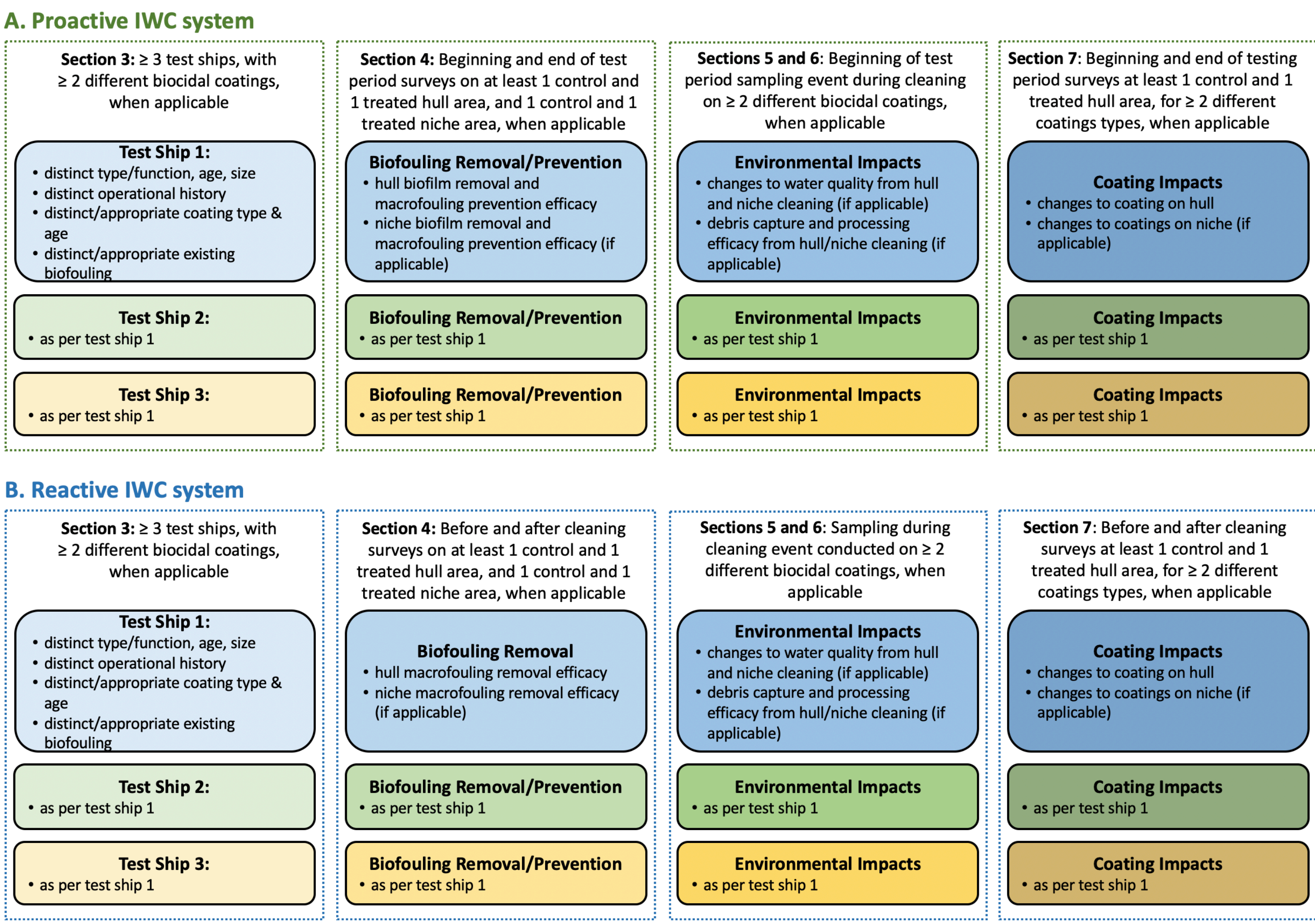

Figure 1. Basic testing components for a Proactive IWC system (A) and a Reactive IWC system (B). Details for each component are provided in Sections 3 – 7.

The ships selected, and specific tests conducted, should align with the claims of the IWC service provider, to the extent possible. For example, if it is claimed the IWC system can be used on all coating types, then ships with different biocidal and non-biocidal coatings should be included in testing. If the IWC system is claimed to be appropriate for use on large cargo ships with extensive macrofouling, then testing should include these ship types that have close to the upper limit of the claimed biofouling type and coverage. Likewise, if the IWC system is intended for use on both relatively flat hulls as well as intricate niche areas, then examples of both ship surface types, and distinct equipment used for different surface types, should be included in testing.

While it may not be feasible to directly examine all IWC service provider claims in one set of independent tests, extrapolation or prediction of performance and safety (beyond the specific conditions and parameters tested) should be avoided.

3.1. Classification of IWC system application for testing

Test trials, on diverse replicate test ships (*n* ≥ 3, varying in size, age, routes, operational profile, etc.) should incorporate specific evaluations of performance and environmental safety (Figure 1 and Sections 4 – 7). These should be based on the IWC system design, function and application, and the basic categories of key variables presented in Table 2.

Table 2. Simplified considerations for testing based on IWC system design and function.

Surface type	Fouling type	Coating type	Debris capture and processing
• Planar and curved **hull surfaces** (sides, bottom, wind-and-water line) vs • Angled and complex **niche areas** (protrusions and recesses) or • **All** surface types	• **Biofilm** (micro-organisms) vs • **Macrofouling** (macro-organisms) or • **All** biofouling types	• **Non-biocidal** coatings vs • **Biocidal** coatings or • **All** coating types	• **No debris capture** vs • **Debris capture and processing** (waste collection, treatment, and/or disposal)

The following two examples represent experimental designs that incorporate replicate test ships and the considerations provided in Tables 1 and 2 (fouling rating [FR] is described in Section 4.1).

- A Proactive IWC system could be tested for use only on hull surfaces with specific biocidal coatings on:
  a) a large, transcontinental tanker with a four-year-old biocidal coating, and existing FR 20 (with small infrequent patches of FR 30) biofouling;
  b) a modern roll-on/roll-off ship, just out of drydock with the same biocidal coating, and FR 0 to 10 biofouling; and
  c) a regional cruise ship, with a second appropriate biocidal coating, and FR 0 to 20 biofouling.
- A Reactive IWC system, with debris capture/processing, could be tested for broad applications on:

a) a large, transcontinental bulk carrier, with the same four-year-old biocidal coating on both hull and niche areas, and with existing biofouling ranging from FR 40 to 60;
b) a regional container ship, with one-year-old fouling release coating on the hull and second type of biocidal coating on niche areas, and with biofouling ranging from FR 20 to 40;
c) a local passenger ferry with a hard non-biocidal coating on both hull and niche areas, and with existing biofouling of FR 30; and
d) a local barge with no niche areas, a third type of biocidal coating, and with heavy biofouling ranging from FR 60 to 90.

The following sections on testing protocols and methods apply to all categories of IWC systems and applications. The exception is Section 6 (debris capture and processing), which only applies to systems that attempt debris capture/disposal and/or waste treatment. Additionally, where multicomponent systems have been developed to clean the hull and external niches, the different primary components (e.g., different cleaning units) should be tested to assess the complete IWC system's efficacy and safety.

3.2. Duration and extent of testing

All test trials should be conducted over at least a 90-minute cleaning event (see Section 5.3, and accommodations for niche areas), with the IWC system operating in a normal, defined cleaning mode for the conditions presented. Sampling for the various performance measures described below can take place in smaller designated subsections of the test ship's cleaned areas, or during a series of smaller time periods (minimum 90-minutes) of a full cleaning event. However, at least one test trial, on one test ship, should involve a substantial cleaning area (e.g., at least 1/3 of the test ship) over a realistic timeframe (e.g., several hours), representative of the expected typical application of the IWC system being tested.

Proactive systems - Proactive IWC is a biofouling management system, designed for periodic removal of biofilms and incipient macrofouling in order to inhibit the development of mature macrofouling growth over time. To account for the temporal aspects of the Proactive IWC approach, the test period for each replicate test ship ($n \geq 3$) should be at least 12 months. This period allows for an appropriate determination of IWC system performance regarding macrofouling prevention (comparing biofouling in control and treated test locations, see Section 4). A statement of test duration and results should be explicitly reported as performance may change beyond the period tested (e.g., performance at 12 months may not be indicative of performance at 24 or 48 months).

During the test period, the IWC system should be operated (e.g., cleaning locations on ship and frequency) as determined by the service provider, based on test ship's characteristics, operational profile, and environmental conditions. All test ship routes, operations, and IWC activities (mode, frequency, locations, etc.) should be documented and reported for at least six months prior to (when applicable) and during the testing period (≥ 12 months). Biofouling surveys (Section 4) and assessments of coating impacts (Section 7) should be conducted at a minimum of two timepoints during the testing period – at the beginning and end. Water quality sampling for environmental impacts (Section 5) and debris processing (Section 6, when applicable), should be conducted for at least one timepoint (i.e., at the beginning of the test period).

Reactive systems - Reactive IWC is a biofouling management system designed to remove established/existing macrofouling. Individual test trials (associated with a cleaning event) can, therefore, take place on each replicate test ship ($n \geq 3$) during a relatively short period of a few days to a week. All testing (Sections 4, 5, 6 when applicable, and 7) should take place during combined, comprehensive test trials of normal IWC system activities on individual test ships. This allows for the appropriate assessments of test conditions, system performance, and environmental safety before, during, and after IWC activities.

4. Quantification of Biofouling Removal and/or Prevention

The primary function of all IWC systems is to prevent or remove biofouling (either biofilms, macrofouling, or both) from the submerged surfaces of ships. Therefore, quantification of ship biofouling in response to IWC is essential for any assessment of system performance. A Before-After-Control-Impact (BACI) sampling design should be used to quantify or estimate change in biofouling assemblages associated with cleaning events/activities for each test ship. The BACI sampling design compares designated control (i.e., not cleaned) to treated (i.e., cleaned) test areas associated with a single cleaning event (for Reactive IWC) and/or over time (for Proactive IWC).

Proactive systems - To account for rapid changes in microfouling growth, before and after IWC surveys of biofilm removal (see Proactive systems under Section 4.3) should preferably be carried out on the day of test cleaning event, but no more than 24 hours before or after (note surveys for coating impacts in Section 7 have different timing considerations). However, the assessments of macrofouling prevention, over ≥ 12-months, do not need to be associated with a specific cleaning event.

Reactive systems - To avoid potential influence of natural fluctuations in biofouling communities, before and after IWC surveys of macrofouling should not be conducted more than three days prior to, or after, a test cleaning.

4.1. Fouling level

Testing should only be conducted on ship surfaces with a level of biofouling appropriate to the IWC system's intended use (i.e., at or just below maximum levels and to the extent possible to match the claims of the IWC service provider). There are several methods of rating ship biofouling type and percentage coverage, each having strengths and limitations. For consistency in measures of biofouling within and among tests, at minimum, the following categories of biofouling type and estimates of percentage cover should be used. However, other proven/accepted approaches can also be incorporated in testing, as needed (e.g., Naval Sea Systems Command, 2006; ASTM 6990, 2020). For comparative purposes, the approach chosen should be consistent over the replicate ($n \geq 3$) test vessels.

These Testing Guidelines use two categories of biofouling (microfouling and macrofouling) relevant to the two fundamental types of IWC (Proactive and Reactive). Microfouling is essentially a biofilm that comprised of bacteria, fungi, microalgae, protozoans, and other microscopic organisms not visible by eye. Macrofouling are individual, multicellular organisms (e.g., barnacles, mussels, tubeworms, tunicates, and seaweeds), and colonies of organisms (e.g., bryozoans, hydroids, corals, and sponges), larger than 5 mm (in any dimension) or visible by eye.

Biofouling type categories (Naval Sea Systems Command, 2006):
- Microfouling (fouling rating [FR] 20 or less);
- Moderate (soft) macrofouling (FR 30);
- Moderate (hard) macrofouling (FR 40 – 80); and
- Heavy (hard) macrofouling (FR 90 or greater).

Percentage cover categories (Floerl et al., 2005):
- Absent (0%);
- Light (1 – 5% of the visible submerged surface);
- Considerable (6 – 15%);
- Extensive (16 – 40%); and
- Very heavy (41 – 100%).

Proactive systems - Specific types and levels (FR) of biofilms (i.e., characterized by diver observations, photographs, and/or videos, Section 4.3) for Proactive IWC system assessments include:
- No microfouling (biofilm absent) (FR 0);
- Light microfouling (biofilm thin and light in color, FR 10); and
- Full microfouling (biofilm thicker and darker in color, FR 20).

The presence of any observed macrofouling (> FR 20) should be avoided during the testing of standard Proactive IWC systems. If a Proactive IWC system is specifically designed to avoid unexpected macrofouling, that operational feature can be incorporated into the testing and reported. In the case of a Proactive IWC system claiming to safely and effectively remove both biofilms and some minimal level of unexpected macrofouling (e.g., small rare patches of soft growth), that system should be tested as a Reactive IWC system. However, the testing of such a hybrid system for efficacy and safety in removal and prevention of both micro- and macrofouling should be measured over time (at least 12 months).

Reactive systems - Biofouling surveys (Section 4.3) should be used to provide a coarse level of macrofouling taxonomy (e.g., barnacles, bryozoans, tubeworms, macroalgae, etc.) and, when obvious, estimates of physiological state (i.e., live or dead). However, only determinations of presence or absence of macrofouling, irrespective of species origins or physiological state, should be used in the testing of IWC systems. The straightforward determinations of presence/absence and magnitude of macrofouling addresses the primary ship operational concern (i.e., drag and fuel consumption). Such determinations are also far less prone to error and uncertainties compared to attempts at appraising the origins of individual organisms and if they are alive or viable. Further, they provide a conservative/environmentally protective approach for assessing IWC systems (see Tamburri et al., 2021). Residual baseplates, or basal shell material remaining after cleaning (when clearly devoid of the prior macro-organisms), should be recorded and reported as a separate category from the biofouling described above.

4.2. Control and treated test locations

Pairs of control and treated locations on a test ship are needed to ensure possible confounding issues are accounted for during testing. These control and treated areas should be distinct but as similar to each other as possible (e.g., same coating type/age, depth below the surface, size, shape, and side of a ship). These locations should also be representative of the range of surface types that the specific IWC system claims to be able to safely and effectively clean. Selection of test locations can be accomplished by the IWC service provider designating where they can and cannot clean based on the test ship design drawings and fouling type and coverage. If the service provider claims to clean only relatively flat hull surfaces, then one set of large control and treated locations (e.g., 20 – 30 m length of the ship, from the waterline to the keel) may suffice. However, if there are claims that other surface types can be cleaned (such as the flat bottoms of the keel, rudders, and sea chest grates, etc.) then similar paired control and treated locations should be designated for testing at a smaller appropriate scale (see Section 4.3). For example, to remain consistent with the BACI design, one half of a designated niche area can be cleaned (treated) and the other half not cleaned (control), or one niche area (e.g., bow thruster) is completely cleaned and another comparable paired niche area designated as the uncleaned control. Similarly, if the IWC system claims it can be used on multiple coating types (e.g., biocidal and fouling release), paired control and treated test locations for different coating types can be designated on one individual test ship, when possible.

4.3. Biofouling dive survey sampling methods

Biofouling dive surveys should be designed to quantify and document both biofilms and macrofouling in the designated control and treated locations of each test ship and surface type (Table 2). Quantitative surveys should be conducted by trained divers, to provide comprehensive, robust, and repeatable measures.

Hull areas - Biofouling surveys of hulls (and other relatively flat surfaces) should use a quadrat method to delineate 1 m^2 plots. Each 1 m^2 quadrat should be vertically divided into four bands using five equally spaced straps. Each strap should be demarcated to create a 50-point grid (Figure 2). Four photographic images should be captured within each band. Each image (i.e., “sub-plot”) will be 18 x 24 cm in size. A total of 16 sub-plots should be imaged covering the entire area within each 1 m^2 plot.

Estimates of fouling rating and percentage cover should be made from composite photographs of each plot (Figure 2). If poor visibility prohibits photography, the full area of sample plots should be visually inspected using point counts as described in Section 4.4. Any additional video collected by the IWC system itself, during surveys or cleaning events, should be provided to the testing team. An example of a dive survey scheme can be found in Figure 3, including the defined surface types within a single treated (cleaned) or control area as described in Table 3.

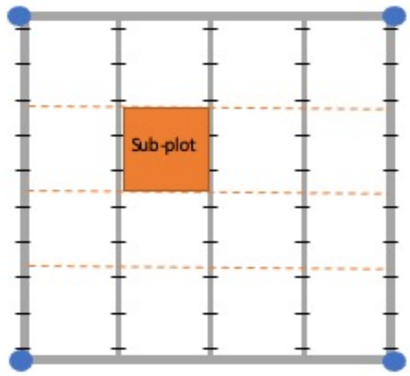

Figure 2. Example of a quadrat frame containing four survey bands used to survey each 1 m^2 plot. A total of 16 sub-plots (bounded by the dashed lines between bands) can be imaged and/or 50-point counts defined. Blue circles represent magnets to attach the quadrat for sampling.

Condition of hull surfaces should be captured using video and/or digital still imaging and/or visual observations stratified by the surface types (Figure 3). Within each surface type, divers should evaluate at least six, randomly placed, 1 m^2 plots, corresponding with recommendations in Morrisey et al. (2015) and Tamburri et al. (2020). If the IWC system is claimed to clean angled surfaces (edges), linear transects along edges should be photographed immediately adjacent to the edge (i.e., not a full 1 m^2 quadrat around the edge, but a single 25 cm x 1 m band centered along the edge).

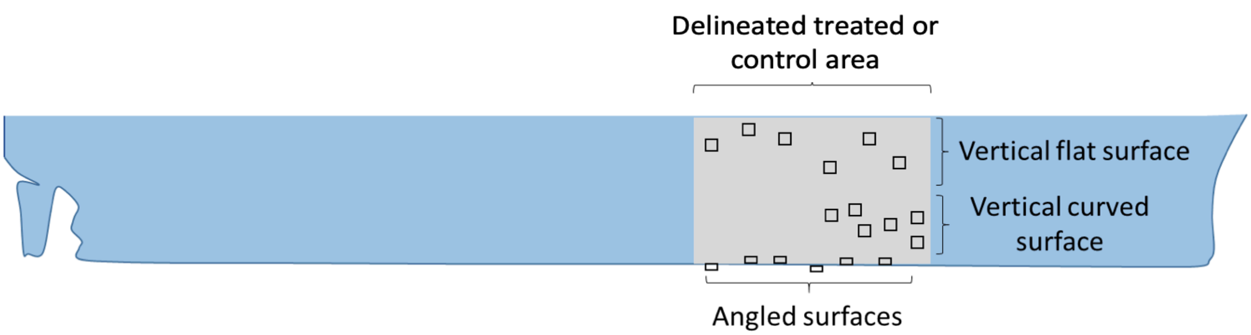

Figure 3. Pictorial representation (not to scale) of an example delineating a treated or control area (grey), surface types within the test area, and a stratified, randomized six replicate plots within each surface stratum (black squares). The bottom area, not visible in this figure, should also be sampled using six replicate plots.

Table 3. Example of a dive survey biofouling quantification scheme for one of the control or treated test areas. In this example, four total surface types are to be tested.

Surface Type	Number of Plots	Number of Images Within One Plot	Total Photos
Vertical flat	6	16	96
Horizontal flat	6	16	96
Vertical curved	6	16	96
Angled surfaces	6	4*	24

* Six plots, of the same width/dimensions, but photos would only be taken of angled surfaces (e.g., edge of bilge keel) within each plot.

Niche areas - For relatively small niche areas (e.g., paired control and treated small gratings), quantification of biofouling in the entire test areas is recommended. For larger niche areas, representative quadrat sub-sampling maybe needed. While there is no requirement for specific quadrat sizes or shapes (e.g., square, rectangular, circular), the sampling design for niche areas should be standardized and provide consistent data across tests and replicate ships. Therefore, quadrats or even transect samples can be fit to the niche area shape and size. Sampling and replication approaches can be scaled down appropriately for use on smaller niche areas (e.g., Morrisey et al., 2015). For example, quadrats of 0.01 m^2 have been employed successfully for the quantification of biofouling percentage cover and composition in ship niche areas (e.g., Frey et al., 2014). Sampling can also be further stratified into sub-niche areas. For example, rudders can be divided into trailing edge, bottom edge, leading edge, hinge gap, and side face (Figure 4), or thrusters can be divided into rim, grate, tunnel, and mechanism case (Davidson et al., 2014).

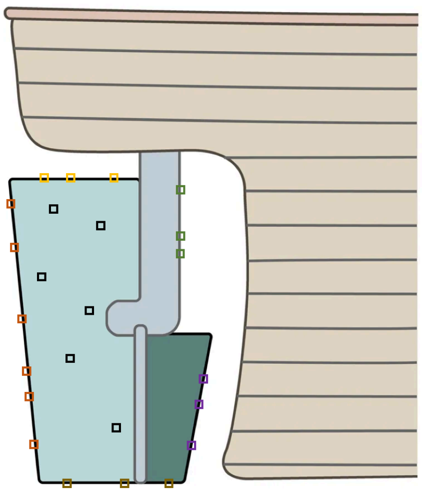

Figure 4. Pictorial representation (not to scale) of an example delineating a treated or control niche area, and sub-niche areas. In this example, one side face of the rudder can be treated/cleaned and the other designated as the control. The randomized three to six replicate plots within each sub-niche area of the rudder are designated by different colored squares.

Hull and niche areas - Differences in biofouling percentage cover and biofouling composition should be determined among areas sampled using appropriate statistical analysis (e.g., non-parametric Kruskal-Wallis tests and PERMANOVA test, respectively; Tamburri et al., 2020). If macrofouling is found to be rare or extremely patchy, well described and validated methods for lower resolution video transects examining larger areas can be considered in addition to the methods described here.

Qualitative biological samples may also be collected at the end of the sampling period to provide better determinations of dominant macrofouling taxa present. However, these samples should only be collected in a way that does not influence any testing assessments of biofouling or coating condition.

Proactive systems - In addition to the presence, level and percentage cover of biofilms measured at the beginning and end of each test ship's testing period (as described above), an assessment should also be conducted to qualitatively estimate the efficacy of biofilm removal at least once, on the hull of each test ship. The assessment consists of two surveys:

- One pre-cleaning survey should occur within 12 hours before in-water cleaning begins; and
- One post-cleaning survey should occur within 12 hours after in-water cleaning ends.

The two surveys should be: (a) conducted in a location on the test ship that is scheduled to be cleaned; and (b) consider the different surfaces that the IWC system can clean. A survey within the predesignated treated test area would be preferred, but not required. Randomly placed 1 m^2 quadrats (locations determined in the water by individual divers) should be used to document the biofilm in the designated area, with at least 12 replicates sampled before and 12 replicates sampled after cleaning activities. After the quadrat is attached to the hull, a diver should remove the biofilm from two of the four quadrat bands (Figure 5).

Existing biofilm should be removed by hand using one or more clean sponge per wiped band. This will enable observers to visually compare biofilm on the ship surface to a set standard/baseline (bare surface) in a photo comparison. The single-use sponge(s) per band should be photographed for observations of any collected biofilm material. The dive team should then document the biofilm within each plot by taking at least three randomly placed photographs of a small 18 x 24 cm sub-section. For a direct comparison, each image should capture both wiped (cleaned by hand) and unwiped (undisturbed) spaces side-by-side (Figure 5). Once the plot has been photographed, the quadrat should be removed from the surface and placed at another location (selected at random) within the designated area on the ship surface and the survey repeated. This method has been designed for relatively flat hull surfaces. If the Proactive IWC system is claimed to clean angled surfaces (edges) and other niche areas, modification to the method can be made, such as linear transects along edges or scaling down to appropriate dimensions and image number (as discussed above).

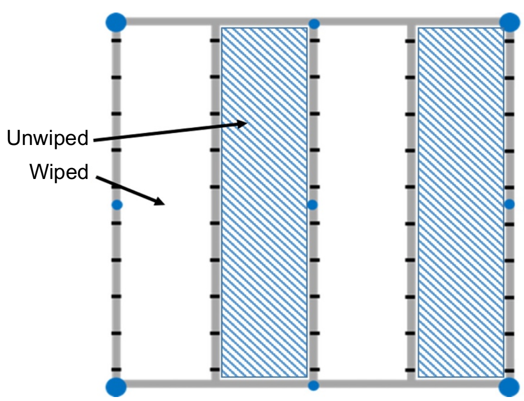

Figure 5. Examples of a 1 m^2 quadrat used to determine biofilm cover by using percentage cover visual estimates and fouling ratings within the four bands. The two quadrat bands (0.25 m^2 each) identified in white will be sponge-wiped of biofilm. Side-by-side photos will compare wiped versus unwiped sections (blue). In addition, several single photographs including both wiped and unwiped sections in the same image should be captured.

While individual Proactive IWC systems may put forward claims with respect only to biofilm removal efficacy, or only to macrofouling prevention efficacy, it is recommended that testing of both indicators of performance (presence/absence of biofilms before and after cleaning events and presence/absence of macrofouling growth over time, as described above) be included for comprehensive, standardized testing.

4.4. Dive survey sampling method during low visibility

Hull areas - In low visibility conditions, when still images are unreliable for biofouling surveys, an *in-situ* method should be used. Similar, comparable analyses (for quantifying biofouling type and extent) can be conducted directly by the diver, rather than during post-dive image analysis (e.g., Tamburri et al., 2020). For each hull area sampled, 1 m^2 quadrats should be placed in the test areas for at least 6 plots per surface type. The quadrats should be used to determine biofouling cover (biofilm and/or macrofouling) by: 1) using a point count method of the 50 evenly spaced points delineated on the bands of the 1 m^2 area; and 2) using percentage cover visual estimates within each of the four bands (Figure 6). Biofouling should be identified to FR rating as described in Section 4.1. Divers should use data sheets and dive slates to record data in the field. After a quadrat is positioned, one diver uses an underwater light to illuminate the sampling area while the other records the data.

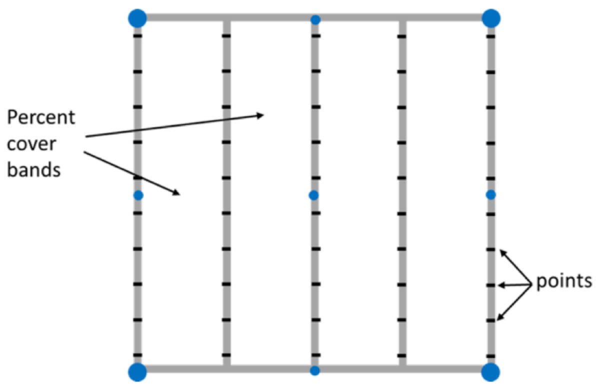

Figure 6. Example of a 1 m^2 quadrat that should be used to determine biofouling cover in low visibility conditions.

Differences in biofouling percentage cover obtained from point counts should be determined among areas sampled (before/after, treated/control, by surface type) using appropriate statistical analysis (e.g., non-parametric Kruskal-Wallis tests and PERMANOVA test).

Other low visibility biofouling survey methods, such as photographs using a camera water-box system on relatively flat surfaces (e.g., Hearin et al., 2016; Ralston et al., 2022), can be considered for use if validated.

Niche areas - The quantification of biofouling in niche areas may be particularly challenging under low visibility conditions. As described above, quadrats and transect samples can be fit to the niche area shape and size and sampling approaches can be scaled down appropriately for use on smaller niche areas (e.g., Morrisey et al., 2015). However, the determination of type and extent of biofouling in niche areas should only be conducted if the appropriate level of data quality can be confirmed (e.g., low visibility biofouling surveys of hull areas, Tamburri et al., 2020).

4.5. Environmental characteristics to quantify

In conjunction with all biofouling dive surveys, the minimum background environmental characteristics that should be observed or measured and reported are provided in Table 5.

Table 5. Environmental characteristics to be recorded during biofouling dive surveys.

Environmental characteristic	Method of measurement
Water temperature	Single or multiparameter instrument/thermometer
Salinity	Single or multiparameter instrument/salinity meter
Water clarity/turbidity	Secchi disc and/or turbidity sensor
Wind speed and direction	Hand-held anemometer or a nearby weather station
Current speed and direction	Current meter
Tide	Local tide tables and visual observations
Sea state	Beaufort scale (www.weather.gov/pqr/beaufort)
Air temperature	Thermometer or data from a nearby weather station
Weather	Visual observations (precipitation, cloud cover, etc.)

Note: additional measures (e.g., water dissolved oxygen and pH) may be informative, or required to test service provider claims, can be added as needed.

5. Quantification of Changes to Water Quality

5.1. Water quality measures as proxies for broad environmental impacts

All testing should be coordinated with local, regional, or national authorities, as necessary, as such entities may have specific water quality standards or threshold requirements regarding the release of contaminants (chemical, physical, and biological) during IWC. As these standards can vary greatly among locations, all testing of IWC systems should report total or absolute values (e.g., means and standard deviations) of the measured water quality parameters (Section 5.2) during and adjacent to IWC activities (Section 5.3). Those values should also be compared statistically (e.g., Student's *t*-test) for significant differences from normal ambient or background ranges of the same parameters at the location of testing.

Proactive systems - For each of the replicate test ships ($n \geq 3$), samples should be collected and analyzed as part of at least one specific cleaning trial at the beginning (T0 or beginning of testing) of the ≥ 12-month test period. For each ship, the water quality sampling should be repeated during trials for hull and niche and/or coating type (i.e., biocidal coating and non-biocidal coating) cleaned. For systems that clean biocidal coatings, a minimum of two distinct biocidal coating types should be tested (e.g., a different biocidal coating on two of the test ships) and samples analyzed for all relevant biocides and co-biocides (i.e., "booster" biocides).

Reactive systems - Samples should be collected and analyzed during trials for each of the replicate test ships ($n \geq 3$) and repeated for each surface type (e.g., hull and niche) and/or coating type (i.e., biocidal coating and non-biocidal coating) cleaned For systems that clean biocidal coatings, a minimum of two distinct biocidal coating types should be tested and samples analyzed for all relevant biocides/co-biocides.

5.2. Water quality parameters to quantify

Environmental impacts from IWC include potentially unacceptable changes to local water quality and sediment conditions and the potential release of live organisms. The following parameters should be analyzed (from collected water quality samples) as practical, measurable, and conservative proxies for environmental impact (Table 6; Tamburri et al., 2021).

Table 6. Water quality parameters to be measured for IWC systems and their purpose.

Parameter	Purpose
Total suspended solids (TSS)	Represents the mass of particulate material, in background water and water near cleaning activities, which will include possible biofouling organisms, coating material, and IWC associated fragments.
Particle size and distribution (PSD)	Indicative of the type/characteristics of the solid material found in TSS analyses.
Particulate organic carbon (POC) Dissolved organic carbon (DOC)	Indicative of the amount of biological material present.
Test ship coating total and dissolved biocide(s) (e.g., copper- and/or zinc-based compounds)	Measure of possible ship coating associated biocide release, when applicable.
Microplastics (MP) Nanoplastics (NP)	Measure of possible ship coating associated polymer release.

The appropriate volumes, for all subsamples and various analyses, should be placed in suitable, cleaned bottles (e.g., glass containers and sample bottles for assessment of MP/NP). All sample bottles should be labelled with unique identification numbers prior to sampling. To ensure analytical validity, all samples should be stored and transported within the appropriate time, temperature, and light requirements.

To the extent possible, certified, standardized, and/or validated analytical methods should be used. Table 7 provides some examples of analytical methods that can be employed.

Table 7. Sample type and examples of accepted analytical methods (and limits of detection) for measuring water quality parameters. Any analytical method used should be validated and accepted by relevant IWC approval or permitting authority.

Sample Type	Analytical Method	Method Detection Limit	QA Reporting Limit
TSS	NASLDoc-030, SM208 E, SM2540D, EPA 160.2	2.4 mg TSS/L (2019)	2.4 mg TSS/L (2019)
POC	NASLDoc-033, EPA 440.0	0.0633 mg C/l (2019)	0.0633 mg C/l (2019)
DOC	NASLDoc-014, SM5310B/C	0.16 mg/L DOC (2019)	0.50 mg/L DOC (2019)
PSD	ISO-13322-1	>10 – 1000 µm (2020)	>10 – 1000 µm (2020)
Particulate and Dissolved Copper (Cu)	EPA 200.8/EPA 6020A	0.1 $\mu g\ L^{-1}$ (2018)	0.5 $\mu g\ L^{-1}$ (2018)
Particulate and Dissolved Zinc (Zn)	EPA 200.8/EPA 6020A	Diss. 0.5 $\mu g\ L^{-1}$ Part. 0.1 $\mu g\ L^{-1}$ (2018)	Diss. 1.0 $\mu g\ L^{-1}$ Part. 1.0 $\mu g\ L^{-1}$ (2018)
Microplastics (MP) Nanoplastics (NP)	ISO/DIS 24187, ASTM D83302-20		

5.3. Water quality sample collection

Discrete and continuous, time-integrated water samples should be collected at two stations for every IWC system test trial (i.e., during a cleaning event). One of the two stations is designated for measuring background/ambient conditions adjacent to the test ship. This station should be at ≥ 50 meters away from, and clearly not impacted by, the test cleaning activities (e.g., mid-depth, up current, and/or opposite side of the test ship). The second station should be directly on the IWC system's cleaning unit, at a point designated as the most likely to be the greatest source of possible environmental release (Table 8). At both stations, during each test trial, a series of three sequential, continuous time-integrated samples should be collected simultaneously over at least a 90-minute sampling period (Figure 6 and 7).

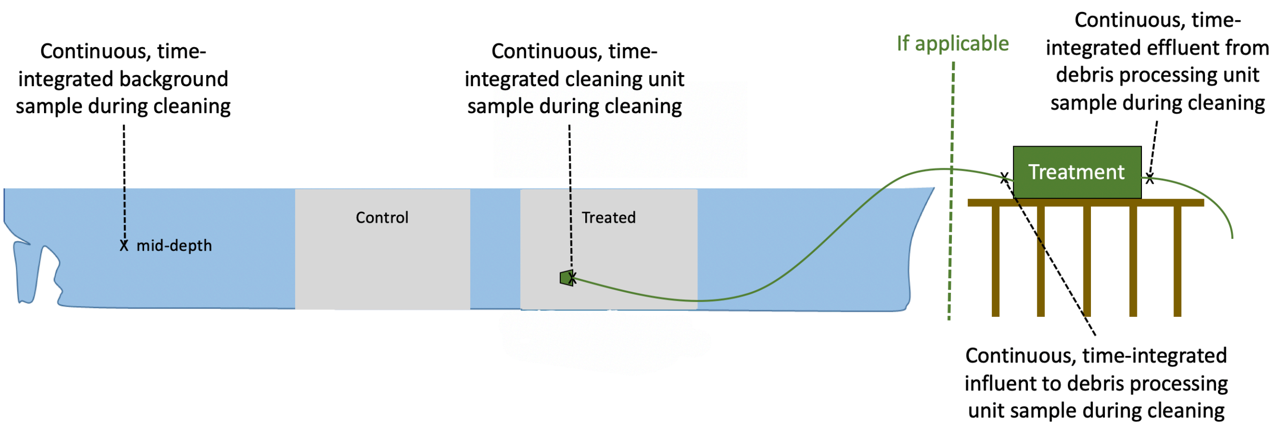

Figure 6. Pictorial representation (not to scale) of water quality sampling locations during a test cleaning event. Debris processing and effluent impacts are described in Section 6. Not pictured are the discrete samples of background/ambient conditions taken before and after a test trial at the same location.

It may not always be possible to identify—and position a single sample collection point on—the part of the IWC system cleaning unit with the largest potential for environmental release. The TO should work with the IWC system service provider to understand the cleaning unit design and operations (e.g., computational fluid dynamics models or video assessments of debris plumes) to optimize sample collection point placement to the extent possible.

Both samples, one for ambient/background and second on the cleaning unit, should be collected with the appropriate pump and hose systems (e.g., sufficient power and flow rates) into a series of sample collection containers or carboys. Care should be taken to ensure sampling equipment and methods do not introduce artifacts (e.g., sample contamination with metals or polymers), including appropriate controls and blanks (See Section 8). Both 90-minute (or more) continuous time-integrated sample events (background and cleaning unit) should then be divided into three 30-minute (or more) sequential samples. This provides a level of sample replication and insights into possible temporal changes in water quality during a test cleaning event (Figure 7, Table 8).

Three appropriately sized sample containers for each sequential sample should be placed at the end of the sampling hose for both the background and cleaning unit and filled sequentially at a standardized flow rate suitable to collect the needed analytical volumes (e.g., 20 to 40 L) over a 30-minute sampling period. After the first container is filled (at the 30-minute mark), the hose can be moved to the second container, and then to the third container at the 60-minute mark (Figure 7). Each sample container should then be uniformly mixed prior to sub-sampling for analysis of specific water quality parameters (Section 5.2).

While 90 minutes (or more) is typically required for IWC of large areas of a ship's hull, smaller niche areas may take far less time. When possible, at least single 30-minute continuous, time-integrated samples should be collected (background and cleaning unit) to assess discharges during niche area cleaning.

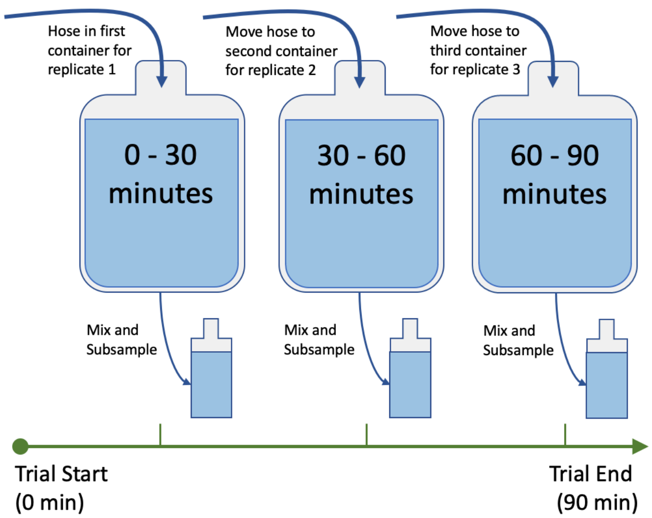

Figure 7. Pictorial representation of three-replicate, continuous, sequential, time-integrated water quality sampling, at each sample location, during a test cleaning event.

Table 8. Description of minimum water quality sample collection during an individual IWC system test trial.

Purpose	Station	Number of sample events	Number of continuous samples per event
Background or ambient conditions during cleaning	≥ 50 m upstream of system	1 per test ship and per each test cleaning location on test ship > 90-minute samples*	3 at minimum intervals of: 0-30 minutes 30-60 minutes 60-90 minutes
Potential environmental release from cleaning unit during cleaning	On IWC system cleaning unit	1 per test ship and per each test cleaning location on test ship > 90-minute samples*	3 at minimum intervals of: 0-30 minutes 30-60 minutes 60-90 minutes

*Sampling duration can be reduced for niche areas, dependent on size of area being cleaned.

As water quality conditions will vary over time at a single location (e.g., based on season, tides, storms, local ship traffic), ambient background water quality should also be characterized by discrete sampling at the testing location before and after a test cleaning event (Table 9). This background station should be located near the test ship or test berth/anchorage, at an appropriate depth (e.g., mid-depth of the test ship), and collected using a water sampling device such as a van Dorn or Niskin bottle, or rapid pump sampling system (Table 9).

Table 9. Description of minimum background water quality sampling before and after an individual IWC system test trial. Background sampling should be designed to capture, to the extent possible, the range of relevant ambient water quality parameters at the testing location.

Timing relative to IWC system test	Number of samples
One day before Three different times (at least 2 hours apart)	3
One hour before	1
One hour after	1
One day after Three different times (at least 2 hours apart)	3

5.4. General environmental characterization

In conjunction with all assessments of water quality, background environmental characteristics should be observed or measured and reported as per Section 4.6; Table 5.

6. Quantification of Debris Processing and Effluent

This section describes methods for: 1) quantifying debris processing efficacy; and 2) any potential changes to water quality from debris processing effluent. Therefore, it only applies to IWC systems that attempt some form of debris capture and treatment or removal. Debris processing can include treatments such as particle settling, filtration, flocculation, selective binding media (e.g., to remove metals), and disinfection treatments (e.g., UV, heat, chlorination). The waste processing units for the majority of existing IWC systems that are designed to capture, process, and dispose of biofouling and coating debris are located above the water surface on support boats, barges, or dockside.

Some IWC systems may only include a simple form of debris capture as part of the submerged cleaning unit (e.g., coarse filtration with periodic emptying of nets above the surface). This section does not apply to such IWC systems, as the discharge from of this approach can be assessed, with targeted sampling, as described in Section 5.

6.1. Sampling the debris processing unit

To estimate biofouling and coating related waste processing efficacy, the debris processing unit influent (i.e., water and material entering the unit) and effluent (i.e., water and material released from the unit back into the environment) should be sampled. Sampling should be conducted using a series of sequential, continuous time-integrated water samples as part of any IWC system test (Figure 7). While percent reductions (i.e., effluent compared to influent) in various water quality parameters can provide insight on the performance of the debris processing unit, they do not allow for an appropriate assessment of possible effluent impacts on local waters (Tamburri et al., 2021). Therefore, the priority assessment of environmental impacts should be a statistical comparison of: 1) effluent water quality parameters versus simultaneous measure of background/ambient conditions; and 2) discrete background samples collected before and after test cleaning events (as described in Section 5.1).

For each test area cleaned during each test trial, simultaneous, continuous time-integrated samples should be collected: 1) at a suitable influent sample port; and 2) at a suitable effluent sample port (i.e., to produce representative samples). Sampling should occur over at least a 90-minute period for a hull cleaning event and target at least 30-minutes for a niche area cleaning event. Again, both 90-minute continuous time-integrated sample locations (influent and effluent) should then be divided into three 30-minute sequential samples (Figure 7) to provide sample replication and insights into possible temporal changes in water quality. Care should again be taken to ensure sampling equipment and methods do not introduce artifacts (e.g., sample contamination with metals or polymers), including appropriate controls and blanks (See Section 8).

For most IWC systems tested, it will be necessary to allow sufficient time between the start of the cleaning of each section of the hull and the collection of samples of effluent to allow residual effluent in the system to be flushed through. This can be determined by calculating the volume of water required to flush the residual effluent or by running aliquots of an environmentally safe dye (e.g., rhodamine or fluorescein) through the system at appropriate times.

The three appropriately sized sample containers should be placed at the end of the sampling hose and filled at a standardized flow rate suitable to collect the needed analytical volumes (e.g., 20 to 40 L) over a 30-minute sampling period. After the first container is filled (at the 30-minute mark), the hose can be moved to the second container, and then to the third container at the 60-minute mark (Figure 7 and Table 10). Each sample container should be uniformly mixed prior to sub-sampling for analysis of specific water quality parameters (Table 6).

Table 10. Description of minimum water quality sample collection for testing IWC system debris processing unit.

Purpose	Station	Number of sample events	Number of continuous samples per event
Quantify the captured dissolved and particulate material removed from vessel	Processing unit influent	1 per test ship and per each test cleaning location on test ship > 90-minute samples*	3 at minimum intervals of: 0-30 minutes 30-60 minutes 60-90 minutes
Potential environmental release from debris processing unit	Effluent release point	1 per test ship and per each test cleaning location on test ship > 90-minute samples*	3 at minimum intervals of: 0-30 minutes 30-60 minutes 60-90 minutes

*Sampling duration can be reduced as needed, dependent on size of area being cleaned.

6.2. Water quality parameters to quantify

Discharge of debris processing effluent should be assessed through a direct statistical comparison of the same water quality parameters (e.g., TSS, PSD, POC, DOC, biocides, MP, and NP) in effluent water and in background water, as described in Section 5.1.

The appropriate volumes, for all subsamples and various analyses, should be placed in suitable, cleaned bottles (e.g., glass containers and sample bottles for assessment of MP/NP). All sample bottles should be labelled with unique identification numbers prior to sampling. To ensure analytical validity, all samples should be transported and stored within the appropriate times, temperatures, and light requirements.

To the extent possible, certified, standardized, and/or validated analytical methods should be used. Table 7 provides examples of analytical methods that can be employed.

The IWC system service provider should supply a standard operating procedure (SOP) for the debris processing unit, including the frequency of activities (e.g., changing or cleaning of filters and filter cartridges) to prevent failure of the unit. The use of the debris processing unit during the test shall be audited against this SOP. The parts of the waste treatment system above the water surface shall be monitored for leaks, overflows, or alarms A log of system operations/performance shall be kept, noting any problems, such as blocked or ruptured filters, or leaks. Contingency plans to manage the risks and rectify system failures should be included in the SOPs. Video recordings (five minutes or more) of the debris processing unit operations and functioning (e.g., control panel, solid waste separation and collection, effluent discharge) should be captured at the beginning, middle, and end of each test trial as supporting documentation of service provider claims.

Some IWC system debris processing units may include an effluent disinfection stage to kill or render organisms "non-viable" prior to environmental release. Where this is the case, additional data should be collected to document proper dose and/or operations of the disinfection process, rather than measuring or estimating live or viable organisms (see Tamburri et al., 2021). Measured water quality values (Section 5.2) will also provide fundamental insight on the possible effluent release of macro-organisms of concern (e.g., very few, if any, particles > 10 µm in size may indicate reduced biosecurity risk from macro-organisms).

The IWC system provider should supply details of the disinfection method employed (e.g., UV, heat, biocide) and existing data to verify its efficacy on various aquatic organisms (including propagules and pathogens) that may be found within the effluent. This should include results of pre-treatment of the effluent (e.g., filtration to a specified size) required to achieve an efficacious secondary process. Efficacy can be deduced from scientific literature demonstrating doses known to effectively treat ship's ballast water, effluent from land-based aquaculture or seafood processing facilities, or municipal wastewater (Georgiades et al., 2021).

During each IWC system test it should be confirmed that the treatment system is meeting specifications (e.g., concentration, time, temperature) via independent observations or measurements, as appropriate. A log of disinfection system operations/performance shall also be kept, noting any problems with these systems. Methodologies will vary dependent on the type of treatment system.

The total volume of wastewater produced, processed, and/or discharged during a test cleaning trial should be documented/reported and linked directly to the total area and duration of the cleaning event. This can be estimated via repeated measurements using a flow meter attached to the system or through siphoning off the flow for direct volumetric measures under timed conditions.

6.3. Solid waste disposal

For each test cleaning trial, the total wet mass and/or volume of solid waste material removed during debris processing should be quantified and characterized to the extent possible. This should also be directly linked to the total area and duration of the specific cleaning event, as well as the type and abundance of biofouling on the surface before cleaning. All documentation associated with the appropriate disposal of solid waste should also be reported. While waste disposal requirements are determined by each port or local jurisdiction, proof of compliance should be provided.

7. Quantification of Ship Coating Impacts

Observations of coating physical condition on various ship surface types before and after cleaning should be recorded (photos and/or videos) and reported. For comparative purposes, the approach chosen should be consistent over the replicate ($n \geq 3$) test ships and incorporate existing approaches for coating assessments (e.g., Paint Deterioration Rating Scale in NSTM, 2022). These should be done as part of the diver biofouling surveys described in Section 4, noting considerations for Proactive IWC systems. Observations regarding physical condition of the coating can include, but are not limited to, visible scratches, brush marks, paint flakes, pitting, bare metal/polish through, and blemishes.

For a more quantitative estimate of potential IWC system impacts on ship coatings from relatively flat hull locations, dive surveys of both control and treated areas should include repeated measures of coating dry film thickness (DFT). Digital DFT gauges, with sensors designed for underwater use, are an accepted (e.g., Tribou and Swain, 2017), non-destructive means to measure total coating thickness on submerged ship surfaces. There are multiple types of sensors or gauges (e.g., electromagnetic induction, eddy current, ultrasonic), each designed to take measurements on specific hull substrates (e.g., ferrous or non-ferrous).

The gauge used should be:

a) appropriate for the hull substrate;
b) appropriate for underwater measurements;
c) calibrated, operated, and maintained to manufacturer's specification; and
d) have a specified accuracy of at least ± 3%.

Divers collecting DFT measurements should also carry a reference surface (i.e., a clean piece of bare metal) to verify zero (blank reading) while at each sampling location. DFT sampling locations should be within the designated control and treated test areas described in Section 4.2 and Table 11.

Unlike the stratified, randomized design described above for biofouling assessments, the use of specific predesignated sampling locations for evaluation of coating condition or thickness allows one to confidently sample the same area before and after cleaning. If sampling is not completed at the same locations, variation in coating thickness across relatively small scales (< 0.5 m) may impact the results. DFT measurements should not be taken through any more than a light biofilm (FR 10), as this will impact results. There can be a latent change in DFT immediately after cleaning, which can also influence results. When possible, it is recommended not to take post-cleaning DFT measurements on the same day as the cleaning event (note Section 4 for biofouling assessments for Proactive IWC systems). Finally, DFT sampling locations should not be over high build protective or fairing compounds, such as the area around Impressed Current Cathodic Protection (ICCP) systems.

Proactive systems - For each test ship ($n \geq 3$), DFTs should be measured at least once before the first test cleaning at the beginning of the testing period and once at the end of the testing period (i.e., after ≥ 12 months) after the last test cleaning. In both the designated control and treated hull locations on each test ship, 50 individual gauge measures of DFT should be collected in at least 10 replicate 1 m^2 quadrats. Both the individual quadrat means and standard deviations, and the overall control and treated location mean and standard deviation, should be calculated and compared statistically for each survey and over time.

Reactive systems - On each test ship ($n \geq 3$), DFTs should be measured within three days before and three days after each test cleaning event (but not the same day, if possible). In both the designated control and treated hull locations on each test ship, 50 individual gauge measures of DFT should be collected in at least 10 replicate 1 m^2 quadrats. Both the individual quadrat means and standard deviations, and the overall control and treated location mean and standard deviation, should be calculated and compared statistically for each survey.

Table 11. Example of before and after dry film thickness (DFT) survey scheme for control and treated test areas, for each test ship ($n \geq 3$), and for both Proactive (blue shading) and Reactive (green shading) IWC systems.

IWC System Testing	Number of Plots	DFT Measures/Plot	Total DFT Measures/Survey
Proactive IWC control - beginning of testing period	10	50	1,000
Proactive IWC treated - beginning of testing period	10	50	
Proactive IWC control - end of testing period	10	50	1,000
Proactive IWC treated - end of testing period	10	50	
Reactive IWC control - before a cleaning event	10	50	1,000
Reactive IWC treated - before a cleaning event	10	50	
Reactive IWC control - after a cleaning event	10	50	1,000
Reactive IWC treated - after a cleaning event	10	50	

8. Data Management

8.1. Data management system

A formal data management system should be implemented, which encompasses and traces the path of the data from their generation to their final use or storage. For example, from field measurements and sample collection/recording through transfer of data to computers (laptops, data acquisition systems, etc.), laboratory analysis, data validation/verification, quality assessments, and reporting of data of known quality to the clients and sponsors. The data management system should also include control mechanisms for detecting and correcting errors.

Data quality objectives - Data quality objectives (DQO) are qualitative and quantitative statements that clarify study objectives, define the appropriate types of data, and specify the tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions. DQOs are typically expressed in terms of acceptable uncertainty associated with a point estimate at a desired level of statistical confidence. Only data that meet or exceed these criteria should be deemed valid.

The development of DQOs should follow US EPA's *Guidance for the Data Quality Objectives Process* (EPA QA/G-4, 2006), or a similar accepted framework.

Data quality indicators - DQOs are supported by Data Quality Indicators (DQIs), which are supported by Measurement Quality Objectives (MQOs). During test planning, DQIs should be considered, and specific MQOs are set to ensure that data are of appropriate quality for their intended use. The traditional DQIs considered are:

- accuracy, which is a measure of the closeness of a measured value to the true value;
- precision, which is a measure of the repeatability of a measurement;
- bias, which is the systematic or persistent distortion of a measurement process which causes error in one direction;
- representativeness, which describes how well the data reflect the actual conditions;
- comparability, data are of known quality and can be validly applied by external users;
- completeness, which describes whether valid data are produced for all the submitted samples, or just some fraction thereof; and
- sensitivity, which describes the lowest measure, or increment of measurement, that a technique can detect.

Examples of accepted approaches to determining DQIs include US EPA's *Guidance for Quality Assurance Project Plans* (EPA QA/G-5, 2002a) and *Guidance for the Data Quality Objectives Process* (EPA QA/G-4, 2006).

8.2. Data recording and archiving

Various types of data are acquired and recorded electronically and/or manually during testing by the TO staff. Sample collection data (e.g., date, time, and location of collected samples) and analytical data should be recorded by hand (using indelible ink) on pre-printed data collection forms and/or in bound laboratory notebooks that are uniquely identified and are specific to the system test. All documentation should be:

- made promptly at the time of observation;
- accurate, legible, permanent, clear, and complete;
- dated and initialed by responsible personnel; and
- copied, scanned, and/or photographed.

Chain-of-custody forms should be used as needed.

Accepted data recording and archiving procedures must meet the requirements of ISO/IEC 17025, Section 7.5 and Section 8: Clauses 8.4.1 and 8.4.2 (ISO, 2017).

8.3. Data analysis

Data analysis is the process of systematically applying statistical and/or logical techniques to describe and illustrate, condense, summarize, and evaluate data. An essential component of ensuring data integrity is the accurate and appropriate analysis of field samples and laboratory measurements of environmental and technology performance variables. Examples of relevant data analysis are described by Tamburri et al. (2020).

Quality assurance and quality control - There is potential for variability in any sample collection, analysis, or measurement activity. Field variability generally contributes more than laboratory variability. Total study error can result from among sampling unit variability, and within-sampling-unit variability.

Quality assurance (QA) measures undertaken to assure the reliability of the data collected include duplicate sampling, replicate analysis, calibration and maintenance procedures, schedules, and standards (if applicable) for all equipment used in the test. Quality control (QC) measures are actions to assure that defined standards are met in the analysis of data. These measures should be measurement or method specific and are defined within the relevant SOP. Field QC samples collected should include equipment blanks, trip blanks, field blanks, field duplicate samples, and matrix spike/matrix spike duplicate samples. Routine procedures for laboratory QC include daily instrument calibrations, efficiency and background checks, and standard tests for precision and accuracy.

Guidance on accepted QA/QC measures can be found in ASTM (2017) and US EPA's *Guidance for Quality Assurance Project Plans* (EPA QA/G-5, 2002a).

Measurement uncertainty - Measurement uncertainty should be reported as described in ISO 17025:2017, Section 7.8.3.1c (ISO, 2017) or other similar standard, when:

- it is relevant to the validity or application of the test result;
- instructed by a client; or
- the uncertainty affects compliance to a specification limit.

The IWC testing process may have uncertainties associated with factors such as equipment calibration, operator skill, sample variation, and environmental factors. Measurement uncertainty does not need to be calculated for those standard analytical methods that specify limits to the values of the major sources of uncertainty of measurement and the form of presentation of calculated results. For other methods, the combined standard uncertainty should be calculated as described in guides, such as, ANSI/NCSL (1997), GUM (2008), and A2LA (2014).

9. Quality Assessments

Quality assessments include technical audits and data quality assessments. Fundamental principles of the assessment process include:

- assessments are performed by the QA Manager, who is independent of direct responsibility for performance of the test;
- each assessment is fully documented;
- each assessment should be responded to by the appropriate level of the testing team;
- quality assessment reports require a written response by the person performing the inspected activity, and acknowledgment of the assessment by test lead; and
- corrective action should be documented and approved on the original assessment report, with detailed narrative in response to the assessor's finding.

9.1. Technical audits

Technical audits are systematic and objective examinations of test implementation to determine whether data collection activities and related results comply with the test protocol, are implemented effectively, and are suitable to achieve its data quality goals. Audits of tests should include technical system audits (TSA) and audits of data quality (ADQ).

Technical system audit - A TSA is a thorough, systematic, and qualitative evaluation of the sampling and measurement systems associated with testing. The objective of the TSA is to assess and document the conformance of on-site testing procedures with the requirements of the test protocol, published reference methods, and associated procedures. The TSA assesses test facilities, equipment maintenance and calibration procedures, reporting requirements, sample collection, analytical activities, and QC procedures. Both laboratory and field TSAs should be performed following the U.S. EPA's document *Guidance on Technical Audits and Related Assessments for Environmental Data Operations* (EPA QA/G-7, 2000a), or a similar accepted framework.

Audit of data quality - An ADQ is a quantitative evaluation of the test data. The objective of the ADQ is to determine if the test data were collected according to the requirements of the test protocol and associated procedures and to verify whether the data were accumulated, transferred, reduced, calculated, summarized, and reported correctly. The ADQ assesses data accuracy, completeness, quality, and traceability.

The ADQ should be conducted by the QA Manager following procedures found in the US EPA's *Guidance on Technical Audits and Related Assessments for Environmental Data Operations* (EPA QA/G-7, 2000a), or a similar accepted framework.

9.2. Data quality assessment

The Data Quality Assessment (DQA) is a scientific and statistical evaluation of validated data to determine if the data are of the right type, quality, and quantity to support conclusions on the performance of the IWC systems tested. The DQA process includes consideration of:

- soundness - the extent to which the scientific and technical procedures, measures, and methods employed to generate the information are reasonable for, and consistent with, the intended application;
- applicability and utility - the extent to which the information is relevant for the intended use;
- clarity and completeness - the degree of clarity and completeness with which the data, assumptions, methods, and quality assurance, employed to generate the information are documented; and
- uncertainty and variability - the extent to which the variability and uncertainty (quantitative and qualitative) in the information or in the procedures, measures, and methods are evaluated and characterized.

A DQA should be conducted as described in the US EPA's *Guidance for Data Quality Assessment: Practical Methods for Data Analysis* (EPA QA/G-9, 2000b) or a similar accepted framework and include:

- data verifications;
- data validations; and
- data usability assessment.

9.3. Non-conforming work and corrective action

Non-conforming work can occur during various stages of testing (e.g., incorrect following of documented test procedures, use of uncalibrated test equipment, lack of staff supervision or training, errors in the test report). Procedures should be established for the control of non-conforming work. The procedures should meet the requirements of ISO 17025:2017, Section 7.10 (ISO, 2017) and ensure that:

- the responsibilities and authorities for the management of non-conforming work are designated and actions (including halting of work and withholding of test reports, as necessary) are defined and taken when non-conforming work is identified;
- an evaluation of the significance of the non-conforming work is made, including criteria;
- corrective actions are taken immediately, together with any decision about the acceptability of the non-conforming work;
- where necessary, the client is notified and work is recalled;
- the responsibility for authorizing the resumption of work is defined; and
- all actions are recorded and become part of the permanent project file.

An example of an accepted corrective action procedure is presented in the US EPA's *Control of Non-conforming Work* (EPA SESDPROC-019-R3; USEPA 2016).

9.4. Audit reporting

The QA Manager is responsible for all audit reports. These written reports focus on whether the test activities and related analytical results:

- comply with the test plans and related SOPs;
- are implemented effectively; and
- are suitable to achieve data quality goals.

An audit report usually consists of:

- an introduction describing the date, location, purpose, and scope of the audit;
- a detailed account of the findings and their basis;
- conclusions, including a discussion of any findings requiring corrective action; and
- recommendations (if requested) for resolving problems that affect quality.

Findings of audit results can generally be divided into three categories:

- noteworthy practices or conditions;
- observations, which are neither positive nor negative; and
- non-conformances, which are deviations from standards and documented practices (e.g., test plans, SOPs, reference methods).

Non-conformances can be divided into two subcategories:

- deficiencies, which adversely impact the quality of results; and
- areas of concern, which do not necessarily (but could) result in unacceptable data.

10. Human and Environmental Health and Safety

The IWC service provider and TO personnel should all follow standard laboratory and field work safety procedures, abiding by all health and safety legislation in the location of testing, and wear protective clothing and equipment as required. All parties should have liability coverage from their own institutions or companies. All testing operations should cease if there are any concerns regarding human health and safety, and/or any potential significant environmental impacts, during testing operations. Decisions to terminate testing should be made by the TO in consultation with the service provider and appropriate local authorities.

11. Additional Considerations for Permits and Approvals

While not directly related to the methods presented here to perform tests of IWC system efficacy, there are specific considerations that should apply to the processing and issuing of permits or permission to operate. Permit or approval granting bodies should be aware that a range of factors influence the nature of discharges associated with IWC systems and, thus, their potential for environmental contamination. These include the type(s) and age of the antifouling coating systems cleaned, the size and frequency of vessels cleaned, the amount and type of biofouling present, the IWC method, personnel responsible for cleaning operations, and the receiving environment (e.g., hydrodynamics, habitat; Morrisey et al., 2013; Scianni and Georgiades, 2019; Tamburri et al., 2021).

The importance of independently collected and reported data (see Section 1.6) is paramount to a proper evaluation of the suitability of an IWC system for obtaining permission or a permit to operate. Although the practice of vendor or permittee-derived data may be acceptable during preliminary assessments of system maturity and during ongoing monitoring to show that the system continues to operate at expected levels, initial permission should also consider independent third-party data as a foundation for determining system efficacy.

Permit or approval granting bodies should also be aware of the human factor when considering the authorization of IWC activities. A technology can perform at a certain level under one individual operator but use by a different operator may produce different results. We recommend requiring a combined evaluation of the IWC system, overall service provider, and the individual operator seeking a permit.

While effects on water quality can be monitored in real time, some potential impacts associated with IWC can occur over longer temporal and larger spatial scales (e.g., accumulative impacts within sediments or local organisms, or the establishment of non-indigenous species). As such, permit or approval granting bodies should consider the institution of time-limited approvals and long-term monitoring programs to inform future decisions.

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13. Abbreviations and Glossary

Anti-fouling system	A coating, paint, surface treatment, surface, or device that is used on a vessel or submerged equipment to control or prevent the attachment of organisms.
ADQ	Audits of data quality
BACI	Before-after-control-impact
Cleaning of biofouling	The physical removal of biofouling organisms from a surface.
Cu	Copper
DFT	Dry film thickness
DOC	Dissolved organic carbon
DQA	Data quality assessment
DQIs	Data quality indicators
DQOs	Data quality objectives
FR	Fouling Rating: a scale used to rate the type and level of biofouling present on vessels.
Independent testing organization	An appropriately qualified, scientific contractor approved to conduct the test (Section 1.6).
IWC	In-water cleaning
Macrofouling	Distinct multicellular biofouling organisms, both individuals and colonies, that are visible to the human eye, such as barnacles, tubeworms, hydroids, and fronds of algae. Does not include microscopic organisms that comprise biofilms.
MQOs	Measurement quality objectives
Microfouling	A layer of microscopic organisms, such as bacteria and diatoms, and the slimy substances that they produce.
MP	Microplastics

Multicomponent system	A system reliant on two or more individual components to achieve the required IWC. The use of each component is to be specified in the system SOP. For example, the use of a hand tool for addressing niche areas after main cleaning unit use on hull surfaces.
NP	Nanoplastics
Niche areas	Niche areas are protrusions or recesses in a ship's hull, and generally include any surface that is not relatively flat (i.e., curves, complex structure, angles, etc.) or cannot be cleaned by standard hull IWC devices. Niche areas are exposed to the external marine environment and therefore subject to different hydrodynamic forces. They are prone to coating damage, or being inadequately coated, can be difficult to access, and often more susceptible to biofouling.
NIS	Non-indigenous species
Pathogens	Microorganisms (e.g., viruses, bacteria, protists, and fungi) that cause disease in other organisms.
Performance data	The results produced by following the testing procedures outlined within this document.
POC	Particulate organic carbon
PSD	Particle size and distribution
Propagules	Any non-adult biological material that is used for the purpose of propagating an organism to the next stage in its life cycle. May include dispersive gametes, seeds, spores, or regenerative tissue.
QA/QC	Quality assurance/quality control
Secchi depth	A Secchi disk is a weighted circular disk (20–30 cm in diameter), divided into quadrants painted alternately black and white, used to measure water transparency in bodies of water. The disk is mounted on a pole or line and lowered slowly down through the water column. The depth at which the disk is no longer visible ("Secchi depth") is related to water color and turbidity.
TO	Testing organization
TSA	Technical system audit
TSS	Total suspended solids
Zn	Zinc