Ministry for Primary Industries
Manatū Ahu Matua

Technical advice:

Evaluation of in-water systems to reactively treat or remove biofouling within vessel internal niche areas

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This technical advice supports the development of criteria and processes for biofouling removal or treatment approvals for the internal niche areas of vessels.

Executive summary

Vessel biofouling is a major pathway for the introduction and spread of non-indigenous marine species. In-water systems for the reactive removal or treatment of biofouling from vessel internal niche areas (i.e. sea chests and internal pipework systems) have been proposed as a measure to manage the biosecurity risks from biofouling. However, the development and regular use of these systems for reactively cleaning or treating biofouling for in-service vessels can create residual biosecurity risks that may require assessment and management.

This document informs the development of testing requirements and standards for the operation and performance of in-water systems to obtain MPI approval for their use in New Zealand. The test methodologies advised are based on the outcomes of a companion review of existing in-water systems available globally, or currently in development (Growcott *et al*. 2016; Growcott *et al.* 2017). The objective of the technical advice is to assist consenting authorities (both government and non-government) in making informed decisions regarding the acceptability of in-water systems for removal or treatment of biofouling within vessel internal niche areas, based on their efficacy.

The test methodologies and performance criteria on the internal surfaces of vessels have been divided into the following system categories:

- removal ("cleaning") systems;
- chemical treatment systems;
- thermal treatment systems;
- co-treatment systems (i.e. combinations of the above);
- filtration or treatment systems for removed waste and effluent.

The level of testing will vary depending on in-water system type. For treatment systems, the level of testing is influenced by the quality of available efficacy data. Pre-testing considerations need not apply for thermal treatments that reach 60 °C and are held at that temperature for 60 minutes or more, or for physical removal systems.

The performance criteria for each system category are summarised below.

Table 1. In-water system performance criteria.

System	Performance criteria
All systems (removal and treatment)	*All macroscopic biofouling in the cleaned or treated internal niche area should be removed or rendered non-viable (i.e. not capable of living and developing to reproductive maturity).*
Filtration or treatment of biofouling waste (effluent) created during application of the system.	*Maximum particle size in the filtered effluent should be 12.5 µm or all biological material of macroscopic biofouling should be rendered non-viable.*
Containment of effluent or biofouling	*Effluent should not be released to the environment unless filtered or treated to the above criteria (≤ 12.5 µm or non-viable).* *There should be no release of macroscopic biofouling > 0.5 cm in diameter during system mobilisation, operation or demobilisation (e.g. by divers, hoses or application of sea chest blanks).*

For all systems, full system testing should be completed on biofouling present on actual vessels (hereafter known as vessel testing) and the outcomes of the testing should be assessed against defined performance criteria. The tests should be realistic simulations of the intended use of the full system on a vessel. Further, reporting should include the results of all test runs – failing and passing.

The system developer should specify the category (or categories) of internal surface that their system can be applied to.

For each system category and its application, guidance is given on:

- providing system information, including its:
    - method of operation and technical specifications;
    - intended application(s);
    - standard operating procedure(s) (SOP);
- test conduct, including:
    - oversight by appropriately independent qualified personnel;
    - choice of vessels;
    - the level of replication of the test(s);
    - the environmental conditions under which the test(s) should occur;
- methods to assess system efficacy with respect to:
    - different internal niche area types, volumes and configurations;
    - different types and levels of biofouling;
    - effects on the anti-fouling coatings, marine growth prevention systems and vessel structure;
    - waste capture or containment and treatment;
- data collection and reporting on the outcomes of the test.

The report also contains discussion on the information base and rationale used to develop the performance criteria, test methodologies and guidance on the likely costs of undertaking the tests. Example templates and guidance documents are appended to assist data collection and reporting.

Contents

Abbreviations and definitions

Antifouling 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.
Biological material	Adults, tissues or propagules of macroscopic fouling organisms.
Cleaning of biofouling	The physical removal of biofouling organisms from a surface.
Containment system	An object such as a sea chest blank or bung that restricts the exchange of water between the enclosed section of the internal niche area and the surrounding environment.
FR	Fouling Rating: a scale used by the US Navy to rate the type and level of biofouling present on vessels.
Independent supervising scientist	An appropriately qualified, scientific contractor approved by MPI to conduct the test.
Internal niche area(s)	Internal areas of a vessel hull that are more susceptible to biofouling accumulation due to different hydrodynamic forces, susceptibility to antifouling coating wear or damage, or absence of antifouling coatings or marine growth prevention systems. These areas include cavities (e.g. sea chests, box coolers), internal pipework and enclosures (including crossovers and cofferdams), and associated seawater systems (e.g. air conditioning, engine cooling, fire-fighting, freshwater making) and components (e.g. seals, valves, plate coolers).
LD_{100}	The acute, single dose or concentration of the treatment that is lethal to 100 % of the relevant organisms.
Lethal agent	The method used by treatment systems to render relevant organisms non-viable. This could be a biocide, de-oxygenation or a physical treatment such as elevated temperature.
Level of testing	The type and amount of testing that provides the minimum amount of robust evidence for assessment of system efficacy.
LT_{100}	The period of exposure needed to achieve 100 % mortality of the relevant organisms for a single, acute concentration, dose or temperature.
$LTemp_{100}$	The temperature lethal to 100 % of relevant organisms.
$LpHV_{100}$	The pH and volume of acid descaler that is lethal to 100 % of relevant organisms.

Macroscopic biofouling ("macrofouling")	Distinct multicellular biofouling organisms visible to the human eye, such as barnacles, tubeworms and hydroids. Does not include microscopic organisms that comprise the slime layer.
Manual systems	The physical removal of biofouling organisms by hand or using small hand held tools. Manual removal may include use of hand-held scrapers, brushes or pads.
Mechanical systems	The physical removal of biofouling organisms using powered tools or equipment. Mechanical systems may include use of powered rotary brushes, pads, and blades; or high-pressure water or cavitational jets and may be operated by divers or mounted on remotely operated vehicles (ROVs).
Marine Growth Prevention System	Antifouling system used for the prevention of biofouling accumulation in internal sea water cooling systems and sea chests. Various operating mechanisms exist that typically include either the electrolysis of copper, aluminium and iron anodes, biocidal injection dosing systems or the electrolysis of seawater to produce chlorine compounds.
MPI	[New Zealand] Ministry for Primary Industries.
Non-viable	Biological material of macroscopic fouling (adults, tissues or propagules) that is not capable of living and developing to reproductive maturity in marine or estuarine environments.
Performance criteria	Specific criteria and endpoints met for a system to be considered to be biosecure.
Performance data	The results produced by undertaking the test methodologies outlined within this document.
Propagules	Any non-adult biological material 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.
Relevant organisms	Organisms typically observed in vessel internal niche areas. Includes macroscopic biofouling and mobile organisms, such as crabs, sea stars and fish.
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. The disk is mounted on a pole or line, and lowered slowly through the water column. The depth at which the disk is no longer visible ("Secchi depth") is related to water colour and turbidity.
Slime layer	A layer of microscopic organisms, such as bacteria and diatoms, and the slimy substances that they produce.
SOP	Standard Operating Procedure: detailed, written instructions on the method of operation of the system to achieve consistency in its performance for removing or treating biofouling.

Treatment of biofouling	Systems that kill biofouling organisms *in situ*.
Treatment systems	Treatments that are applied directly to the fouled area of the vessel to kill biofouling organisms *in situ*, but which do not physically remove the organisms. Treatments may include, but are not limited to, systems that apply heat, biocides or ultrasound to fouled areas.
Viable	Biological material of macroscopic fouling (adults, tissues or propagules) that is capable of living and developing to reproductive maturity in marine or estuarine environments.

1 Background

1.1 PURPOSE OF THIS DOCUMENT

This document contributes to the scientific background for approving in-water cleaning or treatment systems under the Craft Risk Management Standard for Biofouling for Arriving Vessels, and within New Zealand's domestic biofouling pathway management approach. The Ministry for Primary Industries (MPI) will consider this document along with other information in determining proposed measures that are practical to implement and align with all applicable legislation, while ensuring the biosecurity risk does not exceed New Zealand's appropriate level of protection.

The test methods within this technical advice document ensure that the performance data generated are fit-for-purpose and of appropriate accuracy and precision. This document is informed by the Australian and New Zealand in-water cleaning guidelines (Department of the Environment and the Ministry for Primary Industries 2015) and the New Zealand transitional facilities regulations (Ministry for Primary Industries 2013).

This document is a companion to, and borrows heavily from, the MPI commissioned report developed for external hull surfaces: "Procedures for evaluating in-water systems to remove or treat vessel biofouling" (Morrisey *et al.* 2015).

1.2 SCOPE OF THIS DOCUMENT

1.2.1 General scope

The development of performance criteria and test methods for in-water systems to remove or treat vessel biofouling and mobile species within vessel internal niche areas is focussed on management of biosecurity risks, both in the testing of systems for approval and in their use once they have been approved. Potential chemical contamination resulting from the application of in-water systems, while an important environmental risk, is outside the scope of this document. Nevertheless, the relevant consent(s) for in-water system use should be obtained from the relevant jurisdiction prior to testing commencement (Appendix 8.4).

The application of reactive in-water systems necessitates the assessment of system use on marine growth prevention systems (MGPS), antifouling system(s) and in relation to the safe operation of the vessel. The assessment includes specification of any damage (e.g. physical, structural damage, corrosion, warping) to the internal niche area and the type, age and condition of the MGPS and antifouling system(s) before and after system application.

1.2.2 Specific scope

This document provides technical advice for the testing of the following categories of in-water treatment (rendering non-viable) and cleaning ("removal")[1] systems:

- oxidising and non-oxidising chemicals;
- thermal treatment;
- physical removal;
- co-treatments.

[1] For the purpose of this document, in-water cleaning is defined as the physical removal of biofouling organisms from a surface. In-water treatment is defined as the killing of biofouling organisms *in situ*.

The document applies only to in-water systems that are used to reactively treat or remove biofouling and mobile species associated with vessel internal niche areas. Treatment or removal of biofouling on the general hull area and external surfaces, including sea chest gratings[2], is covered by Morrisey *et al.* (2015) and therefore outside the scope of this document.

The in-water systems tested should be capable of the treatment or cleaning of heavily fouled internal surfaces. Therefore, vessel testing for the majority of internal niche areas should be completed on biofouling of at least Fouling Rating (FR) 90 and 100 % cover over minimum total coverage of 4 800 cm^2 (Floerl *et al.* 2005; Naval Ships' Technical Manual 2006; Morrisey *et al.* 2015; Appendix 8.1) that covers a specified portion of the internal niche area (Section 3.5.2) and includes the use of settlement plates and relevant organisms, where appropriate (Section 3.5.8). FR and coverage for narrow or smaller internal niche areas should be determined on a case-by-case basis with a rationale provided.

2 Performance criteria and test methods

The performance criteria for all in-water systems should be validated as effective in meeting the following stated outcomes:

2.1 PERFORMANCE CRITERIA FOR IN-WATER SYSTEMS (PHYSICAL REMOVAL AND TREATMENT SYSTEMS, INCLUDING TREATMENT OF EFFLUENT)

The performance criteria for all in-water systems is that all macroscopic biofouling should be removed or rendered non-viable.

2.2 PERFORMANCE CRITERIA FOR EFFLUENT FILTRATION

The performance criteria for filtration of effluent from in-water cleaning systems or effluent release prior to completion of treatment is a maximum particle size of 12.5 µm in the filtered effluent (Morrisey *et al.* 2015).

Alternative or additional treatments to filtration, such as irradiation with ultra-violet (UV) light, heat or addition of biocides, should render all biological material of macroscopic biofouling non-viable (see Abbreviations and definitions). These systems are, however, typically reliant on prior filtration of the waste water to improve their efficacy.

No treatment criteria should be necessary if waste is discharged to a sewer that incorporates secondary treatment.

2.3 PERFORMANCE CRITERIA FOR EFFLUENT CAPTURE AND BIOFOULING CONTAINMENT

The performance criteria for effluent capture (e.g. suction device) or containment (e.g. sea chest blank) are: no effluent should be released into the marine environment during system mobilisation, application or demobilisation, unless it has been subject to the minimum filtration or treatment criteria as set out in Section 2.2.

[2] Unless the in-water system can isolate biofouling located on external areas of the sea chest grate while at the same time treating internal fouling, for example, via the use of sea chest blanks with a raised edge.

Any release of macroscopic biofouling > 0.5 cm in diameter or larger into the marine environment during system mobilisation, operation or demobilisation is considered to represent a failure to meet the performance criteria (Morrisey *et al.* 2015).

2.4 EVALUATION OF SYSTEM SUITABILITY BY AN APPROPRIATELY QUALIFIED PERSON

In-water systems may damage (e.g. via corrosion, warping or physical impact) components of internal seawater systems (e.g. plate coolers, valves and seals), affecting the safe operation of the vessel. Discussions of system application with an appropriately qualified person (e.g. vessel surveyor, marine engineer) early in the initial design phase would help to mitigate any potential issues which may impact upon the physical integrity of a vessel.

Failure to adequately investigate system suitability may limit its uptake within certain vessel classes.

Appropriately qualified persons or companies who may complete such an evaluation include:

- vessel surveyors[3];
- international classification societies (e.g. Lloyd's Register, Bureau Veritas, DNV GL etc.).

Evidence of system suitability should be included as part of the data package (Appendix 8.3.5).

2.5 TESTING PATHWAYS

The level of testing is dependent on the mode of action of the proposed in-water system. For treatment systems, the level of testing is dependent on the quality of efficacy data, if any, supplied by the developer. By contrast, pre-testing considerations of efficacy data do not apply for physical removal systems (Section 2.5.4).

It is in the best interests of developers of treatment systems to systematically review the relevant scientific data to inform the development of the proposed treatment system and exposure endpoints.

2.5.1 Determination of testing pathway

The quality of all treatment data used to satisfy the efficacy data assessment (Section 2.5.2) should be systematically evaluated using a modified Klimisch *et al.* (1997) approach. Click here for a spreadsheet of this modified scoring system.

For those in-water treatment systems that have inadequate efficacy data, data generation (e.g. LD_{100}, $LTemp_{100}$, $LpHV_{100}$, LT_{100}) by way of land-based testing (Section 2.5.3) should be conducted prior to the commencement of vessel testing (Section 2.5.4).

The assessment of efficacy data and associated recommendations should be conducted by an independent scientist and supplied in the form of a report (Appendix 8.3.1; 8.3.2).

[3] https://www.maritimenz.govt.nz/commercial/safety/safety-management-systems/recognised-surveyors/recognised-surveyors.asp

The results of vessel testing of in-water treatment systems can be assured to be transparent and robust if the system satisfies the efficacy data assessment and follows the test methods described.

A regulator review of the efficacy data and independent recommendation report prior to the commencement of land-based or vessel testing should help to ensure the appropriate testing pathway is selected.

2.5.2 Assessment of efficacy data for treatment systems

The presence or absence of the following factors should be reported (Appendix 8.3.1; 8.3.2) for each study presented to determine treatment efficacy (i.e. LD_{100}, LT_{100}, $LTemp_{100}$, $LpHV_{100}$):

- quality of methodology, e.g.:
  - presence of controls;
  - level of replication;
  - test chemical (e.g. purity, speciation, formulation, stability over treatment duration);
  - modified Klimisch score (Section 4.1.7);
- treatment efficacy:
  - the lethal dose (LD_{100}) or temperature ($LTemp_{100}$) or pH and volume of acid descaler ($LpHV_{100}$) and duration of treatment (LT_{100}) necessary to achieve 100 % mortality of relevant organisms (Section 3.1.1; Section 4.1.5.2);
  - physical and water chemistry parameters (e.g. water temperature, salinity, pH, concentration of organic and inorganic matter);
- test organism(s):
  - collection source (e.g. supplier, wild collection, settlement plates, collection location and site description (e.g. intertidal, subtidal));
  - organism type(s) (e.g. bivalve, barnacle, bryozoan, crustacean):
    - length of acclimation period and water quality parameters (e.g. temperature, pH, dissolved oxygen);
    - size range(s);
    - confirmation of attachment (e.g. byssal threads);
    - % coverage of biofouling on each settlement plate, where applicable (see Section 3.1.1);
    - measurement of biomass;
- discussion:
  - appropriateness of test data (e.g. methodology, appropriate measurement methodology and measurement interval of test chemical(s) concentration(s));
  - range of relevant organisms tested:
    - justification of species tested (e.g. relationship of tested organisms to the most resilient relevant organisms to the treatment agent as documented in the literature; Section 4.1.5.2).

The below considerations should be discussed when assessing and reviewing all the systematically evaluated data:

- treatment efficacy:
  - the range of lethal dose(s) (LD_{100}) or temperature(s) ($LTemp_{100}$) or lethal pH and volume ($LpHV_{100}$) and duration of treatment(s) (LT_{100}) necessary to achieve 100 % mortality of relevant organisms;

- treatment agent considerations:
  - if environmental discharge of treatment effluent is to occur:
    - can the treatment agent be deactivated (e.g. dechlorination);
    - persistence or bioaccumulation potential of agent(s) or metabolites in the environment;
    - are discharge permits needed (e.g. effluent concentration or temperature allowed under a resource consent);
- conclusions:
  - recommendations, considerations or data gaps regarding the proposed treatment system.

2.5.3 Land-based testing

For in-water treatment systems (chemical, thermal and co-treatments) that do not meet the specifications of the efficacy data assessment, land-based testing should be conducted by an independent scientist to generate the necessary data prior to vessel testing. The results of all test runs – failing and passing – should be reported.

Land-based testing is not necessary for thermal treatments that reach 60 °C and are held at that temperature for 60 minutes or more.

2.5.4 Vessel testing

Consideration of vessel testing results for an in-water system is contingent upon:

- the provision of appropriate efficacy data:
  - based on a report indicating a sufficient amount of data exist for the particular treatment, supplied by an appropriately qualified independent scientist (Section 2.5.2; Appendix 8.3.1; 8.3.2); or
  - following land-based testing, supervised and reported by an appropriately qualified independent scientist (Section 2.5.3); or
  - a physical removal system is used.

Physical removal systems are likely to only be used to clean biofouling on internal niche areas that are safe to access (i.e. near the entrance of the sea chest or internal seawater system). In order to meet the performance criteria (Section 2.1), physical removal systems may have to be used as part of a co-treatment.

Vessel testing is important to assess the performance of the full proposed system in a realistic simulation of its intended use (i.e. the full system test should be conducted on internal niche areas of appropriate size, configuration, with relevant fouling extent and type). In addition, the possibility of biofouling dislodgment, escape (e.g. mobile organisms) or release (e.g. effluent) by divers, hoses, and the in-water system should be assessed during set-up, application and demobilisation (Morrisey and Woods 2015).

The system developer should specify which internal niche area configuration(s) and volume(s) their system is designed to treat or clean, including whether internal niche areas are interconnected and non-independent of other systems (e.g. sea-chests, box coolers, crossovers, cofferdams). The full system is tested under the conditions appropriate to that category, as set out in Sections 3.6.2, 3.7.2, 3.8.2 and 3.9.

Because testing should be conducted on multiple similar internal niche areas, it may be necessary to test the system *on more than one vessel*.

The results of all test runs – failing and passing – should be reported.

The optimal data to be generated for the different system categories are summarised in Figure 1.

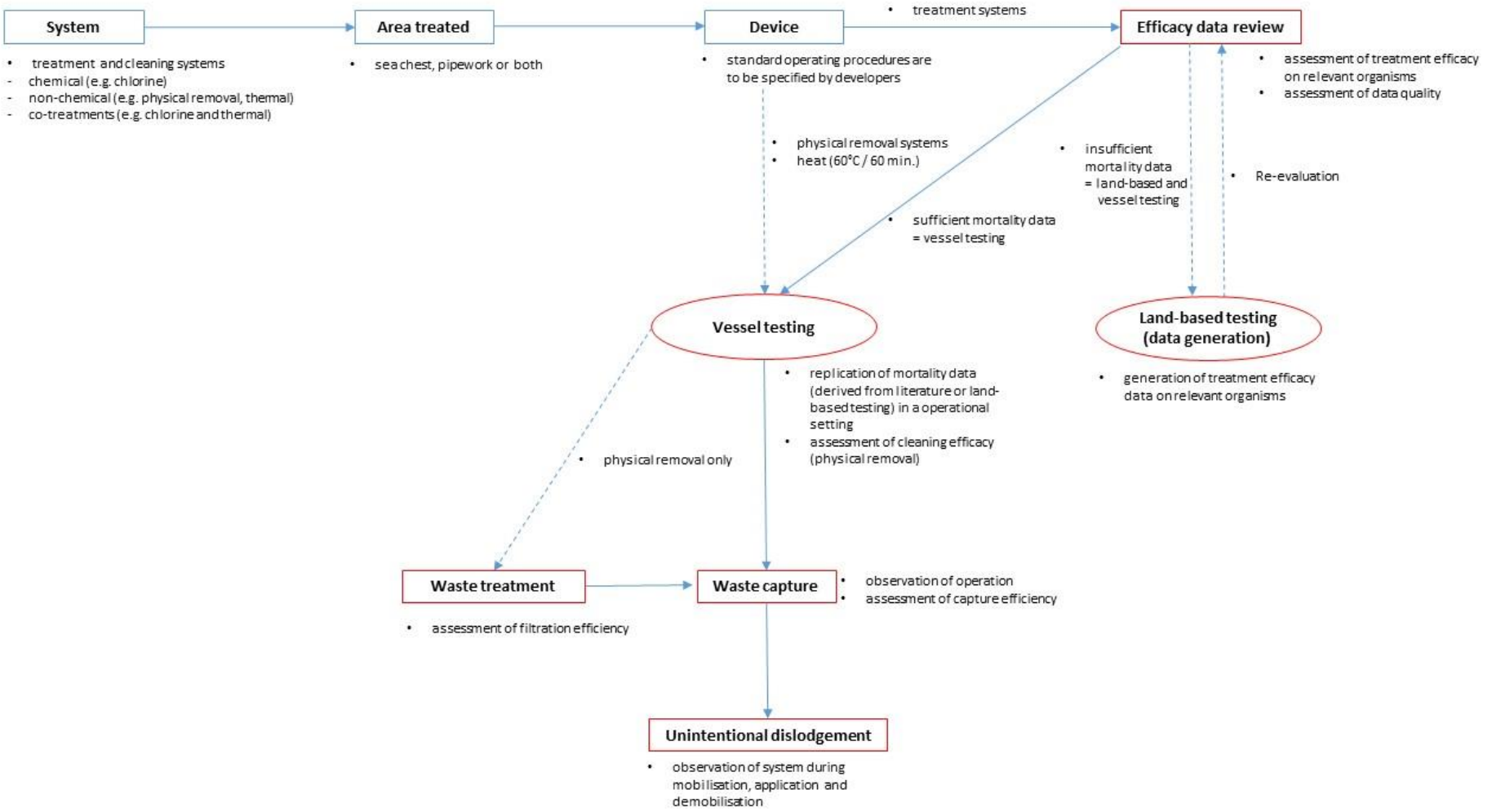

Figure 1. Summary of biosecurity data that should be generated for in-water systems that reactively treat or clean biofouling within vessel internal niche areas. Blue features are those that are common to all in-water systems, with the red circles showing the mode of testing (land-based or vessel testing) and red rectangles showing testing or assessment components. Broken arrows show test or assessment components that are only relevant for particular in-water systems.

3 Testing

3.1 GENERAL CONSIDERATIONS FOR LAND-BASED TESTING (CHEMICAL, THERMAL AND CO-TREATMENTS)

The following considerations should be documented by the developer with the test data (Sections 3.2.4; 3.3.4; 3.4.4), where applicable:

- description of the treatment type:
  - mechanism of action to treat relevant organisms (Section 3.1.1);
  - method of exposure (e.g. how is the treatment to be introduced to the test system);
- data generation from land-based testing, including:
  - for chemical treatments and co-treatments that use chemicals:
    - the lethal dose (LD_{100}) or lethal pH and volume ($LpHV_{100}$), temperature and duration of treatment (LT_{100}) that achieves 100 % mortality of relevant organisms;
  - for thermal treatments:
    - the temperature ($LTemp_{100}$) and duration of treatment (LT_{100}) that achieves 100 % mortality of relevant organisms;
  - for co-treatments that use heat:
    - the lethal dose (LD_{100}), or lethal pH and volume ($LpHV_{100}$), temperature and duration of treatment (LT_{100}) that achieves 100 % mortality of relevant organisms;
- details and qualification of independent scientific organisations and personnel performing and supervising the test.

The following factors should be considered by the developer prior to testing:

- test conduct (e.g. relevant organisms, experimental design and level of replication; see OECD[4] or ASTM[5] ecotoxicology testing methods for guidance), including the date and location and any modifications to the methods set out in Section 3.1;
- settlement plate construction material, size, shape, length of deployment and deployment location[6];
- test conduct and supervision (e.g. by appropriately qualified, independent scientists).

3.1.1 Determination of relevant organisms for testing

The in-water systems tested should be capable of treating heavily-fouled internal surfaces, therefore, for the majority of internal niche areas testing should be completed on viable biofouling of at least FR 90 and 100 % cover (Section 3.5.2) and include the use of settlement plates and relevant organisms, where appropriate (Floerl *et al.* 2005; Naval Ships' Technical Manual 2006; Morrisey *et al.* 2015; Section 3.5.8; Appendix 8.1). FR and coverage for narrow or smaller internal niche areas should be determined on a case-by-case basis with a rationale provided.

[4] http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761

[5] http://www.astm.org/BOOKSTORE/BOS/1106.htm

[6] Optimising settlement arrays for surveillance of non-indigenous biofouling species: Literature review (*/document-vault/15067*) may be used as guidance.

The selection of test organisms (i.e. relevant organisms, settlement plates or a combination of both) is governed by the availability and quality of information present in the scientific literature. An independent supervising scientist may consider the 12 broad taxonomic groups identified in the Risk Analysis: Vessel Biofouling (Bell *et al.* 2011) as a starting point for their review of relevant organisms.

The species chosen as relevant organisms for land-based testing should be justified using scientific literature (i.e. shown to be resilient to the treatment compared to other fouling or mobile species (Bell *et al.* 2011)) and be identified to *species* level.

For settlement plates, the immersion time necessary to achieve a relevant type, age, level and coverage of biofouling is dependent on the rate of colonisation and growth, which may take longer in temperate climates. Deployment of settlement plates early on in the testing process should allow sufficient time for biofouling to accumulate. The relevance of the species on the settlement plates to the intended purpose of the system should be documented. Additionally, settlement plate dimensions and source material should be justified using scientific literature.

The type of biofouling on settlement plates can be described in broad taxonomic and morphological categories of biofouling organisms (such as hydroid, erect bryozoans, barnacles, and colonial ascidians), and should be determined by a suitably qualified independent scientist (Section 4.2.3).

All organisms that survive exposure testing on trial surfaces should be identified to *species* level by a suitably qualified independent scientist (Section 4.2.3).

3.1.1.1 Measurement of water quality parameters

For each test, the salinity, pH, temperature, dissolved organic carbon and dissolved oxygen level of the sea water should be measured prior to the introduction of the treatment, during treatment exposure and after the exposure period has occurred. The filtration level of the sea water is to be reported, as applicable. For acid descalers, measurement of water quality parameters, with the exception of pH, should only be necessary prior to the introduction of the treatment. The volume of descaler needed to achieve efficacy per biomass treated should be recorded.

3.1.1.2 Measurement of treatment agent

For chemical treatments, the concentration of lethal agent or pH (acid descalers) in exposure tanks should be measured at intervals over the test duration relevant to the breakdown of the chemical.

For acid descaler treatments, pH may be measured using titration or spectrophotometry, as applicable.

For thermal treatments, the experimental design should factor in the rate of temperature increase, as this can influence the temperature at which mortality occurs. Continuous real-time temperature recording should occur in all exposure tanks to ensure that the desired temperature is achieved and maintained for the duration of the test.

Quality assurance and quality control procedures should be provided and followed for all measurements of treatment agent.

3.1.2 Assessment of test organism viability

For land-based testing, the presence of any viable organisms following exposure represents a failure to meet the performance criteria (Section 2.1). Viability should be determined using visual assessment techniques (Section 3.1.2.1).

3.1.2.1 Assessment of viability

Immediately after exposure to the treatment, the test organisms should be moved to sorting trays and covered with sea water. Organism viability should be assessed by signs of active feeding or movement by using either a handheld magnifying glass or lamp or a dissecting microscope (Appendix 8.2).

If viability is uncertain, organisms should be placed in a holding tank for two days and monitored at regular intervals to see if active feeding or movement occurs (Appendix 4.1.3).

Photographic or videographic images documenting each viability assessment should be provided.

3.1.2.2 Assessment of test organism(s) biomass

The biomass of all test organism(s) should be measured prior to all treatments and, for acid descalers, after completion of the treatment.

3.1.2.3 Photographic recording

All photographic images should include a scale object (such as a tape measure) and be to a resolution (minimum 300 pixels per inch or 5 megapixels) that allows:

- FR, percentage cover and viability to be allocated to images of settlement plates taken prior to treatment;
- Type and viability of relevant organisms to be determined, as possible.

A minimum of three photographic images and one videographic recording should be provided for verification of the use of settlement plates, and relevant organisms, and their placement within the test area.

All images should be provided, together with a key defining the test, replicate number and date on the labels included in each image and the image number.

3.1.3 Personnel for land-based testing

Independent, scientific supervision of testing ensures transparency and robustness of the testing methods followed. The independent supervising scientist nominated by the developer to undertake the land-based testing should be appropriately qualified from a reputable scientific organisation. The developer and an independent supervising scientist should not originate from the same organisation. Any potential conflicts of interest should be disclosed prior to testing.

To protect confidentiality and intellectual property, it may be necessary to draw up contracts between the developer, independent scientist(s) and regulator (upon receipt of the data).

3.1.4 General reporting of land-based testing

The results of all test runs – failing and passing – should be reported. All test results should be reported using the templates in Appendix 8.3.3.

Reporting of the test outcomes should include the following information (in addition to detailed results specified in Sections 3.2.4, 3.3.4 and 3.4.4):

- details (names and affiliations) of the personnel who conducted the testing and authored the report;
- any potential conflicts of interest.

3.2 LAND-BASED TESTING FOR CHEMICAL TREATMENT SYSTEMS

In addition to the general reporting outlined in Section 3.1, this test methodology is intended to assist the generation of data to define the lethal dose (LD_{100}) or lethal pH and volume ($LpHV_{100}$) and duration of treatment (LT_{100}) for chemical treatment systems (Section 2.1).

3.2.1 Before treatment

Prior to the commencement of the trial:

- measure the water quality parameters at appropriate intervals during the acclimation period and just prior to testing (Section 3.1.1.1);
- visually assess test organism viability (and attachment) (Appendix 8.2);
- for settlement plates, record and photograph the FR and percentage coverage (Section 3.1.1; 3.1.2.3);
- record all test organism(s) biomass (Section 3.1.2.2);
- record relevant organism species name, size, condition and attachment (e.g. confirm the production of byssal threads by bivalves and their attachment to the test surface).

3.2.2 During treatment

During the trial:

- measure the water quality parameters and chemical concentration or pH and volume added (Section 3.1.1.1; 3.1.1.2).

3.2.3 After treatment

At the end of the trial:

- record the test duration;
- measure the water quality parameters and chemical concentration or pH and volume needed (Section 3.1.1.1; 3.1.1.2);
- visually assess test organism viability (Section 3.1.2.1; Appendix 8.2);
- for acid descalers, record all remaining test organism(s) biomass (Section 3.1.2.2).

Record all generated data (Appendix 8.3.3) and assess whether the performance criteria have been met (Section 2.1).

3.2.4 Reporting

Using the recommended templates in Appendix 8.3.3; 8.3.4, an independent supervising scientist should report each of the following:

- *general:*
  - a description and specification of the chemical tested;
  - a description of the testing methodology, including choice of relevant organisms tested (Section 3.1.1);
- *before treatment:*
  - test organisms:

        - species, size, biomass and viability of relevant organisms being tested;
        - type, level (FR) and cover (%) of biofouling present on each settlement plate;
        - details of collection (e.g. source, season, location, habitat - intertidal, subtidal), acclimation conditions (e.g. duration, water quality parameters, (Section 3.1.1.1));
- *during treatment:*
    - timing and frequency of water quality and analytical samples were taken (Section 3.1.1.2);
    - the results of chemical analysis (e.g. concentration);
    - water quality parameters (Section 3.1.1.1);
    - pH and volume needed (for descalers).
- *after treatment:*
    - duration of chemical exposure;
    - water quality and analytical parameters (Section 3.1.1.1; 3.1.1.2);
    - the amount and type of viable organisms via direct observation:
        - a description of the general condition, number, size and type of any potentially viable organisms (Appendix 8.2);
        - for acid descalers only, measurement of remaining biomass (Section 3.1.2.2);
    - a description of any variations or deviations in application of the test relative to the testing methodology and test reporting;
- *conclusions for each test undertaken:*
    - chemical efficacy:
        - were the performance criteria met (Section 2.1)?
- *overall discussion and conclusion using all generated data (i.e. all land-based tests undertaken):*
    - testing rationale and potential for vessel application, including:
        - rationale for testing methodology (including treatment measurements);
        - rationale for relevant organisms tested;
        - potential influences on efficacy (e.g. temperature, presence of organic matter, level and type of fouling encountered; volume needed);
        - environmental concerns (e.g. persistence, toxicity, bioaccumulation):
            - considerations for mitigating environmental concerns;
        - engineering concerns:
            - potential effects of system use on a vessel's structural integrity;
    - recommendations, considerations or data gaps regarding the proposed treatment system.

The results of all test runs – failing and passing– should be reported. Example templates for reporting are provided in Appendix 8.3.

3.3 LAND-BASED TESTING FOR THERMAL TREATMENT SYSTEMS

In addition to the general reporting outlined in Section 3.1, this test methodology is intended to assist the generation of data to define the lethal temperature ($LTemp_{100}$) and duration of treatment (LT_{100}) for thermal treatment systems (Section 2.1).

Land-based testing is not necessary for thermal treatments that reach 60 °C and are held at that temperature for 60 minutes or more.

3.3.1 Before treatment

Prior to the commencement of the trial:

- measure the water quality parameters at appropriate intervals during the acclimation period and just prior to testing (Section 3.1.1.1);
- visually asses test organism viability (and attachment) (Appendix 8.2);
- for settlement plates, record and photograph FR and percentage coverage (Section 3.1.1; 3.1.2.3);
- record all test organism(s) biomass (Section 3.1.2.2);
- record relevant organism species name, size, condition and attachment (e.g. confirm the production of byssal threads by bivalves and their attachment to the test surface).

3.3.2 During treatment

During the trial:

- measure the water quality parameters and temperature (Section 3.1.1.1; 3.1.1.2).

3.3.3 After treatment

At the end of the trial:

- record test duration;
- measure the water quality parameters and temperature (Section 3.1.1.1; 3.1.1.2);
- visually assess test organism viability (Section 3.1.2.1).

All generated data should be recorded (Appendix 8.3.3) and used to assess whether the performance criteria have been met (Section 2.1).

3.3.4 Reporting

Using the recommended templates in Appendix 8.3.3; 8.3.4, an independent supervising scientist should report each of the following:

- *general:*
  - a description of the testing methodology including choice of relevant organisms tested (Section 3.1.1);
- *before treatment:*
  - test organisms:
    - species, size, biomass and viability of relevant organisms being tested;
    - type, level (FR) and cover (%) of biofouling present on each settlement plate;
    - details of collection (e.g. source, season, location, habitat - intertidal, subtidal), acclimation conditions (e.g. duration, water quality parameters (Section 3.1.1.1));
- *during treatment:*

  - timing and frequency of when temperature measurements were taken (Section 3.1.1.2);
  - temperature achieved throughout the exposure duration, including the rate of temperature increase;
  - water quality parameters (Section 3.1.1.1);
- *after treatment:*
  - duration of thermal exposure;
  - water quality parameters (Section 3.1.1.1);
  - the amount and type of viable organisms via direct observation:
    - a description of the general condition, number, size and type of any potentially viable organisms (Appendix 8.2);
  - a description of any variations or deviations in application of the test relative to the testing methodology and test reporting;
- *conclusions for each test undertaken:*
  - thermal efficacy:
    - were the performance criteria met (Section 2.1)?
- *overall discussion and conclusion using all generated data (i.e. all land-based tests undertaken):*
  - testing rationale and potential for vessel application, including:
    - rationale for testing methodology (including treatment measurements);
    - rationale for relevant organisms tested;
    - potential influences on efficacy (e.g. rate of temperature increase, presence of organic matter, level and type of fouling encountered);
    - environmental concerns (e.g. thermal pollution):
      - considerations for mitigating environmental concerns;
    - engineering concerns:
      - potential effects of system use on a vessel's structural integrity;
  - recommendations, considerations or data gaps regarding the proposed treatment system.

The results of all test runs – failing and passing – should be reported. Example templates for reporting are provided in Appendix 8.3.

3.4 LAND-BASED TESTING FOR CO-TREATMENT SYSTEMS

In addition to the general reporting outlined in Section 3.1, this test methodology is intended to assist the generation of data to define the lethal dose(s) (LD_{100}) or lethal pH and volume ($LpHV_{100}$) and duration of treatment (LT_{100}) (for co-treatments using more than one chemical treatment); or lethal dose(s) (LD_{100}) or lethal pH and volume ($LpHV_{100}$), temperature and duration of treatment (LT_{100}) (for co-treatments using a chemical(s)-temperature combination) to meet the criteria for treatment systems (Section 2.1).

3.4.1 Before treatment

Prior to the commencement of the trial:

- measure the water quality parameters at appropriate intervals during the acclimation period and just prior to testing (Section 3.1.1.1);
- visually assess test organism viability (Appendix 8.2);
- for settlement plates, record and photograph FR and percentage coverage (Section 3.1.1; 3.1.2.3);
- record all test organism(s) biomass (Section 3.1.2.2);
- record relevant organism species name, size, condition and attachment (e.g. confirm the production of byssal threads by bivalves and their attachment to the test surface).

3.4.2 During treatment

During the trial:

- measure water quality parameters and take analytical samples, as applicable (Section 3.1.1.1; 3.1.1.2).

3.4.3 After treatment

At the end of the trial:

- record test duration;
- measure water quality parameters and take analytical samples, as applicable (Section 3.1.1.1; 3.1.1.2);
- visually assess test organism viability (Section 3.1.2.1).
- record biomass of all remaining test organism(s) (for co-treatments using acid descalers only) (Section 3.1.2.2).

All generated data should be recorded (Appendix 8.3.3) and used to assess whether the performance criteria have been met (Section 2.1).

3.4.4 Reporting

Using the recommended templates in Appendix 8.3.3; 8.3.4, an independent supervising scientist should report each of the following:

- *general:*
  - a description and specification of the chemical(s) tested;
  - a description of the testing methodology including choice of relevant organisms tested (Section 3.1.1);
- *before treatment:*
  - test organisms:
    - species, size and viability of relevant organisms being tested;
    - type, level (FR) and cover (%) of biofouling present on each settlement plate;

        - details of collection (e.g. source, season, location, habitat - intertidal, subtidal), acclimation conditions (e.g. duration, water quality parameters (Section 3.1.1.1));
- *during treatment:*
  - frequency and timing of when analytical samples and temperature measurements were taken (Section 3.1.1.2);
  - where applicable, the results of chemical analysis (e.g. concentration);
  - where applicable, the temperature achieved throughout the exposure duration, including the rate of temperature increase;
  - where applicable, pH and volume needed (for descalers);
  - water quality parameters (Section 3.1.1.1);
- *after treatment:*
  - duration of exposure;
  - water quality parameters (Section 3.1.1.1) and results of chemical analyses, where applicable;
  - the amount and type of viable organisms via direct observation:
    - a description of the general condition, number, size and type of any potentially viable organisms (Appendix 8.2);
    - for co-treatments using acid descalers, measurement of remaining biomass (Section 3.1.2.2).
  - a description of any variations or deviations in application of the test relative to the testing methodology and test reporting;
- *conclusions for each test undertaken:*
  - co-treatment efficacy:
    - were the performance criteria met (Section 2.1)?
- *overall discussion and conclusion using all generated data (i.e. all land-based tests undertaken):*
  - testing rationale and potential for vessel application, including:
    - rationale of testing methodology (including treatment measurements);
    - rationale of relevant organisms tested;
    - rationale and applicability of testing temperature (chemical co-treatments only);
    - potential influences on efficacy (e.g. presence of organic matter, water temperature, level and type of fouling encountered, volume needed);
    - environmental concerns (e.g. persistence, toxicity, bioaccumulation, thermal pollution):
      - considerations for mitigating environmental concerns;
    - engineering concerns:
      - potential effects of system use on the vessel's structural integrity;
  - recommendations, considerations or data gaps regarding the proposed treatment system.

The results of all test runs – failing and passing – should be reported. Example templates for reporting are provided in Appendix 8.3.

3.5 GENERAL CONSIDERATIONS FOR VESSEL TESTING

The following considerations should be documented by the developer and submitted with the test data (Section 3.6.7; 3.7.7; 3.8.5; 3.9):

- description and specification of the system tested:
  - mechanism of action to clean or treat biofouling;
  - equipment design;
  - method of operation;
- description of system applications:
  - internal niche area(s) the system is designed to treat or clean;
  - the minimum and maximum dimensions (L x W x H), volume (m^3) or configuration the system is designed to treat or clean;
  - standard operating procedures (SOP) for the system, which detail:
    - the mode of operation of the system, including how it is to be applied;
    - steps to be taken to ensure that viable organisms are not released during mobilisation, application, and demobilisation of the system (including contingency plans to manage these risks);
    - for chemical treatments and co-treatments that use chemicals, the SOP should specify:
      - the lethal dose (LD_{100}) or lethal pH and volume ($LpHV_{100}$), and duration of treatment (LT_{100}) and temperature necessary to achieve 100 % mortality;
      - how dose and mortality will be verified, including quality assurance and quality control procedures;
    - for thermal treatments and co-treatments that use heat, the SOP should specify:
      - the temperature ($LTemp_{100}$) and duration of treatment (LT_{100}) necessary to achieve 100 % mortality;
      - how temperature and mortality will be verified, including quality assurance and quality control procedures;
    - sea and weather conditions under which the system is intended to be used (e.g. limits on current speed, wave height, water temperature, water clarity, pH, salinity, organic matter, etc. to ensure efficacy, containment and operator safety);
    - the total time, including set-up and demobilisation, it takes to treat or clean an internal niche area;
- details of the test vessel:
  - name, vessel type, length;
  - detailed diagram of each internal niche area to be cleaned or treated including its position on the vessel;
- details and qualification of independent scientific organisations and personnel performing and supervising the test.

The following factors should be considered by the developer prior to testing:

- proposed test methods, including the date and location and any modifications to the methods set out in Sections 3.6, 3.7, 3.8 and 3.9. Modification may relate, for example, to limitations on the intended use of the system (e.g. internal niche area(s) of the vessel that the system is to be used on);
- the area to be cleaned or treated:

  - type of vessel and internal niche area (e.g. sea chest with or without a removable grate);
  - volume of internal niche area (for calculating target chemical concentration, pH and volume or thermal parameters needed);
  - levels and type of biofouling in the test area (Section 3.5.2);
- details on how isolation of the internal niche area occurred, where applicable (e.g. external blanking plates, isolation valves);
- the ability of the vessel to safely operate after system application (Section 3.5.5);
- the size or configuration of an internal niche area that may limit the efficacy of the in-water treatment or cleaning system (e.g. position on the vessel, orientation, shape and size of a sea chest or potential isolation issues between the sea chest and associated internal seawater pipework system);
- test supervision (e.g. by appropriately qualified, independent supervising scientists);
- operator considerations:
  - where the developer does not intend to use their own staff to operate the system during testing, detailed instructions for the system, including schematic diagrams, should be provided to the personnel performing the test. The instructions should be sufficiently detailed that testing can be done safely and in accordance with the developer's instructions and the intended use;
  - consultation and cooperation with the ship's engineer to ensure a safe operating environment;
  - divers carrying out and observing test operations should have communication with the surface supervisor. Those operating the system in-water should have the means, on the system, to stop, start and manoeuvre the system, and the surface supervisor should be able to shut down the system independently in cases of equipment or system failure;
  - testing operations should comply with safe diving codes of practice and appropriate health and safety legislation (Section 4.2.1).

3.5.1 General conditions for test implementation

The general conditions necessary for testing include:

- conduct during periods of slack water, with current speeds of no more than 1 kn (~ 50 cm s^{-1}), in order to aid an independent supervising scientist in observing system operation (Section 4.3.2);
- conduct at locations and times when water clarity (measured as vertical Secchi disk depth) is at least 2 m (Section 4.3.3).

3.5.2 Level, cover and origin of biofouling on the test surface

3.5.2.1 Treatment systems

The treatment systems tested should be capable of emergency use on biofouling from heavily fouled internal surfaces, therefore testing for the majority of internal niche areas should be completed on viable biofouling of at least FR 90 and 100 % cover that occurs over at least 12 × 400-cm^2 (20 × 20 cm) sample areas (Floerl *et al.* 2005; Naval Ships' Technical Manual 2006; Morrisey *et al.* 2015; Appendix 8.1; 8.2). The minimum total coverage should be 4 800 cm^2, however the fouled sample areas maybe continuous or consist of separate sample areas, or a combination of the two. FR and coverage for narrow or smaller internal niche areas should be determined on a case-by-case basis with a rationale provided. Some sample areas should be accessible for biofouling removal for viability testing (Section 3.5.11), with other

sample areas accessible using an endoscope. A minimum of three photographic images and one videographic recording should be provided for each determination of FR and % cover.

Chemical and thermal in-water systems should be tested on biofouling assemblages that include relevant organisms known to be resilient to the treatment (e.g. for chlorine treatments, biofouling assemblages should include organisms with the ability to isolate themselves from the surrounding environment, particularly bivalves and barnacles (Section 4.1.5.2)).

The type of biofouling present can be described in broad taxonomic and morphological categories (such as, hydroids, tubeworms, erect bryozoans, bivalves and barnacles) and determined by a suitably qualified independent scientist (Section 4.2.3). All surviving organisms should be identified to *species* level and photographed.

For treatment systems, settlement plates and other relevant organisms should be used to provide assurance of treatment efficacy throughout the internal niche area being assessed (Section 3.5.8). A minimum of three photographic images and one videographic recording should be provided for verification of the use of settlement plates (e.g. biofouling type, cover and viability) and relevant organisms (e.g. type and viability).

3.5.2.2 Physical removal systems

The physical removal systems tested should be capable of emergency use on all accessible heavily fouled internal surfaces. For the majority of internal niche areas, testing should be completed on viable biofouling of at least FR 90 and 100 % cover that occurs over at least 12 × 400-cm^2 (20 × 20 cm) sample areas (Floerl *et al.* 2005; Naval Ships' Technical Manual 2006; Morrisey *et al.* 2015; Appendix 8.1; 8.2). The minimum total coverage should be 4 800 cm^2, however the fouled sample areas maybe continuous or consist of separate sample areas, or a combination of the two. FR and coverage for narrow or smaller internal niche areas should be determined on a case-by-case basis with a rationale provided. A minimum of three photographic images and one videographic recording should be provided for each determination of FR and % cover.

3.5.2.3 All systems

To minimise biosecurity risk during the test, biofouling should be regionally derived (Department of Environment and the Ministry for Primary Industries 2015). To ensure this, the vessel's operational history, including biofouling management, should be known. If a vessel with regional biofouling[7] is not available, a vessel with non-regional biofouling may be used only if the associated biosecurity risk can be shown to be minimal, for example by examining the vessel's operational history or by having a diver and taxonomic inspection of the hull and internal niche areas. In the latter case, the costs and time delays of using a vessel with non-regional biofouling (including the costs associated with obtaining a resource consent) should be balanced against those of waiting for a suitable vessel to arrive or of relocating the system to another port where a regionally fouled vessel is available (Morrisey *et al*. 2015).

[7] Biofouling that has been acquired in the same location where in-water system testing is proposed. "Regional" is as specified by the relevant local government authority in New Zealand. This category may be defined on the basis of the known distribution of established aquatic invasive species or ongoing pest management, or the location of high-value environments. Such delineation is the responsibility and prerogative of the local government.

3.5.3 Personnel for system testing

As operation of the system would typically necessitate specific training or expertise, it is reasonable that the developer would use their own staff or contractors. Therefore, operation of the system during testing may be done by the developer's staff or a contractor(s) nominated by the developer.

Independent, scientific supervision of testing ensures transparency and robustness of the testing methods followed. The independent supervising scientist nominated by the developer to undertake the vessel testing should be appropriately qualified from a reputable scientific organisation. The developer and an independent supervising scientist should not originate from the same organisation. Any potential conflicts of interest should be disclosed prior to testing.

To protect confidentiality and intellectual property, contracts may be drawn up between the developer, contractor(s), independent supervising scientist(s) and regulator (upon receipt of the data).

3.5.4 General reporting of testing

The results of all test runs – failing and passing – should be reported. All test results should be reported using the templates in Appendix 8.3.5.

Reporting of the test outcomes should include the following information (in addition to detailed results specified in Sections 3.6.7, 3.7.7, 3.8.5 and 3.9):

- details of the system tested, including areas for which use of the system is intended;
- details (names and affiliations) of the personnel who operated the system;
- details (names and affiliations) of any independent scientist(s) conducting the test (if these are different from personnel conducting the test), and any potential conflicts of interest;
- the environment (location, weather and sea conditions, vessel draft, location of test areas, water clarity at the time of testing (as Secchi depth)) in which the test was done;
- diagram of the internal niche area the in-water system was tested on, including: information of its location on the vessel, volume, configuration, location of any valves and seals, and material(s) the internal niche area is constructed from.

The test reporting described in Sections 3.6.7, 3.7.7, 3.8.5 and 3.9 includes assessments of the amount and type of biofouling on the surface of the test areas before and after the test, and viability of biofouling organisms following treatment application (Appendix 8.2).

3.5.5 Condition of vessel internal structures

Vessel internal structures to which the test is applied should be inspected by an appropriately qualified person (Section 2.4). Inspections should occur prior to and after system application to the internal niche area. Observations of any detrimental effects (e.g. corrosion, warping or physical damage) to any vessel material or components (e.g. plate coolers, valves and seals) should be recorded, including those as a result of using a containment system (e.g. sea chest blank). A minimum of three photographic images and one videographic recording should be provided for each area assessed.

System application should not negatively affect the operational condition of the vessel.

3.5.6 Sample size and internal niche area type(s) for testing in-water system efficacy

3.5.6.1 Physical removal systems

During the test, the in-water system should be used in the manner in which it is intended under normal operation. The in-water system should be tested on at least *six* accessible internal niche areas (Section 4.2.4):

At least *one* of the above internal niche areas should be at the upper size limit the system is intended to be used on.

The level of replication *may necessitate testing on more than one vessel* (Table 3-1).

If the in-water system is to be used on only external areas, such as sea chest grates, then it should be assessed using the most up-to-date test methods based on Morrisey et al. (2015).

3.5.6.2 Chemical, thermal and co-treatments

During the test, the in-water system should be used in the manner in which it is intended under normal operation.

The efficacy of the in-water system should be tested on *six* vessel internal niche areas (Section 4.2.5).

The containment ability of the in-water system can be examined on the above *six* niche areas following efficacy testing (Section 4.2.5).

Three of the tested internal niche areas should be at the upper size limit that the system is intended to be used on. *Three* of the tested internal niche areas should be at the lower size limit that the system is intended to be used on.

The tested internal niche areas should be similar to each other, for example, the first two internal areas tested cannot be sea chest and pipework with the remaining area consisting of pipework only.

The level of replication *may necessitate testing on more than one vessel* (Table 3-1).

3.5.6.3 Type of internal areas needing assessment to determine efficacy of treatment systems

To assess the efficacy of treatment systems, it is necessary to take into account the physical complexity of the surfaces within the internal niche area(s) of the test vessel(s).

Assessment of sea chests (and cavities) should consist of sampling areas including, but not limited to:

- internal walls and corners;
- weld seams;
- baffles;
- recesses;
- other difficult to access areas, depending on specific design (e.g. cooling veins in the case of box coolers).

Assessment of internal seawater systems should consist of sampling areas including, but not limited to:

- internal pipe walls and bends;
- weld seams;
- baffles (e.g. cofferdams);
- heat exchangers;
- valves.

3.5.6.4 Type of internal areas that should be assessed to determine efficacy of physical removal systems

To assess the efficacy of physical removal systems, it is necessary to take into account the accessibility and physical complexity of the surfaces within the internal niche area(s) of the test vessel(s).

The particular internal niche areas that may be assessed include, but are not limited to:

- internal walls and corners;
- weld seams;
- baffles;
- recesses;
- internal pipe walls and bends;
- other difficult to access areas, depending on specific design (e.g. cooling veins in the case of box coolers).

Table 3-1. Summary of recommended replication and sampling regime for in-water system testing on internal niche areas.

System	Physical removal	Chemical, thermal and co-treatments
Test area	**Internal niche area**	**Internal niche area**
Number of test areas needing system application for efficacy testing (replication)	*n* = 6	*n* = 3 (per upper and lower size limit, respectively)
Number of test areas needed for assessment of containment	N/A	*n* = 3 (per upper and lower size limit, respectively)

3.5.7 Suitable vessels for testing

The vessels used in testing should fulfil the following criteria:

- have internal niche areas of size and configuration relevant to the intended use of the system:
    - removal systems – at least *one* replicate internal niche area should be at the upper size limit the system is intended to be used on;
    - treatment systems – *three* internal niche areas should be at the upper size limit the system is intended to be used on, and *three* internal niche areas should be at the lower size limit the system is intended to be used on;
- type and coverage of biofouling that the system is intended to be used on (Section 3.5.2).

The developer, or the contractor carrying out the test, should arrange access to vessels to assess their suitability for testing, and should use their own contacts for arranging this (Section 4.2.6). Possible options include:

- commercial or recreational vessels waiting to be slipped or dry-docked;

- New Zealand Defence Force vessels;
- oil and gas industry vessels (Morrisey *et al.* 2015).

3.5.8 Determination of additional relevant organisms for vessel testing (treatment systems only)

In addition to Section 3.5.2, the use of settlement plates and other relevant organisms should be used, where practical (Section 3.5.2.1). This provides further confidence that the performance criteria for the treatment system have been met (Section 2.1). Relevant organisms (e.g. bivalves) should be placed in areas of the internal niche area that can be accessed but do not contain the appropriate fouling (e.g. within areas of pipework that have access ports). Settlement plates should be placed in accessible areas of the internal niche area that are identified as poor mixing zones (e.g. corners, curves, obstructed or sheltered areas). The use of additional organisms and settlement plate fouling, however, does not replace the testing on biofouling within the vessels' internal surfaces (Section 3.5.2) and does not replace any of the sampling recommended for test areas (Section 3.5.6.3).

The type of biofouling on settlement plates can be described in broad taxonomic and morphological categories (such as, hydroids, tubeworm, erect bryozoans, bivalves, barnacles and colonial ascidians) and should be determined by a suitably qualified independent scientist. However, all surviving organisms should be identified to *species* level and photographed.

The rationale for determining relevant organisms for vessel testing should be justified using scientific literature (i.e. specific systems should be tested where possible on the most tolerant species or taxonomic group). Relevant organisms tested should be identified to *species* level.

A rationale for placement of settlement plates and relevant test organisms throughout the test area and the number of replicates should be provided in the report.

A minimum of three photographic images and one videographic recording should be provided for verification of the use of settlement plates (e.g. biofouling type, cover and viability) and relevant organisms (e.g. type and viability) and their placement within the test area.

3.5.9 Measurement of the treatment agent (chemical treatments)

For chemical treatments, measurements should be taken to demonstrate that the necessary lethal chemical concentration is delivered by the in-water system throughout the internal niche area for the appropriate exposure period. For descalers, this would be a combination of pH and volume needed. Methods used to measure the chemical concentration or pH should be appropriate to the lethal agent and be measured at intervals over the test duration relevant to the breakdown of the chemical.

For acid descaler treatments, pH may be measured via titration or spectrophotometry, as applicable.

Depending on internal niche area configuration and chemical type(s), triplicate measurements or continuous real-time measurements should be taken from at least *three* different locations within the internal niche area, taking into account:

- distance from the point of injection (e.g. end of relevant pipework);
- poor mixing zones (e.g. corners, curves, obstructed or sheltered areas, or bends).

The location of each measurement should be recorded. The rationale for each location chosen for analytical measurements should be provided.

All measurements should be repeated at a minimum of *three* intervals:

- immediately after the containment system is in place, taking into account time needed for adequate mixing;
- mid-way through the treatment;
- immediately prior to the end of treatment.

The temperature of the sea water should also be measured on the day of testing using an appropriate method.

Any deviation from the above measurement parameters should be justified.

Quality assurance and quality control procedures should be provided and followed for all measurements of treatment agent.

3.5.10 Measurement of the treatment agent (thermal treatments)

For thermal treatments, real-time temperature data loggers should be used to demonstrate that the necessary lethal temperature is achieved throughout the test areas for the appropriate period of time.

Real-time temperature data loggers should be located in at *least* three different locations within the internal niche area, taking into account:

- distance from the point of injection or heating element (e.g. end of relevant pipework);
- poor mixing zones (e.g. corners, curves, obstructed or sheltered areas, bends).

The location of each measurement should be recorded. The rationale for each location chosen for measurement should be provided.

Quality assurance and quality control procedures should be provided and followed for all measurements of treatment agent.

3.5.11 Assessing viability of biofouling

For all in-water systems, the presence of any remaining viable macroscopic organisms in the cleaned or treated internal niche area represents a failure to meet the performance criteria (Section 2.1). Viability *before* and *after* testing should be evaluated visually and recorded by video imagery. A minimum of one videographic recording should be provided for each determination of organism viability.

Following system application, an independent supervising scientist should take a representative sample of the remaining cleaned or treated biofouling from a minimum of *six* 25-cm^2 areas within accessible sections of the internal niche area (Section 3.5.2.1).

Representative samples of biofouling assemblages should be removed manually (i.e. by hand or using a paint scraper) from the treated surface, taking care to ensure that the organisms and the surface are not damaged during removal. The samples should be placed into sea water in labelled, sealable water-tight bags (e.g. zip-lock bags) or containers for transfer to shore.

Samples should be appropriately stored and rapidly assessed so that viability is not confounded by handling or environmental variables (e.g. exposure to air, temperature fluctuations, sunlight).

Each biofouling assemblage sample collected from the treated areas should be placed separately into a sorting tray and covered with sea water. The types of organisms in each sample and their structural integrity (intact or exhibiting some degree of damage; Appendix 8.2) should be recorded (Appendix 8.3.7.5). Each major taxonomic group (i.e. barnacles, tubeworms, hydroids, bryozoans, etc.) should be sorted into separate dishes, covered with sea water, and left undisturbed for 20–30 minutes. The organisms in each sorting dish should be examined under magnification using either a handheld magnifying glass or a dissecting microscope for signs of active feeding or movement (Appendix 8.2). If viability is uncertain, organisms should be placed in a holding tank for two days and monitored at regular intervals to see if active feeding or movement occurs (Appendix 4.1.3).

For settlement plates and other relevant organisms that are added to an internal niche area, the ideal sampling regime is dependent on, for example, the size of the niche area(s) and settlement plates or number of individual species used per trial. The sampling rationale and level of replication are therefore unique, and should be justified using scientific literature. Viability of biofouling communities on settlement plates and relevant organisms can be assessed using the above methods. A minimum of one videographic recording should be provided for each determination of organism viability.

3.5.11.1 Videographic and photographic assessment

The developer and an independent supervising scientist carrying out the testing should use the imaging as described below. If access to surfaces is difficult, a pipe inspection camera (endoscope) with its own light source could be used.

All images should be to a resolution that allows:

- the confirmation of biofouling viability, FR and percentage cover in images taken prior to treatment (Appendix 8.1; 8.2);
- broad assessment of the surface physical and structural condition of the area before and after system application (video or still imaging);
- assessment of viability post-treatment (video only) (Morrisey *et al.* 2015).

For each image(s), the following metadata should be included:

- the date of the test;
- the name of the system under test;
- the name of the test vessel;
- the internal niche area being sampled (e.g. sea chest or pipework and location on the vessel);
- the location of still images within the internal niche area, or the location of pass over the internal niche area if video images are not continuous for the whole area.

The developer and independent supervising scientist should determine the best method for meeting the above conditions. These are dependent on the equipment used and the environmental conditions (particularly water clarity) at the time of testing. Diver swimming speed should not exceed 30 cm s^{-1} (0.6 kn) to prevent blurring of the image in individual frames (Section 4.3.2).

After system testing, video images of organism viability should be obtained within two days of completion of the test.

All images should be provided and, if applicable, a key explaining the text in the metadata of each image (e.g. codes for location on hull, location within the niche area, image number) and listing any viable biofouling present in each image (in the case of post-treatment images). A diagram should be provided showing the locations of:

- each internal niche area on the hull;
- the location of each image within the internal niche area.

A minimum of one videographic recording should be provided for each determination of organism viability.

3.5.12 Assessing containment and biofouling dislodgement (chemical, thermal and co-treatments)

The ability to contain internal sea water, mobile organisms and biofouling without dislodgement should be assessed by video recording the exterior of the sea chest or pipework outlet(s), discharge points or shrouding system by an underwater observer (Section 4.3.4). A visible, non-toxic tracer dye (e.g. fluorescein sodium salt, Basic Blue 3 or Rhodamine WT Red at a concentration of 4 g L^{-1} or an appropriate equivalent) could be used (Morrisey *et al.* 2015).

To ensure that the assessment of containment does not negatively influence the assessment of viability (i.e. the use of dyes impeding the ability of an independent supervising scientist to assess viability), the containment ability of the in-water system should be tested on *three* vessel internal niche areas (Section 4.2.5) *after* they have been used for efficacy testing.

3.5.13 System testing approval by regulatory authorities

A resource consent may be needed depending on the location in which the test is done. This is particularly so if biocides, chemicals or heated water are used to treat biofouling, or if any effluent is to be released back into the marine environment. Obtaining this, or any other necessary approval, is the responsibility of the developer.

Where resource consent is needed, several test variables may reduce the biosecurity and chemical contamination risks:

- presence of regional biofouling within the test areas (e.g. negligible biosecurity risk);
- containment, collection or treatment of waste water;
- for removal methods:
    - presence and effectiveness of the antifouling system (e.g. aged coatings may have reduced levels of biocide and thus represent a lower contamination risk than those systems recently applied; Morrisey *et al.* 2015);
    - size of test area(s) compared to cleaning a whole vessel.

3.6 VESSEL TESTING FOR CHEMICAL TREATMENT SYSTEMS

Vessel testing is necessary to best assess the performance of the full proposed system in a realistic simulation of its intended use. This methodology allows assessment of the effects of scale and environmental conditions on system efficacy, waste containment, escape of mobile species and of biofouling dislodgement by divers and equipment while in operation.

3.6.1 Vessel selection

A chemical treatment system should be tested on at least one vessel (Section 4.3.1). *If the vessel does not contain the appropriate number of internal areas* (Section 3.5.6.2; Table 3-1), *then testing on more than one vessel should be undertaken.*

Three of the replicate internal niche areas should be at the upper size limit the system is intended to be used on. *Three* of the replicate internal niche areas should be at the lower size limit the system is intended to be used on.

3.6.2 Testing method

During the test, the in-water system should be used in the manner in which it is intended under normal operation. The efficacy of the in-water system should be tested on *six* replicate internal areas (Section 3.5.6.2).

The containment ability of the in-water system can be tested on the same *six* internal niche areas (Section 4.2.5) after efficacy testing has occurred (Section 3.5.12).

Before testing proceeds, an independent supervising scientist should determine the biofouling viability, rating (FR), percentage cover and area fouled in each internal area (Section 3.5.2.1). The physical and structural conditions of the entire area should be assessed by a suitably qualified independent professional (Section 3.5.5).

Each internal area should be recorded by video and digital still imaging to allow auditing of biofouling viability, rating and cover (Section 3.5.11.1; 4.3.4).

Systems that release effluent (e.g. draining) from the internal niche area prior to, or during exposure to the lethal agent, should meet the criteria for testing effluent treatment systems (Section 2.2; 3.10).

3.6.2.1 Assessing treatment containment

If the sea water within the internal niche area is to be contained, then the sea chest grate and all water overflow discharge outlets should be sealed. The process of installing the containment system should be recorded on video by the underwater observer to determine if any biofouling is dislodged or if there is any escape of mobile species (Section 3.5.12).

3.6.3 Monitoring conditions achieved by the system

An independent supervising scientist should measure and record the concentration of the chemical agent or pH and volume required by treatment system at appropriate intervals. Methods used to measure the concentration or pH should be appropriate to the system (Section 3.5.9).

3.6.4 Assessing chemical treatment efficacy

After the system has undergone testing, images of the treated internal area (Section 3.5.6.3) should be obtained by video photography (Section 3.5.11.1). An independent supervising scientist should examine each video in its entirety to assess the viability of any residual organisms (Appendix 8.2).

Any viable organisms detected in the video should be recorded against the image identifier (file name) and a description of the location within the treated internal area (e.g. by reference to the timestamp in the video at which the viable organism is detected). An independent supervising scientist should also describe the general condition of the biofouling present, including signs of bleaching, physical damage, morbidity and whether there are any indicators of viability (Appendix 8.2).

After video photography occurs, representative samples (Section 3.5.11) of biofouling from accessible sections of the treated internal niche area should be collected to determine viability (Appendix 8.2).

Relevant organisms and settlement plate fouling communities added to the internal niche area (Section 3.5.11) should be assessed for viability (Appendix 8.2) in addition to the above sampling recommendations.

The type (i.e. taxonomic group) and condition (including viability) of biofouling observed in samples taken from the treated internal area should be recorded against the sample identifier.

Information should be recorded directly to an electronic spreadsheet version of the data sheet template (Appendix 8.3.7.5), or to a paper version and later transferred to an electronic version.

The presence of any viable biofouling within the treated internal area constitutes a failure to meet the performance criteria (Section 2.1). All viable biofouling should be identified to *species* level by a qualified independent scientist.

The internal niche area should be assessed to identify any physical or structural damage caused by system deployment or removal of sea chest blanking plates (Section 3.5.5).

3.6.5 Assessing containment efficacy and biofouling dislodgement

A diving observer should record on video the test process, including set-up and demobilisation, to assess material dislodgement from the vessel, the escape of mobile organisms and release of untreated effluent from the internal niche area. The video may be recorded by an independent supervising scientist (using Underwater Breathing Apparatus) or by a diver under the direction of an independent supervising scientist using surface-to-diver communications.

For systems that use a sea chest blanking plate or other water-blocking equipment (e.g. bungs, corks) to seal water discharge outputs or overflows, their ability to contain the internal sea water should be assessed by the use of a suitable dye (Section 3.5.12). Effective containment would be indicated by the absence of visible dye in the water column near the mouth of the sea chest and outputs or overflows of the other relevant sealed-off areas.

After completion of the trial, the video should be assessed for evidence of material being dislodged from the vessel over the entire test and for leakage and escapes from the internal niche area tested. This assessment should be included in the reporting template (Appendix 8.3.5).

The beginning of each video recording should include a label indicating:

- the date of the test;
- name of the system being tested;
- name of the vessel;
- organism type (e.g. bivalve, bryozoan, crustacean) niche area type (e.g. sea chest grate), position on vessel and replicate number.

Organism escapes or leakage of untreated effluent from the treated internal area or dislodgement of any macroscopic particles > 0.5 cm diameter during system set-up or demobilisation represents a failure to meet the performance criteria (Section 2.3).

3.6.6 Post-treatment

Once the necessary chemical concentration (LD_{100}) or pH and volume ($LpHV_{100}$) and exposure time (LT_{100}) has been achieved, the treated water should be:

- discharged on-shore; or
- neutralised prior to release; or
- if permitted by resource consent, discharged directly into the marine environment.

All data should be recorded (Section 3.6.7).

3.6.7 Reporting

Using the recommended template in Appendix 8.3.5, an independent supervising scientist should report each of the following:

- *general:*
  - a description and specification of the system (Section 3.5);
  - a description of the standard operating procedure (SOP) for the system (Section 3.5);
  - evaluation of system use on vessel materials, including (as appropriate) a list of materials the system is incompatible with (Section 2.4);
  - a description of how the test was undertaken, including:
    - the location, type of vessel used, internal niche area (e.g. sea chest, internal pipework), internal niche area material, dimensions of internal niche area (e.g. length, width, breadth, volume), and environmental conditions during the test (Section 3.5.4);
    - a description of the procedures followed during set-up, testing of the system and demobilisation;
  - the total time, including set-up and demobilisation, it takes to treat each internal niche area;
- *before treatment:*
  - type, viability, level (FR), cover (%) and area of organisms present in each internal niche area (Section 3.5.2);
  - for settlement plates and relevant individual organisms:
    - species, size and viability of individual organisms being tested;

        - type, level (FR) and cover (%) of biofouling present on each settlement plate;
        - details of collection (e.g. source, season, location, habitat - intertidal, subtidal), acclimation conditions (e.g. duration, water quality parameters);
    - type and condition of internal niche area to be treated:
        - the video or still image(s) on which these assessments were made should be provided with the report;
- *during treatment:*
    - the results of samples taken to monitor the chemical concentration or pH achieved and appropriate volume, including where and when the samples were taken (Section 3.5.9);
- *after treatment:*
    - the amount and type of viable organisms observed in video recordings of the treated internal niche area including:
        - a description of the general condition of the organisms present, including signs of physical damage, change in pigmentation and morbidity (Appendix 8.2);
        - a description of the number, size and type of organisms (Section 3.5.11.1) that exhibited indications of potential viability (Appendix 8.2) and their location within the treated internal niche area;
        - relevant video time-stamp and video identifier (file name);
    - the amount and type of viable biofouling recorded in each replicate sample of biofouling removed from the accessible treated internal areas, including:
        - a description of the number, size and type of biofouling organisms (Section 3.5.11) that exhibited indications of potential viability (Appendix 8.2);
        - relevant sample identifier (i.e. test replicate identifier);
        - location of the internal niche area on the vessel;
    - physical and structural condition of the treated internal niche area:
        - the video or still images on which these assessments were made should be provided with the report (Section 3.5.11.1);
    - qualitative assessment of:
        - loss of material by dislodgement from the vessel and escape of mobile organisms during set-up, operation and demobilisation;
        - leakage from the system:
            - the video images from which this assessment was made should be provided with the report;
    - a description of any variations or deviations in application of the test relative to the SOP, test methods and quality control and quality assurance procedures;
    - a discussion of the system efficacy, including whether the performance criteria were met;
    - recommendations for system or SOP improvement.

The results of all test runs – failing and passing – should be reported. Example templates for reporting are provided in Appendix 8.3.

3.7 VESSEL TESTING FOR THERMAL TREATMENT SYSTEMS

Vessel testing is necessary to best assess the performance of the full proposed system in a realistic simulation of its intended use. This methodology allows assessment of the effects of scale and environmental conditions on system efficacy, waste containment, escape of mobile species and of biofouling dislodgement by divers and equipment while in operation.

3.7.1 Vessel selection

A thermal treatment system should be tested on at least one vessel (Section 4.3.1). *If the vessel does not contain the appropriate number of test areas* (Section 3.5.6.2; Table 3-1) *then testing on more than one vessel should be undertaken.*

Three of the replicate internal niche areas should be at the upper size limit the system is intended to be used on. *Three* of the replicate internal niche areas should be at the lower size limit the system is intended to be used on.

3.7.2 Testing method

During the test, the in-water system should be used in the manner in which it is intended under normal operation. The efficacy of the in-water system should be tested on *six* replicate test areas (Section 3.5.6.2).

The containment ability of the in-water system can be tested on the same *six* internal niche areas (Section 4.2.5) after efficacy testing has occurred (Section 3.5.12).

Before testing proceeds, an independent supervising scientist should determine the biofouling viability, rating (FR), percentage cover and area fouled in each internal niche area (Section 3.5.2). The physical and structural conditions of the entire area should be assessed by a suitably qualified independent professional (Section 3.5.5).

Each internal niche area should be recorded by video and digital still imaging to allow auditing of biofouling viability, rating and cover (Section 3.5.11.1; 4.3.4).

Systems that release effluent (e.g. draining) from the treated internal niche area prior to, or during exposure to the lethal agent, should meet the criteria for effluent treatment systems (Section 2.2; 3.10).

3.7.2.1 Assessing treatment containment

If the sea water within the internal niche area is to be contained, then the sea chest grate and all water overflow discharge outlets should be sealed. The process of installing the containment system should be recorded on video by the underwater observer to determine if any biofouling is dislodged or if there is any escape of mobile species (Section 3.5.12).

3.7.3 Monitoring conditions achieved by the system

An independent supervising scientist should measure and record the temperature that is achieved by the treatment system at appropriate intervals. Methods used to measure the temperature should be appropriate to the system (Section 3.5.10).

3.7.4 Assessing thermal treatment efficacy

After the system has undergone testing, images of the internal niche area (Section 3.5.6.3) should be obtained by video photography (Section 3.5.11.1). An independent supervising

scientist should examine each video in its entirety to assess the viability of any residual organisms (Appendix 8.2).

Any viable organisms detected in the video should be recorded against the image identifier (file name) and a description of the location within the treated internal niche area (e.g. by reference to the timestamp in the video at which the viable organism is detected). An independent supervising scientist should also describe the general condition of the biofouling present, including signs of bleaching, physical damage, morbidity and whether there are any indicators of viability (Appendix 8.2).

After video photography occurs, representative samples (Section 3.5.11) of biofouling from accessible sections of the treated internal niche area should be collected to determine viability (Appendix 8.2).

Relevant organisms and settlement plate fouling communities added to the internal niche area (Section 3.5.11) should be assessed for viability (Appendix 8.2) in addition to the above sampling recommendations.

The type (i.e. taxonomic group) and condition (including viability) of biofouling observed in samples taken from each treated internal niche area should be recorded against the sample identifier.

Information should be recorded directly to an electronic spreadsheet version of the data sheet template (Appendix 8.3.7.5), or to a paper version and later transferred to an electronic version.

The presence of any viable biofouling within the treated internal niche area constitutes a failure to meet the performance criteria (Section 2.1). All viable biofouling should be identified to *species* level by a qualified independent scientist.

The treated internal niche area should also be assessed to identify any physical or structural damage caused by system deployment or removal of sea chest blanking plates (Section 3.5.5).

3.7.5 Assessing containment efficacy and dislodgement of biofouling

A diving observer should record on video the test process, including set-up and demobilisation, to assess the amount of material dislodged from the vessel, the escape of mobile organisms and release of untreated effluent from the internal niche area. The video may be recorded by an independent scientist (using Underwater Breathing Apparatus) or by a diver under the direction of an independent supervising scientist using surface-to-diver communications.

For systems that use a sea chest blanking plate or other water-blocking equipment (e.g. bungs, corks) to seal water discharge outputs or overflows, their ability to contain the internal sea water should be assessed by the use of a suitable dye (Section 3.5.12). Effective containment would be indicated by the absence of visible dye in the water column near the mouth of the sea chest and outputs or overflows of the other relevant sealed off areas.

After completion of the trial, the video should be assessed for evidence of material being dislodged from the vessel over the entire test and for leakage and escapes from the treated internal area. This assessment should be included in the reporting template (Appendix 8.3.5).

The beginning of each video recording should include a label indicating;

- the date of the test;
- name of the system being tested;
- name of the vessel;
- organism type (e.g. bivalve, bryozoan, crustacean), niche area type (e.g. sea chest grate), position on vessel and replicate number.

Organism escapes or leakage of untreated effluent from the treated internal area or dislodgement of any macroscopic particles > 0.5 cm diameter during system set-up or demobilisation represents a failure to meet the performance criteria (Section 2.3).

3.7.6 Post-treatment

Once the necessary temperature ($LTemp_{100}$) and exposure time (LT_{100}) has been achieved, the treated water should be:

- discharged on-shore; or
- neutralised prior to release; or
- if permitted by resource consent, discharged directly into the marine environment.

All data should be recorded (Section 3.7.7).

3.7.7 Reporting

Using the recommended template in Appendix 8.3.5, an independent supervising scientist should report each of the following:

- *general:*
  - a description and specification of the system (Section 3.5);
  - a description of the standard operating procedure (SOP) for the system (Section 3.5);
  - evaluation of system use on vessel materials, including (as appropriate) a list of materials the system is incompatible with (Section 2.4);
  - a description of how the test was undertaken, including:
    - the location, type of vessel used, internal niche area (e.g. sea chest, internal pipework), internal niche area material, dimensions of internal niche area (e.g. length, width, breadth, volume), and environmental conditions during the test (Section 3.5.4);
    - a description of the procedures followed during set-up, testing of the system and demobilisation;
  - the total time, including set-up and demobilisation, it takes to treat each internal niche area;
- *before treatment:*
  - type, viability, level (FR), cover (%) and area of organisms present in each internal niche area (Section 3.5.2);
  - for settlement plates and relevant individual organisms:
    - species, size and viability of individual organisms being tested;
    - type, level (FR) and cover (%) of biofouling present on each settlement plate;
    - details of collection (e.g. source, season, location, habitat - intertidal, subtidal), acclimation conditions (e.g. duration, water quality parameters);
  - type and condition of internal niche area to be treated:

        - the video or still image(s) on which these assessments were made should be provided with the report;
- *during treatment:*
  - the results of samples taken to monitor the temperature achieved, including where the samples were taken (Section 3.5.9);
- *after treatment:*
  - the amount and type of viable organisms observed in video recordings of the treated internal niche area including:
    - a description of the general condition of the organisms present, including signs of physical damage, change in pigmentation and morbidity (Appendix 8.2);
    - a description of the number, size and type of organisms (Section 3.5.11.1) that exhibited indications of potential viability (Appendix 8.2) and their location within the treated internal niche area;
    - relevant video time-stamp and video identifier (file name);
  - the amount and type of viable biofouling recorded in each replicate sample of biofouling removed from the accessible treated internal niche area, including:
    - a description of the number, size and type of biofouling organisms (Section 3.5.11) that exhibited indications of potential viability (Appendix 8.2);
    - relevant sample identifier (i.e. test replicate identifier);
    - location of the test replicate on the vessel;
  - physical and structural condition of the treated internal niche area:
    - the video or still images on which these assessments were made should be provided with the report (Section 3.5.11.1);
  - qualitative assessment of:
    - loss of material by dislodgement from the vessel and escape of mobile organisms during set-up, operation and demobilisation;
    - leakage from the system:
      - the video images from which this assessment of loss of material during the test was made should be provided with the report;
  - a description of any variations or deviations in application of the test relative to the SOP, test methods and quality assurance and quality control procedures;
  - a discussion of the system efficacy, including whether the performance criteria were met;
  - recommendations for system or SOP improvement.

The results of all test runs – failing and passing – should be reported. Example templates for reporting are provided in Appendix 8.3.

3.8 VESSEL TESTING FOR PHYSICAL REMOVAL SYSTEMS

Vessel testing is necessary to best assess the performance of the full proposed system in a realistic simulation of its intended use. This methodology allows assessment of the effects of scale and environmental conditions on system efficacy, waste containment, escape of mobile species and of dislodgement of biofouling by divers and equipment while in operation.

3.8.1 Vessel selection

A physical removal system should be tested on at least one vessel (Section 4.3.1). *If the vessel does not contain the appropriate number of internal niche areas* (Section 3.5.6.1; Table 3-1) *then testing on more than one vessel should be undertaken.*

At least *one* of the replicate internal niche areas should be at the upper size limit the system is intended to be used on.

3.8.2 Testing method

During the test, the in-water system should be used in the manner in which it is intended under normal operation. The in-water system should be tested on *six* replicate internal niche areas (Section 3.5.6.1).

Before testing proceeds, an independent supervising scientist should determine the biofouling viability, rating (FR), percentage cover and area fouled in each internal niche area (Section 3.5.2). The physical and structural conditions of the entire area should be assessed by a suitably qualified independent professional (Section 3.5.5).

Each internal niche area should be recorded by video or digital still imaging to allow auditing of biofouling viability, rating and cover (Section 3.5.11.1; 4.3.4).

The performance criteria for physical removal systems is that all macroscopic biofouling be removed or rendered non-viable (Section 2.1). The equipment for capture and treatment of biofouling removed during cleaning should be operated and tested during the cleaning trials (Section 3.10) to establish if the performance criteria for effluent filtration and biofouling containment are met (Section 2.3). The developer may choose to perform preliminary tests of cleaning ability without capture *in addition* to full testing.

3.8.3 Assessing physical removal system efficacy

After the system has undergone testing, images of the entire cleaned internal niche area (Section 3.5.6.3) should be obtained by video or still photography (Section 3.5.11).

Any detected biofouling in the video should be recorded against the image identifier (file name) and a description of the location within the cleaned internal niche area (e.g. by reference to the timestamp in the video at which the biofouling is detected). An independent supervising scientist should also describe the general condition of the biofouling present, including signs of physical damage, morbidity and whether there are any indicators of viability (Appendix 8.2).

After video photography occurs, representative samples (Section 3.5.11) of biofouling, if present, from the cleaned internal niche area should be collected to determine viability (Appendix 8.2).

Information should be recorded directly to an electronic spreadsheet version of the data sheet template (Appendix 8.3.7.5), or to a paper version and later transferred to an electronic version.

The cleaned internal niche area should also be assessed by a qualified professional to identify any physical or structural damage caused by system deployment (Section 3.5.5).

3.8.4 Assessing waste capture efficacy and dislodgement of biofouling

A diving observer should record on video the test process, including set-up and demobilisation, to assess the amount of material dislodged from the internal niche area and the amount of material removed but not captured (Section 4.3.5). The video may be recorded by an independent supervising scientist (using Underwater Breathing Apparatus) or by a diver under the direction of an independent supervising scientist using surface-to-diver communications.

For systems that use suction to capture waste, the area of effective capture around the system should be estimated by video recording the use of a visible, non-toxic tracer dye, such as fluorescein sodium salt, Basic Blue 3 or Rhodamine WT Red. During each replicate test, 50 mL aliquots of the dye (at a minimum concentration of 4 g L^{-1}) should be released slowly from a syringe at 10, 25 and 50 cm from system operation (where possible). Effective capture would be indicated by strong directional movement of the dye toward the point of suction. The area of effective capture should be tested at least six times for each prescribed distance starting from the greatest distance (n = 6 per distance for total n = 18). An independent supervising scientist should make visual observations of the dye movement from each position and should ensure that each dye capture experiment is recorded on video.

After completion of the trial, the video should be assessed for evidence of material being dislodged from the vessel over the entire test, subsequent capture of this material, and leakage from the system itself. This assessment should be included in the reporting template (Appendix 8.3.5).

The beginning of each video recording should include a label indicating:

- the date of the test;
- name of the system being tested;
- name of the vessel;
- organism type (e.g. bivalve, bryozoan, crustacean), internal niche area type, position on vessel and replicate number.

Dislodgement of any macroscopic particles > 0.5 cm diameter during system set-up or demobilisation represents a failure to meet the performance criteria (Section 2.3).

3.8.5 Reporting

Using the recommended template in Appendix 8.3.5, an independent supervising scientist should report each of the following:

- *general:*
  - a description and specification of the system (Section 3.5);
  - a description of the standard operating procedure (SOP) for the system (Section 3.5);
  - evaluation of system use on vessel materials, including (if appropriate) a list of materials the system is incompatible with (Section 2.4);

- a description of how the test was undertaken, including:
    - the location, type of vessel used, internal niche area (e.g. sea chest, internal pipework), internal niche area material, dimensions of internal niche area (e.g. length, width, breadth, volume), and environmental conditions during the test (Section 3.5.4);
    - a description of the procedures followed during set-up, testing of the system and demobilisation;
- the total time, including set-up and demobilisation, it takes to clean each internal niche area;

- *before cleaning:*
- type, viability, level (FR), cover (%) and area of organisms present in each internal niche area (Section 3.5.2);
- type and condition of area to be treated:
    - the video or still image(s) on which these assessments were made should be provided with the report;

- *after cleaning:*
- the amount and type of viable organisms observed in video recordings of the cleaned internal niche area including:
    - a description of the general condition of the organisms present, including signs of physical damage, change in pigmentation and morbidity (Appendix 8.2);
    - a description of the number, size and type of organisms (Section 3.5.11.1) that exhibited indications of potential viability (Appendix 8.2) and their location within the cleaned internal niche area;
    - relevant video time-stamp and video identifier (file name);
- the amount and type of viable biofouling recorded in each replicate sample of biofouling removed from the accessible cleaned internal niche areas (if present), including:
    - a description of the number, size and type of biofouling organisms (Section 3.5.11) that exhibited indications of potential viability (Appendix 8.2);
    - relevant sample identifier (i.e. test replicate identifier);
    - location of the internal niche area on the vessel;
- physical and structural condition of the cleaned area:
    - the video or still images on which these assessments were made should be provided with the report (Section 3.5.11.1);
- qualitative assessment of:
    - loss of material by dislodgement from the vessel during set-up, operation and demobilisation;
    - leakage from the cleaning system during operation:
        - the video images from which this assessment of loss of material during the test was made should be provided with the report (Section 3.8.4);
- a description of any variations or deviations in application of the test relative to the SOP, test methods and quality assurance and quality control procedures;
- a discussion of the system efficacy, including whether the performance criteria were met;
- recommendations for system or SOP improvement.

The results of all test runs – failing and passing – should be reported. Example templates for reporting are provided in Appendix 8.3.

3.9 VESSEL TESTING FOR CO-TREATMENT SYSTEMS

Vessel testing of chemical(s) and thermal co-treatments should be assessed according to the testing methodology outlined below.

For co-treatments involving physical removal systems, the type of assessment undertaken is dependent on the order of the co-treatment procedure. The first co-treatment procedure should meet the performance criteria for effluent and biofouling containment (Section 2.2, 2.3) and should not have to render all macroscopic biofouling non-viable (Section 2.1). It is only after the application of the second co-treatment that assessment of macroscopic biofouling viability should be undertaken. For example:

- where a treatment system (e.g. chemical or thermal) is applied prior to the physical removal system, the containment and effluent treatment abilities of the treatment system should be assessed according to Sections 2.2, 2.3, 3.6 and 3.7, as applicable. The physical removal system would then be tested according to the physical removal methodology and criteria (Section 2.1, 2.2, 2.3, 3.8 and 3.10).
- where the physical removal system is applied prior to the treatment system, the containment and effluent treatment ability of the physical removal system should be assessed according to Sections 2.2, 2.3, 3.8 and 3.10. The application of the co-treatment would then be tested according to the relevant methodology and treatment criteria (Section 2.1, 2.2, 2.3, 3.6 or 3.7).

The results of all test runs – failing and passing – should be reported. Example templates for reporting are provided in Appendix 8.3.

3.10 VESSEL TESTING FOR WASTE TREATMENT SYSTEMS

Waste treatment is the available option for systems that do not render fouling non-viable (e.g. physical removal systems or treatment systems that drain effluent from the area prior to, or during exposure to the lethal agent) or discharge waste into a sewer system that does not contain a secondary treatment system[8]. Before in-water cleaning waste is discharged back into the environment, any propagules should be removed or rendered non-viable. The performance criteria for effluent filtration from physical removal systems is a maximum particle size in the filtered effluent of 12.5 µm (Section 2.2; 4.1.2). Alternative or additional treatments to filtration, such as irradiation with ultra-violet (UV) light, heat or addition of biocides, should render all biological material of macroscopic biofouling non-viable (Section 2.1; 4.1.3).

The volume of waste material removed from a hull during cleaning, together with water entrained with it by the waste capture process, is likely to be very large. Lewis (2013) estimated that a volume of 350 000 L of effluent was generated from cleaning a 45 m vessel using a brush cart fitted with a suction system for waste capture. While the volumes of entrained water are likely to be less for internal niche area cleaning, the volume of waste would still place great demands on effluent treatment systems, creating the risk of failure in terms of discharge of untreated or partially treated waste during the treatment process, or of inadequately treated final effluent. Contingency plans for the aforementioned risks should be included within the waste treatment system SOPs.

Removal of biological material of macroscopic biofouling from the effluent may involve some form of filtration. The most likely cause of failure of filtration systems is overloading due to the volume of effluent and the concentration of particles larger than the filter pore size. Overloading may cause effluent to bypass the filter and overflow back into the environment, or cause the filter to rupture. It is therefore important that the capacity of the filtration system is matched to the effluent delivery rate.

As a further safeguard, the final effluent may be treated by a secondary process (e.g. UV light, heat or a biocide) before discharge into the environment. Discharge of biocides may need a resource consent, and obtaining this or any other necessary approval should be the responsibility of the developer (Section 3.5.13).

An alternative option for waste treatment is to discharge cleaning waste to a sewer in which effluent is secondarily treated. No treatment criteria should be needed to be met in this case. Discharge of liquid trade wastewater to sewer systems is likely to need a registration or resource consent, and obtaining this or any other necessary approval is the responsibility of the developer.

Any system intended to be used for capture and treatment of biofouling and effluent should be operated and tested during vessel testing as part of the full cleaning or treatment system. The developer may, however, choose to perform preliminary testing without capture and treatment *in addition to* full system testing.

[8] These systems involve biological processes to biodegrade the organic contaminants in the wastewater. Secondary treatment processes can include wastewater aeration, treatment and filtering media, disinfection, and other technologies (https://www.mfe.govt.nz/publications/rma/proposed-national-environmental-standard-site-wastewater-discussion-document-6).

3.10.1 Test conditions and vessel selection

The equipment for capture and treatment of biofouling and effluent removed during cleaning or treatment should be operated and tested during the vessel trials (Section 3.6; 3.7; 3.8; 3.9). The developer may, however, choose to perform preliminary tests of waste treatment systems *in addition* to full system testing.

3.10.2 General conditions for test implementation

The developer should provide a standard operating procedure (SOP) for the waste capture and treatment systems, including the frequency of changing or cleaning filters and filter cartridges to prevent system overload, and other contingency plans. An independent supervising scientist should audit the use of the waste treatment system during the test against this SOP. The parts of the waste treatment system above the water surface should be monitored for leaks or overflows. A log of system performance should be kept, noting any problems, including blocked or ruptured filters and leaks. A videographic recording of each test replicate should be provided.

The efficacy of waste filtration should be assessed by separately re-filtering samples of the final effluent obtained from each of the test area replicates through filters with a pore size less than 12.5 µm (e.g. Whatman No. 1 (11 µm) or No. 40 (8 µm)). Triplicate 200 mL samples should be taken from the first effluent produced at an appropriate time after the start of cleaning and at two subsequent times during the cleaning process, one approximately halfway through and one at the end. The sampling times during the cleaning process should be recorded. Each sample should be filtered and the filter discs microscopically examined (Section 4.4.1) to determine the presence of objects larger than the pore size of the allowed criteria (Section 2.2; 4.4.2). Evidence should be provided to enable verification of each determination of viability, for example, a minimum of three photographic images and one videographic recording.

In systems where the effluent is treated to kill propagules, rather than filtered to remove them, the viability of organisms or propagules in the effluent should be assessed. Triplicate 200 mL samples should be taken from the first effluent produced after the start of cleaning and at two subsequent times during the cleaning process. The sampling times during the cleaning process should be recorded. Each sample should be filtered and the filter discs microscopically examined to determine the presence and structural integrity of objects larger than the pore size of the allowed criteria (Section 2.2; 4.4.2). Evidence should be provided to enable verification of each viability determination, for example, a minimum of three photographic images and one videographic recording.

For all systems, it is necessary to allow sufficient time between the start of cleaning of each test area and the collection of effluent samples to allow residual effluent in the system to be flushed through. This can be determined by running aliquots of dye through the system at appropriate times (Section 4.4.1).

3.10.3 Reporting

An independent supervising scientist should provide an assessment of how the waste treatment system was operated against the SOP, identifying any deviations from the prescribed method and their consequences for meeting the performance criteria.

The assessment report should list any problems recorded in the performance log and provide recommendations on any improvements that could be made in the SOP based on the outcomes of the trial. The performance log should be included in the report as an appendix.

The report should also state how many particles larger than the performance criteria were present in the samples of the final effluent. The results of the viability assessment (structural integrity) of biological material of macroscopic biofouling present in the effluent should also be presented with appropriate images for verification.

Using the templates in Appendix 8.3.5, an independent supervising scientist is to report each of the following:

- *general:*
  - a description and specification of the system tested (Section 3.5);
  - a description of the SOP for the system (Section 3.5);
  - a description of how the test was undertaken, including:
    - the procedures followed during set-up, system operation, monitoring and demobilisation;
    - the performance of the system, including any deviations from the prescribed SOP and quality control and quality assurance procedures;
  - the results of monitoring the effluent stream, including:
    - the number, size and (where possible) identity of particles > 12.5 µm in dimension; or
    - the viability of biological material of macroscopic biofouling in the effluent;
  - a discussion of the system efficacy, including whether the performance criteria were met;
  - recommendations for system or SOP improvement.

The results of all test runs – failing and passing – should be reported. Example templates for reporting are provided in Appendix 8.3.

4 Rationale for the development of the technical advice

4.1 PERFORMANCE CRITERIA AND TESTING METHODS

Much of the following information has been adapted from Morrisey *et al*. (2015).

4.1.1 Minimum practicable detectable size of biofouling (0.5 cm)

The potential size of biofouling fragments occupies a continuum from microscopic to macroscopic, and includes fragments of colonial organisms and microscopic life stages of larger, solitary adults, such as the gametophyte of *Undaria pinnatifida*. Consequently, a minimum, practical detectable size should be specified for dislodged biofouling. A minimum dimension (diameter or length) of 0.5 cm has been chosen because this is representative of the size of individuals of common calcareous biofouling organisms, such as barnacles and tubeworms, that are able to be readily identified following image capture using available videographic technology (Morrisey *et al.* 2015).

4.1.2 Maximum particle size in treatment system filtered effluent (12.5 µm)

This maximum particle size is a compromise between minimising the biosecurity risk from effluent discharged to the environment and what is practically achievable. A previous review of the pore size of filters needed to remove propagules from effluent from land-based vessel-cleaning facilities recommended a pore size of 60 µm because smaller propagules were unlikely to survive after discharge (McClary and Nelligan 2001). Morrisey *et al.* (2013), however, suggested that survival of smaller propagules was more likely in the case of in-water cleaning because the receiving environment was likely to be more benign. The IMO Ballast Water Convention Regulation D2 has performance criteria of 50 µm and 10 µm[9], and the Australia and New Zealand *Anti-fouling and In-water Cleaning Guidelines* (Department of Environment and the Ministry for Primary Industries 2015) state that "in-water cleaning technologies should aim to, at least, capture debris greater than 50 µm in diameter". Although Morrisey *et al.* (2013) suggested a pore size of 2 µm to eliminate biosecurity risk, 12.5 µm is more realistic with current systems, as indicated by recent testing in Western Australia (Lewis 2013). Systems capable of filtering to 10 µm or smaller are technically possible, but their effectiveness has yet to be demonstrated in this context (Morrisey *et al.* 2015).

4.1.3 Viability of treated biofouling organisms

A viable biofouling organism (adult or propagule) is defined as one that is potentially capable of living and developing normally in the marine environment. This simply means that the organism has survived the treatment process and is in a condition that could *potentially* allow it to grow or produce offspring. The likelihood of successful establishment of populations in New Zealand waters from surviving biofouling is uncertain as it is influenced not only by the physiological condition of the organism, but also by the suitability of the local environment and interactions with resident biota (competitors, predators and parasites) (Morrisey *et al.* 2015).

It may be difficult to determine if a biofouling organism is alive, moribund or non-viable following treatment (e.g. physically intact, non-motile organisms such as macroalgae or sponges). Further, many marine species (particularly macroalgae and clonal invertebrates) are able to regenerate from very small fragments. A precautionary approach is therefore needed

[9] IMO Resolution MEPC.173(58) Guidelines for Ballast Water Sampling (G2), available at www.imo.org/blast/blastDataHelper.asp?data_id=23757&filename=173(58).pdf

to assess viability. Unless an organism can be confidently determined to be non-viable, it should be classified as being 'viable'. Organisms that are moribund (i.e. dying or near death, but which still show signs of mobility or fecundity) should still be regarded as potentially viable (Morrisey *et al.* 2015).

In situ photography or videography of treated macroscopic biofouling provides a guide to the viability of biofouling organisms. Loss of colour (i.e. pigmentation) or mobility can indicate death, but neither is definitive. Also, in structurally complex biofouling assemblages, cryptic organisms may survive in the interstices formed by other biofouling organisms. For this reason, video assessment *in situ* and removal of residual biofouling samples are needed to determine the efficacy of the in-water system (Morrisey *et al.* 2015).

Susceptibility of different organism types to in-water systems (e.g. thermal or chemical) may vary considerably. For example, calcareous organisms such as bivalves and barnacles may have considerably greater tolerance to prolonged treatment at greater intensity than soft-bodied organisms (Forrest *et al.* 2007; Brook 2015). Moreover, some organisms that appear to be non-viable, at least superficially, may still be viable (e.g. Morris and Carman 2012). For these reasons, microscopic examination of representative samples of biofouling is necessary immediately following completion of the surface-treatment and shrouding systems to assess the viability of biofouling organisms reliably.

Despite the difficulties in determining viability, a pragmatic approach is provided that uses *in situ* and laboratory assessments of physiological condition as surrogates for more complex tests of viability. The appended guide for assessing the viability of macroscopic biofouling organisms (Appendix 8.2) is modified from Woods *et al.* (2007), and draws upon other studies that have used pragmatic assessments of biofouling organism viability (i.e. Coutts and Forrest 2005; Forrest and Blakemore 2006; Blakemore and Forrest 2007; Locke *et al.* 2009; Dunmore *et al.* 2011; McCann *et al.* 2013).

Where uncertainty exists, a two-day observation period was introduced to assess the viability of organisms to provide a balance between conservatism, scientific robustness, practicality and costs.

Although propagules (eggs, larvae or spores) released from biofouling organisms during the treatment may be viable, they should be contained by water-blocking equipment (e.g. sea chest blanks), shroud or surface-treatment systems and be treated during operation. It is not technically feasible to distinguish them in the water column from the propagules released by organisms in the surrounding environment. For this reason, attempting to assess their viability in the water column is not recommended.

4.1.4 Vessel testing

Systems designed for cleaning or treating internal niche areas of a vessel may vary in scale and complexity with the size and design of internal niche areas they are intended to be used on. For example, containment of internal sea water within a sea chest needs equipment that is capable of covering sea chest grates which vary in size and shape. The configuration of internal niche areas is also irregular and may necessitate the construction of customised equipment (e.g. to deliver chemicals or heat). Special considerations may apply where systems are only intended for use on particular types of materials. Due to problems likely to be encountered in scale-up, such as maintaining uniformity of temperature or chemical concentration and removing large areas of biofouling, the full system, including waste

treatment systems (if appropriate), should be tested on internal niche areas of the minimum and maximum size and type that the system is intended for use on, and on high levels of fouling biomass.

4.1.5 Land-based testing

For treatment systems using lethal agents for which there is a paucity of data about relevant organism mortality, land-based testing can be used to establish the dose, pH, estimated volumes or temperature (LD_{100}, $LpHV_{100,}$ $LTemp_{100}$) and exposure time (LT_{100}) necessary to meet the performance criteria (Section 2.1). Once these mortality parameters have been established, vessel testing can occur.

The selection of test organisms (i.e. relevant organisms, settlement plates or a combination of both) is governed by the availability and quality of information present in the scientific literature. An independent supervising scientist should consider the 12 broad taxonomic groups identified in the Risk Analysis: Vessel Biofouling (Bell *et al.* 2011) as a starting point.

4.1.5.1 Settlement plates

The use of settlement plates provides an extra level of replication and certainty concerning efficacy for treatment systems (e.g. chemical or thermal).

Advantages of using settlement plates include:

- panels can be deployed within either land-based or field test systems to determine the efficacy of chemical or thermal treatments on a variety of biofouling organisms;
- ability to test treatments on biofouling with three-dimensional structures;
- potential to place settlement plates with internal niche areas where hull fouling is not present;
- ability to identify resilient organism types and species not covered by the literature.

4.1.5.2 Testing of relevant organisms

The most successful types of sessile biofouling organisms that have spread to new geographical locations include bivalves (mussels, oysters and clams) and barnacles (Rajagopal and Van der Velde 2012). This is because they can withstand a wide range of environmental conditions due to their ability to close their shell for an extended period of time and resume feeding once conditions are favourable (Neil and Stafford 2005; Rajagopal 2012). Mobile biofouling organisms (e.g. gastropods, amphipods, crabs, sea stars) which are associated with sea chests may detect the treatment (e.g. chemicals or heat) and actively evade it. To date, studies on biofouling control or elimination have not tended to include mobile organisms (Piola and Hopkins 2012).

Organism size can influence treatment efficacy. For example, Piola and Grandison (2013) found that smaller-sized Australian blue mussels (*Mytilus galloprovincialis planulatus*) were more resilient and survived in higher numbers when exposed to quaternary ammonium compounds compared to larger-sized individuals.

Different populations of the same species may display varying tolerances to abiotic factors (e.g. temperature or chemicals), especially those which occur over a wide latitudinal range or inhabit different habitat types (e.g. intertidal vs. subtidal; urbanised vs. natural environments). When conducting testing, it would be prudent to select representative taxa from intertidal

environments, as these organisms are likely to be, for example, the most thermally tolerant (Piola and Hopkins 2012).

4.1.5.3 Attachment of mussels

If using mussels as test organisms, they should be attached to a surface via byssal threads. Unattached mussels actively attempt to attach to a surface by growing byssal threads. During this process they open their shell to extend their foot thus exposing their soft tissue to the chemical treatment. Conversely, attached individuals appear more resistant to chemical treatments as they can close their shell and recommence aerobic activity once conditions are favourable (e.g. after chlorine treatment has ceased) (Rajagopal *et al.* 2005).

4.1.6 Sea water chemistry

The efficacy of some chemicals is affected by abiotic factors such as temperature, pH and concentration of suspended solids, for example:

- it is known that that non-oxidising chemicals and chlorine are more effective at higher water temperatures (Rajagopal *et al.* 1995; Neil and Stafford 2005);
- the biocidal effect of chlorine is decreased at pH > 8 (Rajagopal 2012);
- it is likely that a higher dose of chlorine is necessary when operating in near-shore environments compared to the open ocean due to the higher concentration of suspended organic and inorganic substances, which reduce the amount of chlorine residual that is available to act as a biocide (Chou *et al.* 1999).

4.1.7 Modified Klimisch score

A systematic approach to evaluating the quality of toxicological and ecotoxicological data was proposed by Klimisch *et al.* (1997). This approach has been modified for evaluating treatment data (Section 2.1).

The ability to systematically evaluate data allows an independent supervising scientist to judge the reliability of results described in the literature, which guides decision-making as to whether land-based testing is necessary prior to vessel testing.

4.2 GENERAL CONSIDERATIONS

4.2.1 Safe diving and codes of practice

Health and safety considerations for the system operators and assessors are of primary importance, but are beyond the scope of this document. Information pertaining to occupational diving in New Zealand is available from WorkSafe New Zealand: https://worksafe.govt.nz/topic-and-industry/occupational-diving/.

When considering diver safety, the following factors related to internal niche areas of a vessel need to be considered, especially if divers are to physically enter a sea chest:

- restricted space for entry and exit;
- difficulties removing or opening and securing sea chest grates for diver access;
- exhaled air can collect at the top of an internal space and restrict diver access during prolonged cleaning unless ‘breather holes’ exist in the shell plate;
- some baffles and internal structures can make areas within sea chests physically inaccessible;
- unless vessel systems can be isolated, sea chests are unsafe for diver cleaning;

- mud and sediment can collect in the base of a sea chest, depending on its shape and configuration. If stirred up, this can restrict visibility impairing cleaning activities.

4.2.2 Level of biofouling on the test surface

The US Navy fouling rating (FR) is more widely used (for example, in testing antifouling coatings) than the biofouling levels of Floerl *et al.* (2005) for defining level of biofouling. However, the FR system does not incorporate percentage cover, as this is assessed separately as a continuous variable for characterising biofouling (Naval Ships' Technical Manual 2006). Therefore the FR system has been integrated with the percentage cover categories of Floerl *et al.* (2005).

4.2.3 Type of biofouling

Assessing the type of biofouling provides a context for the test and the performance of the system under test, and for use in characterising the conditions under which a system may be approved. A high level of taxonomic resolution is unnecessary for these purposes, as this is often a specialised task that would cause unwarranted expense and delay in reporting. However, all viable biofouling following land-based or vessel testing should be identified to *species* level by a qualified independent scientist.

4.2.4 Sample sizes for efficacy testing (physical removal)

A pragmatic approach was taken in specifying the minimum number of internal areas necessary to assess cleaning efficacy and the number of samples of fouling taken to assess viability. The number of internal areas for testing in Section 3.5.6.1 is a compromise between the necessity to scale the assessment for vessels of different sizes and the practicality of undertaking the assessment at reasonable cost (Section 5.2; Morrisey *et al.* 2015).

As it is unlikely that the entire internal area of a vessel would be cleaned due to inaccessibility to all surfaces, the increased replication is justified to offset partial cleaning compared to treatment systems which treat biofouling throughout the entire internal area.

For these reasons, an approach is advised that includes:

- a minimum number ($n = 6$) of internal areas to be cleaned on a vessel;
- the use of an endoscope camera to census the entire cleaned internal area for viable biofouling.

4.2.5 Sample sizes for efficacy testing (treatment systems)

Treatment systems should to be tested on *six* internal areas (Section 3.5.6.2). To ensure efficacy of treatment, systems should be tested on internal niche areas spanning both the upper ($n = 3$) and lower size limits ($n = 3$) (Cahill *et al.* 2019). Further, the internal niche area specified for treatment should be treated in its entirety.

4.2.6 Sources of vessels for testing

Sources of vessels have not been specified because the willingness of the potential providers of test vessels may vary over time or between representatives of each category (for example, different Navy or merchant vessels). Furthermore, developers are likely to have a network of contacts and sources of their own.

4.2.7 Photographic and videographic recording of tests

The maximum swimming speed is based on recently published values for diver swimming speeds during video transect studies (e.g. Holmes *et al.* 2013; Mallet and Pelletier 2014), and the experience of the authors of Morrisey *et al.* (2015).

4.3 TESTING OF SYSTEMS

4.3.1 Vessel selection/number of vessels tested

Different types and sizes of internal niche areas necessitate different system types, or may influence the efficacy of a single system type. Because of this, it is necessary to test a system on multiple examples of each internal niche area. To obtain a sufficient number of replicates, it may be necessary to test the system on more than one vessel or to select a vessel that is likely to have multiple examples of each internal niche area.

To assess the effects of scale on system efficacy, testing should be conducted on vessels which meet the following:

- Removal systems:
    - at least *one* replicate internal niche area should be at the upper size limit the system is intended to be used on;
- Treatment systems:
    - *three* internal niche areas should be at the upper size limit the system is intended to be used on;
    - *three* internal niche areas should be at the lower size limit the system is intended to be used on.

4.3.2 Test conditions – current speed

Faster current speeds make it difficult for an independent supervising scientist to see and record any material knocked off the vessel or not captured, and make it more difficult for the assessor to move around the application area. Strong currents are also likely to make system set-up, deployment and handling, and demobilisation more difficult, increasing the likelihood of material dislodgement. System testing should be conducted during periods of slack water, with current speeds of no more than 1 kn (~ 50 cm s^{-1}), in order to aid an independent supervising scientist(s) in observing system operation. Whether the system can be applied safely in faster current speeds (i.e. without additional hazard to biosecurity or human health) is, to some extent, dependent on the system and the ability of the operator. As guidance, it is suggested that systems may be approved for operation only at current speeds $\leq$ 2 kn (~ 1 m s^{-1}). Current speed may be estimated by releasing a 50 mL aliquot of a non-toxic tracer dye (at a minimum concentration of 4 g L^{-1}) and recording its movement over a fixed distance (e.g. 3 m) or by use of current meters or an acoustic Doppler current profiler.

4.3.3 Test conditions – water clarity

Test conduct, where possible, should occur in water clarity of 2 m or greater Secchi disk reading. Although divers can detect some macro-organisms reliably at visibility < 1 m Secchi disk (Gust *et al.* 2006; Inglis *et al.* 2008), the resolution of the video and still images may be compromised. In poor visibility, it is also more difficult for an independent supervising scientist to see or video material knocked off the vessel during set-up or escaping from the cleaning head/treatment apparatus. Long-term median Secchi depth at 11 ports and marinas around New Zealand (NIWA and MPI unpublished data from Marine High Risk Site Surveillance) ranged from 0.9 – 3.2 m, with all but Lyttelton, Nelson and Opua > 2 m, suggesting that most ports would provide suitable conditions.

4.3.4 Testing method – efficacy testing and conditions to enable assessment for approval

This is to confirm that the FR, percentage cover and area of biofouling comply with the specifications set out in Section 3.1.1 and 3.5.2 for the system under test. This provides

context for system efficacy and the conditions for which the system may be approved. All accessible sections of an internal niche area should be photographed (video or still) prior to system application to allow auditing of FR, percentage allocation and area fouled, and videoed to allow assessment of biofouling viability. This information also provides context for the test (Morrisey *et al.* 2015).

4.3.5 Assessing containment and waste capture efficacy (physical removal systems)

A qualitative method was chosen to assess the waste capture efficacy and accidental dislodgement of biofouling because these aspects of system application are significantly dependent on the skills and motivation of the operator. Operators may, for example, minimise efforts to collect and record material dislodged from the vessel and maximise efforts to recover material escaping from the cleaning head in order to exaggerate the efficacy of the cleaning operation. It is therefore suggested that video recording of the testing process be used to record material dislodged or escaping capture. Large amounts of dislodged or escaped material would be detected by both qualitative and quantitative methods of assessment. Although small amounts of material may not be noticed by the assessor or recorded by the video, the power of quantitative methods to measure them (such as collecting water samples at increasing distances from the test area) is also likely to be small because of background variation in, for example, concentrations of suspended sediments (Morrisey *et al.* 2015).

4.3.6 Assessing containment (treatment systems using a sea chest blank or other water-blocking equipment)

A qualitative method to assess containment was chosen to determine the water isolation capability of a sea chest blank or other water-blocking equipment, and to also determine accidental dislodgement when such equipment is placed on a vessel. Video recording the entire process from mobilisation to demobilisation, in acceptable environmental conditions, provides confidence of detecting material dislodgement. The addition of dye to the internal water body provides assurance that leakage of internal water into the marine environment has not occurred.

4.4 WASTE CAPTURE AND TREATMENT SYSTEMS

4.4.1 Testing method – efficacy of waste filtration

A magnification of up to 400 times is sufficient to see objects of 12.5 µm diameter.

It is necessary to allow sufficient time between the start of cleaning of each internal niche area and the collection of effluent samples to allow residual effluent in the system to be flushed through. This can be determined by running aliquots of dye through the system at appropriate times.

4.4.2 Testing method – viability of organisms or propagules in waste effluent

First and Drake (2013) noted that "with the suite of approaches currently available, it is not possible to determine the viability of organisms rapidly, that is, within minutes of collecting a ballast water sample. Measurements of the photosystem integrity via variable fluorescence and the presence of adenosine triphosphate (ATP) are currently the most promising for rapidly estimating concentrations of living cells in compliance testing of ballast water discharges; however, extensive validation is required to verify the applicability of these approaches for the complexity of real world samples". Given the lack of appropriate methods, it is proposed that structural integrity of organisms and propagules be used as an indicator of viability as per First and Drake (2013) (Appendix 8.2).

5 Feasibility and cost of testing

5.1 GENERAL FEASIBILITY CONSIDERATIONS

5.1.1 Land-based testing

Land-based testing of chemical and thermal treatments should be straightforward to conduct, with the only potentially limiting step being the number of settlement plates and time needed to grow adequate biofouling that meets the specifications in Section 3.1.1.

5.1.2 Vessel testing

The main limitation for testing systems is likely to be the availability of a vessel with adequate biofouling, particularly for assessing efficacy on the largest internal niche area the system is intended to treat or clean. Also, it may be necessary to use more than one vessel to test the system to achieve the necessary level of replication.

A resource consent may be needed if testing involves cleaning areas coated with antifouling paint, or when using a biocide to treat biofouling. There may also be concerns over the potential release of biosecurity contaminants if the vessel has spent time outside the testing location since any previous vessel biofouling maintenance.

5.1.3 Waste capture and treatment systems

Collection of samples from the final effluent should be straightforward (e.g. syphoning effluent from system into sterile plastic bottles prior to discharge).

Discharge of liquid trade wastewater to sewer systems is likely to need a registration or consent (Section 3.5.13). Obtaining these is the responsibility of the system developer.

5.2 ESTIMATED COSTS

The costs associated with vessel testing of in-water systems are likely to be highly variable and dependent on system type. Some indicative costings are reproduced from Morrisey *et al.* (2015) (Table 5-1). In producing these costings, a minimum of two separate sources for the cost of each test item has been averaged. The main assumption is that testing would involve one day for assessing a maximum of six internal niche areas *in situ* on a single vessel. However, more than one vessel is likely to be needed.

Table 5-1. Indicative costs associated with testing in-water systems under realistic operating conditions.

Test item	Indicative cost (NZ$)
Vessel berth/wharf face (20–100 m length)	$65–350 per day
Site power/generator	$50/$525 per day[1]
Crane truck/forklift (< 5 tonne)	$1 408/$800 per day[2]
Dive contractor	$1 960 per day[3]
Scientific contractor (field)	$4 800 per day[4]
Scientific contractor (report)	$14 250[5]
Waste disposal	$5 per kg of solids[6]

Notes

1 For example, 32-amp power cables, power transformer, splitter box or 250-KvA generator. Excludes power/fuel costs.

2 Based on hourly rates of $176 (truck)/$100 (forklift) per hour (includes driver) for an 8-hour day.

3 Based on a single three-diver commercial team for an 8-hour day at $245 per hour. Travel time/costs to/from test location additional. Does not include sampling materials/sundries that may need to be purchased (e.g. sampling quadrats, mesh bags, etc.). Assumes one day to conduct *in situ* testing of the system.

4 Based on a four-person science provider team to ensure independence of testing. This team comprises a three-person in-water field team (one diver, one standby diver and one surface support/skipper) to assess *in situ* system efficacy (via videoing of system in operation for waste capture, knocking off of biofouling, efficacy of waste treatment system, etc.), and one person assessing the efficacy of the waste treatment system, for a 7.5-hour day at $160 per hour, averaged across Technician/Scientist classifications. Travel time/costs to/from test location additional. Does not include sampling materials/sundries that may need to be purchased (e.g. sampling quadrats, mesh bags, etc.). Assumes one day to assess *in situ* efficacy.

5 Based on a single science provider for a 7.5-hour day at $190 per hour for a Senior Scientist to spend a total of 10 days on project set-up/management, client liaison, and production of report (including internal peer-review of report) pertinent to the testing methodology.

6 Based on disposal to landfill by approved waste transporter/handler as industrial/contaminated waste. Minimum weight requirements for collection and disposal by commercial waste companies may apply. Conditional upon Resource Management Act 1991 (RMA) and Hazardous Substances and New Organisms Act 1996 (HSNO). Waste could be regarded as industrial/contaminated waste due to possible antifouling coating contamination, and inherent biological matter.

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8 Appendices

8.1 FOULING RATINGS FOR US NAVAL SHIPS

Sourced from *Naval Ships' Technical Manual (2006). Chapter 081, Waterborne underwater cleaning of Navy ships, Revision 5. S9086-CQ-STM-010.*

CHARACTERISTICS OF FOULING PATTERNS AND CATEGORISATION AT ADVANCED STAGES OF SETTLEMENT

081-1.2.2.2 HARD FOULING. The dominant forms of hard biofouling are barnacles (usually acorn) and tubeworms (serpulids).

081-1.2.2.2.1 BARNACLES. Acorn barnacles have conical hard shells with jagged tops.

081-1.2.2.2.2 TUBEWORMS. Tubeworms form intertwined tubes lying along or projecting out from the hull.

081-1.2.2.2.3 CALCAREOUS DEPOSITS. A result of an active cathodic protection system is the deposition of magnesium and calcium carbonate on bare metal surfaces. The bare nickel-aluminium-bronze-surfaces of a propulsor are highly susceptible to a uniform accumulation of calcareous deposit. The thickness will depend upon the time from the last cleaning and the functionality of the cathodic protection system and although usually more fragile than biological hard-fouling, can still be tenacious and difficult to remove.

081-1.2.2.3 COMPOSITE FOULING. In advance stages of biofouling, mature barnacles and tubeworms may be present along with calcareous bivalve organisms such as mussels or oysters, or hydroids with calcareous cellular structure such as coral or anemones. In advanced stages of biofouling, the ship will be affected by slime, grass, barnacles, and tubeworms. In addition, this stage of biofouling will include soft shell-less animal forms, such as hydroids, anemones, and tunicates (sea squirts).

081-1.2.3 FOULING RATING (FR). The fouling rating scale (Table 081-1-1) describes the 10 most frequently encountered biofouling patterns in order of increasing severity. Representative photographs of each biofouling pattern are provided in Figure 081-1-1.

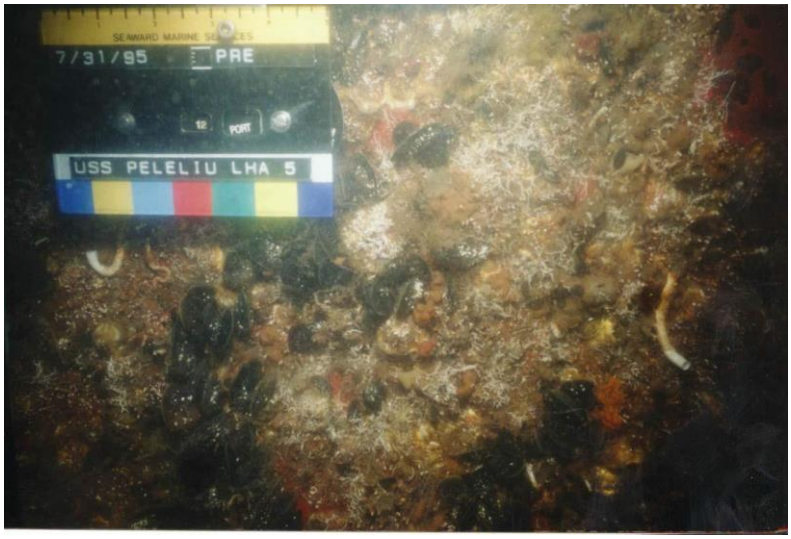
081-1.2.4 FOULING RATING (FR) SCALE. A rating number has been assigned to each of the 10 biofouling patterns on a scale of 0 to 100 in 10-point increments. The lowest number represents a clean hull and the higher numbers represent biofouling organism populations of increasing variety and severity.

081-1.2.5 FOULING PERCENTAGES. The biofouling percentage quantifies the density of biofouling which covers a particular component or area of the hull (i.e. rudder, strut, propeller, stern, port side bow, starboard mid ship, sea chest, etc.).

Table 8-1 Fouling ratings (FR) in order of increasing severity.

Type	Fouling rating (FR)	Description
Soft	0	A clean, foul-free surface; red and/or black antifouling paint or a bare metal surface.
Soft	10	Light shades of red and green (incipient slime). Bare metal and painted surfaces are visible beneath the biofouling.
Soft	20	Slime as dark green patches with yellow or brown coloured areas (advanced slime). Bare metal and painted surfaces may by obscured by the biofouling.
Soft	30	Grass as filaments up to 3 inches (76 mm) in length, projections up to ¼ inch (6.4 mm) in height; or a flat network of filaments, green, yellow, or brown in colour; or soft non calcareous biofouling such as sea cucumbers, sea grapes, or sea squirts projecting up to 1/4 inch (6.4 mm) in height. The biofouling cannot be easily wiped off by hand.
Hard	40	Calcareous biofouling in the form of tubeworms less than ¼ inch in diameter or height.
Hard	50	Calcareous biofouling in the form of barnacles less than ¼ inch in diameter or height.
Hard	60	Combination of tubeworms and barnacles, less than ¼ inch (6.4 mm) in diameter or height.
Hard	70	Combination of tubeworms and barnacles, greater than ¼ inch in diameter or height.
Hard	80	Tubeworms closely packed together and growing upright away from surface. Barnacles growing one on top of another, ¼ inch or less in height. Calcareous shells appear clean or white in colour.
Hard	90	Dense growth of tubeworms with barnacles, ¼ inch or greater in height; Calcareous shells brown in colour (oysters and mussels); or with slime or grass overlay.
Composite	100	All forms of biofouling present, soft and hard, particularly soft sedentary animals without calcareous covering (tunicates) growing over various forms of hard growth.

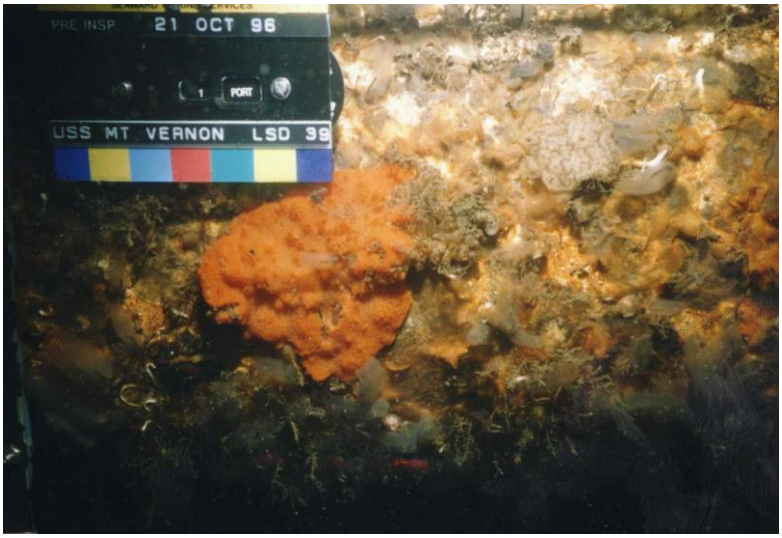
Figure 8-1. Examples of specified fouling rating (FR ≥ 90) for in-water system assessment (4 images).



FR-90, over 90 percent of area.

FR-90, over 90 percent of area.

FR-100, over 50 percent of area.



FR-100, over 100 percent of area.

8.2 GUIDELINES FOR ASSESSING VIABILITY OF MACROSCOPIC BIOFOULING ORGANISMS

These guidelines are modified from Woods *et al.* (2007), and draw upon other studies that have used pragmatic assessments to determine biofouling organism viability (i.e. Coutts and Forrest 2005; Forrest and Blakemore 2006; Blakemore and Forrest 2007; Locke *et al.* 2009; Dunmore *et al.* 2011; McCann *et al.* 2013). Note that indicators related to the desiccation of organisms are not relevant here as biofouling organisms will be wet from in-water sample collection.

Table 8-2: Indicators of the viability of different types of biofouling organisms (Morrisey *et al.* 2015).

Type of biofouling organism	Indicators for potential viability	Indicators for non-viability
Sessile taxa		
Barnacles	• Structure: all shell plates present and intact, opercular plates present. • Feeding/movement: feeding structures (cirri) protrude out of the test and perform sweeping feeding movements. Or: opercular shells closed by muscular action. Feeding or respiration currents visible.	• Structure: shell/opercular plates and/or feeding structures (cirri) broken or missing. • Feeding/movement: feeding structures visible but motionless and slack, and/or no reaction when touched. No feeding or respiration currents visible.
Bivalves	• Structure: both shells present and intact. • Feeding/movement: shells may be locked by muscular action. Shells may also be open (feeding), exposing mantle tissue and siphons (or gaps in mantle), but will close when touched (reaction). Feeding or respiration currents visible.	• Structure: one shell missing or one/both shells significantly cracked or fragmented. • Feeding/movement: shells open but no reaction to touch. No feeding or respiration currents visible.
Encrusting bryozoans	• Structure: colony/fragment contain several intact zooids, and natural colour (pigmentation). • Feeding/movement: filtering apparatus (lophophore) protrude through opening in zooid. Feeding or respiration currents visible.	• Structure: all zooids damaged/smashed, no soft tissues visible or tissues decomposing. Complete loss of pigmentation. • Feeding/movement: zooids' soft tissues and/or feeding structures may be visible but no movement or reaction to touch. No feeding or respiration currents visible.
Erect bryozoans	• Structure: colony/fragment contain several intact zooids, and natural colour (pigment). • Feeding/movement: filtering apparatus (lophophore) protrude through opening in zooid. Feeding or respiration currents visible.	• Structure: all zooids damaged/smashed, no soft tissues visible or tissues decomposing. Complete loss of pigmentation. • Feeding/movement: feeding structures may be visible but no movement or reaction to touch. No feeding or respiration currents visible.
Colonial ascidians	• Structure: colony/fragment in reasonable 'shape', not entirely crushed, and natural colour (pigmentation). Several polyps intact. • Feeding/movement: inhalant and/or exhalant siphons open, but close when touched. Feeding or respiration currents visible.	• Structure: shredded or crushed so that badly damaged, no soft tissues visible or tissues decomposing. No polyps visible (polyps may have 'popped out' from mechanical pressure on colony). Complete loss of pigmentation. • Feeding/movement: siphons open but no reaction to touch. No feeding or respiration currents visible.
Solitary ascidians	• Structure: test (body) intact, no holes or gashes, not crushed flat or severely	• Structure: test badly damaged, crushed or deformed. Branchial basket

Type of biofouling organism	Indicators for potential viability	Indicators for non-viability
	deformed, and natural colour (pigmentation). • Feeding/movement: inhalant and/or exhalant siphons open, but close when touched. Or: siphons closed and resistant to opening. Feeding or respiration currents visible.	exposed and/or damaged, gut system protruding from test, no soft tissues visible or tissues decomposing. Complete loss of pigmentation. • Feeding/movement: siphons open, but no reaction to touch. No feeding or respiration currents visible.
Hydroids	• Structure: body reasonably intact, feeding polyps (often at distal ends of braches) present and natural colour (pigmentation). • Feeding/movement: feeding tentacles exposed. Feeding or respiration currents visible.	• Structure: all polyps damaged/smashed, no soft tissues visible or tissues decomposing. Complete loss of pigmentation. • Feeding/movement: feeding structures may be visible but no movement or reaction to touch. No feeding or respiration currents visible.
Tube-building polychaetes	• Structure: generally intact (body usually within tube), not crushed, no holes or gashes, and natural colour (pigmentation). Care needed, as regeneration from lesser fragmentation is possible with some taxa. • Feeding/movement: worm retracts into tube when touched, and/or feeding structures (tentacular crown) visible and moving. Feeding or respiration currents visible.	• Structure: tube missing, loss of tentacular crown, body badly crushed or lacerated, no soft tissues or tissues decomposing. Complete loss of pigmentation. • Feeding/movement: feeding structures may be visible, but no movement or reaction to touch. No feeding or respiration currents visible.
Sponges	• Structure: fragments retain natural colour, firm texture (don't fall apart). Sponges retain a "fleshy/translucent/shiny" appearance. Look for "translucent" tissue between fibres. • Feeding/movement: extremely difficult to observe. Feeding or respiration currents visible.	• Structure: colony/fragment faded and bleached, falling apart. Complete lack of pigmentation. Sponge a mass of golden fibres/hair-like structures without "translucent fleshy tissue" between the fibres, or decomposing tissues. • Feeding/movement: extremely difficult to observe. No feeding or respiration currents visible.
Macroalgae	• Structure: whole plant or fragments not crushed and natural colour (pigmentation). • Feeding/movement: n/a.	• Structure: badly crushed or fragmented with complete loss of pigmentation. • Feeding/movement: n/a.
Motile taxa		
Crabs	• Structure: several missing limbs no problem unless all are gone. Carapace intact. Natural colour (pigmentation). • Feeding/movement: movement or reaction to touch. Eyes/sensory organs in head region moving. Respiration currents visible.	• Structure: all, or nearly all limbs missing. Carapace significantly damaged (e.g. large holes or parts missing). Complete loss of pigmentation. • Feeding/movement: no movement or reaction to touch. No respiration currents visible.
Molluscs (gastropods, nudibranchs, chitons)	• Structure: body intact (gastropod snails: shell present), and natural colour (pigmentation). • Feeding/movement: movement or reaction to touch.	• Structure: body significantly damaged, crushed or lacerated. Complete loss of pigmentation. • Feeding/movement: no movement or reaction to touch.

Type of biofouling organism	Indicators for potential viability	Indicators for non-viability
Sea stars/brittle stars	• Structure: basal disc or parts of it present (can regenerate). Body (or whatever's present) has natural shape, not crushed, and natural colour (pigmentation). • Feeding/movement: movement or reaction to touch.	• Structure: arm-only without part of basal disc (can't regenerate), body significantly damaged, crushed or lacerated. Complete loss of pigmentation. • Feeding/movement: no movement or reaction to touch.
Amphipods/isopods/tanaids etc.	• Structure: exoskeleton intact. Several missing limbs no problem unless all or nearly all are gone. Natural colour (pigmentation). • Feeding/movement: visible movement/reaction, especially feeding limbs will beat if submerged and alive. Feeding or respiration currents visible.	• Structure: exoskeleton damaged (e.g. large holes or parts missing). All or nearly all limbs or feeding structures missing. Complete loss of pigmentation. • Feeding/movement: no movement or reaction to touch. No feeding or respiration currents visible.
Errant polychaetes	• Structure: generally intact, not crushed, no holes or gashes. Care needed, as regeneration from lesser fragmentation is possible with some taxa. Natural colour (pigmentation). • Feeding/movement: movement or reaction to touch.	• Structure: body badly crushed or lacerated. Complete loss of pigmentation. • Feeding/movement: no movement or reaction to touch.
Fish	• Feeding/movement: movement or reaction to touch.	• Feeding/movement: no movement or reaction to touch. • Complete loss of pigmentation.

8.3 TEMPLATES FOR REPORTING DATA QUALITY AND TEST RESULTS

8.3.1 Example report template for assessment of literature sourced efficacy data (Section 2.5.2)

The following information should be reported for each study used to determine treatment efficacy.

Publication information

- title, author(s), affiliation(s) and year of publication.

Quality of exposure methodology

- method of exposure and system description;
- number of exposure replicates;
- presence of controls;
- modified Klimisch score and rationale.

Treatment efficacy

This section should include information on (as applicable):

- test chemical:
  - purity, speciation, formulation, stability;
- mortality endpoints:
  - lethal dose (LD_{100}) or lethal pH and volume ($LpHV_{100}$) or temperature ($LTemp_{100}$) and duration of treatment (LT_{100}) necessary to achieve 100 % mortality of relevant organisms;
  - method(s) of treatment measurement (including quality assurance and quality control procedures);
- environmental parameters of test:
  - water temperature, salinity, pH, concentration of organic and inorganic matter;
  - behaviour of the treatment agent over duration of the treatment.

Test organism

This section should include information on (as applicable):

- source of test organisms:
  - supplier, wild collection, settlement plates, location collected from, habitat - intertidal, subtidal;
- size ranges of test organisms;
- organism type:
  - identification to relevant taxonomic level (Section 3.1.1);
  - fouling species:
    - attachment (for fouling species) (e.g. byssal threads, fouling of settlement plate);
- length of acclimation period and water quality parameters (e.g. temperature, pH, dissolved oxygen);
- level and cover of biofouling on settlement plates;
- measurement of biomass.

8.3.2 Example of discussion report for assessment of literature sourced efficacy data (Section 2.5.2)

The following discussion points should be addressed when submitting all reviewed data:

- exposure endpoints:
    - LD_{100}, $LpHV_{100,}$ LT_{100} (including exposure temperature); or
    - $LTemp_{100}$, LT_{100} (including rate of temperature increase);
- appropriateness of test data (e.g. methodology, appropriate measurement of lethal agent(s); quality assurance and quality control procedures; Table 8-3);
- range of relevant organisms tested:
    - justification of species tested (e.g. relationship of tested organisms to the most resistant relevant organisms to the treatment agent documented in the literature);
- treatment agent considerations:
    - potential influences on efficacy (e.g. temperature, presence of organic matter, level and type of biofouling);
    - if environmental discharge of treatment effluent is to occur:
        - can the treatment agent be neutralised (e.g. dechlorination);
        - persistence or bioaccumulation potential of agent(s) or metabolites in the environment;
        - are discharge permits needed (e.g. effluent concentration or temperature as allowed under a resource consent);
- recommendations, considerations or identified data gaps regarding the proposed treatment systems and agent.

Appendices

This section should include:

- copy of completed modified Klimisch score spreadsheet for each study assessed;
- PDF copy of each study;
- signed conflict of interest declarations of independent personnel who conducted and supervised the testing.

Table 8-3: Summary of treatment data sourced from the literature

Organism[1]	Dose, volume, Temperature	Exposure time	100 % mortality?	Reference	Klimisch score

[1] All organisms that survive exposure should be identified to *species* level.

8.3.3 Example report template for land-based testing (Section 3.2.4; 3.3.4; 3.4.4)

The results of all test runs – failing and passing – should be reported.

Title

Executive summary

General

This section should include (Section 3.1):

- details and qualification of any independent scientific organisation and personnel performing and supervising the test;
- any potential conflicts of interest;
- a description and specification of the chemical(s) tested (if applicable);
- a description and rationale of the testing methodology used (including treatment measurement) and choice of relevant organisms tested.

The following should be completed for each test replicate

Before treatment

This section should include (Section 3.2.1; 3.3.1; 3.4.1):

- a description of test organisms:
    - species, size and condition of relevant organisms being tested;
    - type, level (FR) and cover (%) of biofouling present on each settlement plate;
    - details of collection (e.g. season, geographic location, habitat - intertidal, subtidal), acclimation conditions (e.g. duration, water quality parameters (Section 3.1.1.1));
    - measurement of biomass (3.1.2.2).

During treatment

This section should include (Section 3.2.2; 3.3.2; 3.4.2):

- the results of chemical analysis (chemical treatments excluding acid descalers) or pH (acid descalers), temperature measurements (for chemical or thermal treatments) and the rate of temperature increase (for thermal treatments);
- method(s) of treatment measurement (including quality assurance and quality control procedures);
- frequency and timing when samples were taken (Section 3.1.1.2);
- water quality parameters (Section 3.1.1.1).

After treatment

This section should include (Section 3.2.3; 3.3.3; 3.4.3):

- the duration of the test;
- water quality parameters (Section 3.1.1.1);
- the amount and type of viable organisms via direct observation:
    - a description of the general condition, number, size and taxonomic identification (to *species level*) of any potentially viable organisms (Appendix 8.2);
- a description of any variations or deviation in application of the test relative to the testing methodology and test reporting;
- Measurement of biomass (acid descalers only; Section 3.1.2.2).

Discussions and conclusion

This section should include:

Conclusion for each test undertaken:

- test chemical efficacy:
  - were the performance criteria met?
  - exposure endpoints:
    - LD_{100}, $LpHV_{100}$, LT_{100} (including exposure temperature); or
    - $LTemp_{100}$, LT_{100} (including rate of temperature increase).

8.3.4 Example discussion report template for land-based testing (Section 3.2.4; 3.3.4; 3.4.4)

The following discussion points should be addressed when submitting all generated test data.

- exposure endpoints:
  - LD_{100} or $LpHV_{100}$ (including exposure temperature), LT_{100}; or
  - $LTemp_{100}$ (including rate of temperature increase), LT_{100};
- test chemical efficacy and potential for vessel application, including:
  - rationale for testing methodology (including treatment measurement and quality assurance and quality control procedures);
  - rationale for relevant organisms tested;
  - potential influences on efficacy (e.g. temperature, presence of organic matter, level and type of biofouling, biomass);
  - environmental concerns (e.g. persistence, toxicity, bioaccumulation):
    - considerations for mitigating environmental concerns;
  - engineering concerns:
    - effect of system use on the vessel's structural integrity;
- discussion of results of failed test runs (if any);
- recommendations, considerations or identified data gaps regarding the proposed treatment system.

Appendix

This section should include:

- copies of each laboratory test report (Appendix 8.3.3) and all associated data (Appendix 8.3.6), including reporting from failed test runs;
- copies of all still images produced during the test and an associated index of the images and sample identifiers;
- signed conflict of interest declarations of any independent personnel who conducted and supervised the testing.

8.3.5 Example report template for vessel testing

The results of all test runs – failing and passing – should be reported.

Title

Executive summary

Introduction

System description and specifications (Section 3.5)
This section should include information on:

- the system(s) and its mechanism of action;
- system design;
- general method of system operation;
- evaluation of system use on vessel materials, including a list of materials the system is incompatible with (as applicable; Section 2.5).

Description of the system application (Section 3.5)
This section should include information on:

- internal niche area(s) that the system may be used on.

Standard operating procedures (SOP) for systems cleaning or treating biofouling (Section 3.5)
This section should include information on:

- how the system is used:
    - for chemical and thermal treatments, this should include describing the lethal dose (LD_{100}), lethal volume ($LpHV_{100}$) or lethal temperature ($LTemp_{100}$) and duration of treatment (LT_{100}) necessary to achieve 100 % mortality of relevant organisms;
    - method(s) of treatment measurement (including quality assurance and quality control procedures);
- steps taken to ensure that viable organisms are not released or dislodged during system set-up, application and demobilisation;
- the physical environment suitable for system application (e.g. internal niche area configuration; proximity of vessel to berth, presence of floating dock, open water, whether the entire fouled area needs to be submerged);
- sea and weather conditions under which the system is intended to be used (e.g. limits on current speed, wave height, water temperature, water clarity to ensure efficacy, biosecure containment and operator safety);
- contingency plans to manage biosecurity risk in the event of system failure.

SOP for waste containment or capture and treatment (Section 3.6.5; 3.7.5; 3.8.4; 3.9 and 3.10). *This section should include detailed information on:*

- the operation of the waste containment system including:
    - the volume of effluent that the containment system is designed to handle;
    - the size of access points (e.g. sea chest opening) the system is designed for;
- effluent treatment system, including (as applicable):
    - the volume of effluent the treatment system can filter:
        - the frequency of changing or cleaning filters and filter cartridges to prevent system overload;

- the volume of effluent the treatment system can treat (e.g. UV, biocide or temperature treatment);
- the fate of the liquid and solid waste streams;

- contingency plans to manage biosecurity risk in the event of system failure.

Methods

Personnel involved in the conduct of the test

- details and qualification of any independent organisations and personnel performing and supervising the test (Section 3.5.3).

Test conditions

This section should include detailed information on:

a) Vessel testing (Sections 3.6.7; 3.7.7; 3.8.5 and 3.9)

- the date and location of the test(s) and the environmental conditions at the time, including:
  - water clarity (Secchi depth);
  - current and tide conditions;
  - wind direction and speed;
  - sea state (Table 8-4);
- the vessel(s) used in the test, including its:
  - size (dimensions and tonnage);
  - design and internal niche area construction materials;
- the internal niche areas that the test was carried out on, including:
  - the type of internal niche area (e.g. horizontal access sea chest, vertical access sea chest, internal pipework);
  - the location of internal niche area(s) on the vessel, including:
    - a schematic of the internal niche area location relative to the whole vessel;
    - a schematic of locations within the internal niche area where assessments of system efficacy (e.g. residual biofouling integrity, viability and measurement(s) of lethal agent(s) or pH) took place;
  - the viability, fouling rating (FR) and percentage cover of biofouling in each accessible area(s);
  - the size, viability and species of additional relevant organisms tested;
  - the viability, fouling rating (FR) and percentage cover of biofouling on each settlement plate (as applicable);
    - details of collection for relevant organisms or settlement plate biofouling (as applicable), including the source, season, location, habitat - intertidal, subtidal and acclimation conditions (e.g. duration, water quality parameters) prior to exposure;
- method of system application to the internal niche area, including:
  - any variations or deviations in application of the test relative to the SOP and test methodology;
  - method(s) of treatment measurement (including quality assurance and quality control procedures);
- methods used by an independent supervising scientist to observe and record the application of the system(s) for containment and waste capture (Section 3.6.5; 3.7.5; 3.8.4; 3.10).

b) Waste containment or capture treatment systems (Section 3.6.5; 3.7.5; 3.8.4; 3.10)

- a description of how the test was undertaken, including:
  - the procedures followed during system set-up, operation, monitoring and demobilisation, including:
    - any variations or deviations in application of the test relative to the SOP and test methodology;
  - the methods used to monitor the effluent stream, including (as applicable):
    - the number, size and identity of particles > 12.5 µm in dimension (as applicable); or
    - the viability of biological material of macroscopic biofouling in the effluent (as applicable)
  - the methods used to monitor containment efficacy.

Results

This section should include detailed information on:

a) All systems

- any loss of material by dislodgement from the vessel during system set-up and demobilisation and from escape during system application, based on examination of video recording (Section 3.6.5; 3.7.5; 3.8.4; 3.10);
- the physical condition of the treated or cleaned internal niche area (Section 3.5.11.1);
- the total time needed for cleaning or treating an internal niche area including set-up, operation and demobilisation.

b) Vessel testing

a. Physical removal

- the amount and type of viable biofouling observed in video recordings of the entire cleaned internal niche area, including:
  - a description of the general condition of the biofouling present, including signs of physical damage and morbidity (Appendix 8.2);
  - a description of the number, size and type of biofouling organisms (Section 3.5.2) that exhibited indications of potential viability (Appendix 8.2) and their location in within the internal niche area;
  - location of the internal niche area on the vessel;
  - relevant image identifier (file name).

b. Chemical, thermal and co-treatments

- the results of samples taken to monitor conditions (e.g. biocide concentration, pH, temperature) achieved during the treatment, including where and when the samples were taken and the total duration of treatment (Section 3.5.9; 3.5.10);
- the amount and type of viable biofouling observed in video recordings within the entire treated internal niche area, including:
  - a description of the general condition of the biofouling present, including signs of physical damage, change in pigmentation and morbidity (Appendix 8.2);
  - a description of the number, size and type of biofouling organisms (Section 3.5.2) that exhibited indications of potential viability (Appendix 8.2) and their location in within the test area;
  - location of the internal niche area on the vessel;
  - relevant image identifier (file name).

c. For all in-water systems
- the amount and type of viable biofouling recorded in each replicate sample of biofouling removed from the selected internal niche areas, including:
  - a description of the number, size and taxonomic identification of biofouling organisms (Section 3.5.2) that exhibited indications of potential viability (Appendix 8.2);
  - location of the test replicate within the internal niche area;
  - relevant sample identifier (i.e. test replicate identifier).

c) Effluent containment systems
- the performance of the equipment, including any deviations from the prescribed SOP;
- the results of monitoring containment, including:
  - identification of any visible leakage (Section 3.5.12).

d) Waste treatment systems
- the performance of the equipment, including any deviations from the prescribed SOP;
- the results of monitoring the effluent stream, including:
  - the number, size and (where possible) identity of particles > 12.5 µm in dimension; or
  - the viability of biological material of macroscopic biofouling in the effluent.

Discussion and conclusions

This section should include detailed discussion of:
- system(s) efficacy, including:
  - whether the system met the performance criteria;
  - exposure endpoints achieved:
    - LD_{100}, $LpHV_{100}$ (including exposure temperature), LT_{100}; or
    - $LTemp_{100}$ (including rate of temperature increase), LT_{100};
- the effects of any variations or deviations in application of the test relative to the SOP(s), test methodology and quality assurance and quality control procedures that may have affected system performance;
- potential influences on system efficacy (e.g. water temperature, pH, salinity, turbidity (i.e. presence of organic matter), level and type of biofouling);
- sea and weather conditions under which the system is intended to be used (e.g. are there limits on factors such as current speed, wave height, turbidity, etc., that may be imposed to ensure system efficacy and biosecure containment?);
- any recommendations for system improvement, its SOP or in the test methodology;
- contingencies in the event of system failure.

Appendices

This section should include:
- copies of consent(s) needed to undertake the test, including:
  - approval by regulatory bodies for in-water testing of the system (Section 3.5.13);
- copies of all records and datasheets (Section 8.3.7), including:
  - measurements of lethal agent(s) or pH;

  - measurements of water quality and environmental parameters;
  - assessment of viability (e.g. copies of video or still images from which assessments were made and the associated index of the images and sample identifiers);
  - the performance log kept during testing of the waste treatment system (Section 3.10.3);
- results of vessel testing runs that failed to meet the performance criteria during independent testing;
- signed conflict of interest declarations of any independent personnel who conducted and supervised the testing;
- a report by an appropriately qualified person confirming that no detrimental effects on safe vessel operation were observed due to system use and the types of materials that the system can be safely used on (Section 3.5.5).

8.3.6 Examples of data reporting templates for land-based testing

8.3.6.1 General.

Test conduct details[1]		Mean water quality parameters[2]	Acclimation period	Start of test	During test	End of test
Date of test		pH				
System developer		Salinity				
Treatment method		Temperature				
Location of test		Dissolved oxygen				
Independent supervising organisation		Dissolved organic carbon				
Independent supervising personnel (including roles and responsibilities)		Level of water filtration				

[1] Section 3.1.3; 3.1.4

[2] Section 3.1.1.1 (include standard deviation, as applicable); for acid descaler treatments water quality parameters can only be measured prior to application

8.3.6.2 Description of test organisms[1] prior to treatment exposure.

Relevant organisms				
Species name	**Replicate identifier(s)**	**Size (mean ± SD)**	**Observations[2]**	**Image identifiers (file name) pre-test**
	e.g. control replicate 1, 2, 3, 4, etc. (include number of organisms per replicate)			
	e.g. exposure replicate 1, 2, 3, 4, etc. (include number of organisms per replicate)			
Settlement plates				
Plate identifier	**Replicate identifier(s)**	**Initial fouling rating, % cover and taxonomic identification**		**Image identifiers (file name) pre-test**
	e.g. control group 1, 2, 3, 4, etc. (include number of replicate plates within group)			
	e.g. exposure group 1, 2, 3, 4, etc. (include number of replicate plates within group)			

[1] Test organisms are separated into "relevant organisms" and those organisms associated with settlement plate testing (Section 3.1.1). Relevant organisms should be identified to *species* level. The type of biofouling on settlement plates can be described in broad taxonomic and morphological categories (such as erect bryozoans, barnacles, and encrusting coralline algae) by a suitably qualified independent scientist. All organisms that survive exposure testing should be identified to *species* level by a suitably qualified independent scientist.

[2] Observations on organism state (e.g. attached, unattached, behaviour, etc.).

8.3.6.3 Measurement of lethal agent(s)[1].

Lethal Agent(s) :_____			
Date:_____ Test replicate	Measurement replicate	Sample identifier	Measurement (e.g. concentration; temperature)
1	1.1.1		
1	1.1.2		
1	1.1.3		
1	1.2.1		
1	1.2.2		
1	1.2.3		
1	1.3.1		
1	1.3.2		
1	1.3.3		
2	2.1.1		
2	2.1.2		
2	2.1.3		
2	2.2.1		
2	2.2.2		
2	2.2.3		
2	2.3.1		
2	2.3.2		
2	2.3.3		
3	3.1.1		
3	3.1.2		
3	3.1.3		
3	3.2.1		
3	3.2.2		
3	3.2.3		
3	3.3.1		
3	3.3.2		
3	3.3.3		

[1] The method used by treatment systems to render relevant organisms non-viable. This could be a biocide, pH, de-oxygenation or a physical treatment such as elevated temperature. Values for the lethal dose (LD_{100}) or lethal pH and volume ($LpHV_{100}$) of the treatment agent or lethal temperature ($LTemp_{100}$) and duration of treatment necessary at that dose, volume or temperature to achieve 100 % mortality (LT_{100}) should be determined by direct measurement (Section 3.1.1.2)

8.3.6.4 Assessment of organism viability.

Test replicate	Sample identifier	Notes on the condition of the test organisms[1]	Number and size of viable organisms[2]
1		Relevant organism testing Species 1 – Species 2 – etc. Settlement plate testing Taxonomic group 1 – Taxonomic group 2 – etc.	Species 1 – Species 2 – etc.
2			
3			
4			
5			
6			

[1] Test organisms are separated into "relevant organisms" and those organisms associated with settlement plate testing. Relevant organisms should be identified to *species* level. The type of biofouling on settlement plates can be described in broad taxonomic and morphological categories (such as erect bryozoans, barnacles, and encrusting coralline algae) by a suitably qualified independent scientist. See Appendix 8.2.for observations on condition of test organisms.

[2] All organisms that survive exposure testing should be identified to *species* level by a suitably qualified independent scientist (Section 3.1.2).

8.3.7 Examples of data reporting template for vessel testing

8.3.7.1 Pre-test summary (Section 3.5).

Pre-test details		**Environmental conditions**		**Vessel characteristics**	
Date of test		Secchi depth[2]		Vessel name	
System developer		Current and tide state[3]		Vessel dimensions (L x W x H) /tonnage	
Type of system		Wind direction/speed		Test areas[5] identity, dimensions (L x W x H), materials, MGPS or paint type	
Removal / treatment method[1]		Sea state[4]			
System operator company/personnel (including roles and responsibilities)		**Water quality parameters**		**Test conditions**	
Location of test		pH		Test area	Fouling rating, type and cover[6]
Independent supervising organisation		Salinity		1	
Independent supervising personnel (including roles and responsibilities)		Temperature		2	
		Dissolved oxygen		3, etc.	
		Dissolved organic carbon/suspended solids			

[1] Specific details regarding the system's mechanism of action for removing or treating biofouling (e.g. brush cart with nylon bristles and filtration unit, steam treatment, etc.).

[2] The 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 colour and turbidity.

[3] Relative assessment of current by divers. Tidal state derived from authoritative source pertinent to location of testing, e.g. New Zealand Nautical Almanac (http://www.linz.govt.nz/sea/nautical-information/new-zealand-nautical-almanac-nz204/nautical-almanac-extracts).

[4] See Table 8-4. Note direction of both wind and sea swell should be recorded.

[5] Identity of test area type: sea chest (vertically or horizontally positioned), internal pipework, both sea chest and internal pipework, dimensions (L x W x H), etc.

[6] List for each of the test areas according to Floerl *et al.* 2005; Naval Ships' Technical Manual 2006; Morrisey *et al.* 2015 and Appendix 8.1 (Section 3.5.2).

8.3.7.2 Efficacy testing of physical removal systems.

Test area(s)[1]	Dimensions of cleaned internal niche area (L x W x H)	Location on vessel[2]	Fouling rating and % cover[3]	Image identifiers (file name)[4]	Residual biofouling cover (%)and type[5]	Image identifiers (file name)	Entire area cleaned? (Yes/No)	Time taken for cleaning[6]
1								
2								
3								
4								
5								
6								

[1] Sea chest, internal pipework or both, including dimensions, in accordance with Section 3.5.6.

[2] This is to be accompanied by a schematic of the location of these test areas on the vessel and where samples and measurements were taken within these areas.

[3] Vessel testing should be completed on biofouling of at least Fouling Rating (FR) 90 and > 40 % cover (Appendix 8.1). Viability should be assessed according to Appendix 8.2.

[4] All images and videos should be recorded according to Section 3.5.11.1 and should be submitted.

[5] Residual biofouling cover and type should be assessed using methods described in Sections 3.5.2 , 4.2.3, and Appendix 8.2.

[6] Including time to set-up, testing of system and demobilisation.

8.3.7.3 Assessments of containment and capture efficacy

8.3.7.3.1 *Assessment of containment (physical removal systems)[1].*

Stage of operations	Estimated biofouling dislodged (amount and type)	Cause of dislodgement (description)	Video identifier (file name and time stamp)
Internal niche area 1			
Set-up			
Mobilisation			
De-mobilisation			
Internal niche area 2			
Set-up			
Mobilisation			
De-mobilisation			
Internal niche area 3			
Set-up			
Mobilisation			
De-mobilisation			
Internal niche area 4			
Set-up			
Mobilisation			
De-mobilisation			
Internal niche area 5			
Set-up			
Mobilisation			
De-mobilisation			
Internal niche area 6			
Set-up			
Mobilisation			
De-mobilisation			

[1] Estimates of the amount of biofouling dislodged from the vessel during system operation should be made using the methods described in Section 3.8.4.

8.3.7.3.2 *Capture and containment ability of cleaning head (physical removal systems)[1].*

Distance from cleaning head (cm)	Replicate number	Capture ability/Evidence of dye leakage from cleaning head	Video identifier (file name and time stamp)
10	1,2,3, etc.		
25	1,2,3, etc.		
50	1,2,3, etc.		

[1] Assessment of leakage from the cleaning head should be made using the methods described in Section 3.8.4.

8.3.7.3.3 *Efficacy of waste treatment system(s) (physical removal and treatment systems)[1].*

Stage of operation	Sampling time during cleaning process	Observations (description)[2]	Were the criteria met? (Yes/No)[3]
Internal niche area 1			
Waste treatment test 1			
Replicate 1			
Replicate 2			
Replicate 3			
Waste treatment test 2			
Replicate 1			
Replicate 2			
Replicate 3			
Waste treatment test 3			
Replicate 1			
Replicate 2			
Replicate 3			
Internal niche area(s) 2-6 [4]			
Waste treatment tests 1-3			
Replicate 1-3			

[1] Waste treatment is the available option for systems that do not render fouling non-viable (e.g. physical removal systems or treatment systems that drain effluent from the area prior to, or during exposure to the lethal agent or acid) or discharge waste into a sewer system that does not contain a secondary treatment system.

[2] To include microscopic observations of particle size, structural integrity and viability, as applicable. Also includes assessment of leakage from the waste treatment system (Section 3.8.4; 3.10).

[3] For effluent filtration systems, the maximum particle size in the filtered effluent is 12.5 µm. For effluent treatment systems (e.g. UV, biocidal, heat), all biological material of macroscopic biofouling is to be rendered non-viable (Section 2.2).

[4] As applicable to system tested (Section 3.5.6).

8.3.7.3.4 ***Testing specific for treatment systems[1].***

Stage of operation	Estimated biofouling dislodged (amount and type)	Cause of dislodgement (description)	Containment equipment	Evidence of dye leakage	Video identifier (file name and time stamp)
Internal niche area 1					
Set-up					
Mobilisation					
De-mobilisation					
Internal niche area 2					
Set-up					
Mobilisation					
De-mobilisation					
Internal niche area 3					
Set-up					
Mobilisation					
De-mobilisation					

[1] Assessment of leakage from the containment system (e.g. sea chest blank, bungs) should be made using the methods described in Sections 3.5.12; 3.6.5, 3.7.5, 3.8.4, 3.9 and 3.10.

8.3.7.4 Measurement of lethal agent(s)[1] (treatment systems).

Lethal agent :_____	Exposure endpoint: LD_{100}:_____ $LpHV_{100}$:_____ $LTEMP_{100}$_____ LT_{100}_____			
Date:_____ Time sampled:	Location on vessel (test area and local within test area)	Replicate number[2]	Sample identifier	Measurement (e.g. concentration; temperature)
1		1.1.1		
1		1.1.2		
1		1.1.3		
1		1.2.1		
1		1.2.2		
1		1.2.3		
1		1.3.1		
1		1.3.2		
1		1.3.3		
2		2.1.1		
2		2.1.2		
2		2.1.3		
2		2.2.1		
2		2.2.2		
2		2.2.3		
2		2.3.1		
2		2.3.2		
2		2.3.3		
3		3.1.1		
3		3.1.2		
3		3.1.3		
3		3.2.1		
3		3.2.2		
3		3.2.3		
3		3.3.1		
3		3.3.2		
3		3.3.3		

[1]The method used by treatment systems to render relevant organisms non-viable. This could be a biocide, pH de-oxygenation or a physical treatment, such as, elevated temperature. Values for the lethal dose (LD_{100}) or lethal pH and volume ($LpHV_{100}$) of the treatment agent or lethal temperature ($LTemp_{100}$) and duration of treatment necessary at that dose or temperature to achieve 100 % mortality (LT_{100}) should be determined by direct measurement (Section 3.5.9; 3.5.10).

[2]When non-continuous measurement methods are not used.

8.3.7.5 Analysis of biofouling viability in samples removed from internal niche areas post-treatment.

Internal niche area[1]	Sample identifier[2]	Notes on the overall condition of the biofouling[3]	Number and size of viable organisms[4]
1		Taxonomic group 1 – Taxonomic group 2 – etc.	Species 1 – Species 2 – etc.
2			
3			

[1] Internal niche area configuration, in accordance with Section 3.5.6.

[2] The sample identifier is to be accompanied by a schematic of the location of the test areas on the vessel, and the locations of the samples and assessments within these areas.

[3] Biofouling condition should be assessed and described using the methods described in Section 3.5.2 and Appendix 8.2.

[4] Any organisms that survive the treatment should be identified to species level by a suitably qualified independent scientist.

8.3.7.6 Analysis of biofouling viability of test areas using video imagery (Section 3.5.11).

	Pre-treatment		Post-treatment		
Test area[1]	**Image identifier(s)[2, 3]**	**Notes on condition of biofouling[4]**	**Image identifier(s)**	**Notes on overall condition of biofouling**	**Identification of viable biofouling organisms[5]**
1		Taxonomic group 1 – Taxonomic group 2 – etc.			Species 1 – Species 2 – etc.
2					
3					

[1] Internal niche area configuration, including dimensions, in accordance with Section 3.5.6.

[2] Imagery to be recorded according to Section 3.5.11.1.

[3] The sample identifier is to be accompanied by a schematic of the location of the test areas on the vessel, and the locations of the samples and assessments within these areas.

[4] Biofouling condition and assessment of viability for each taxonomic group should be assessed and described from image and video analysis using the methods described in Sections 3.5.11.1, 3.5.2, 3.6.4, 3.7.4, 3.8.3 and Appendix 8.2.

[5] All organisms that survive the treatment should be identified to *species* level by a suitably qualified independent scientist.

8.3.7.7 Exposure and analysis of viability of additional test organisms[1].

Relevant test organisms							
Species name	**Position in internal niche area[2]**	**Replicate identifier(s)**	**Size (mean ± SD)**	**Image identifiers (file name) pre- treatment**	**Image identifiers (file name) post- treatment**	**Notes on overall condition of biofouling[3]**	**Identification of viable biofouling organisms[4]**
	e.g. exposure replicate 1, 2, 3, 4, etc. position (include number of organisms per replicate)						
Settlement plates							
Plate identifier	**Position in test area[2]**	**Replicate identifier(s)**	**Initial fouling rating, % cover and taxonomic identification**	**Image identifiers (file name) pre- treatment**	**Image identifiers (file name) post- treatment**	**Notes on overall condition of biofouling[3]**	**Identification of viable biofouling organisms[4]**
	e.g. exposure replicate 1, 2, 3, 4, etc. position (include number of organisms per replicate)						

[1] Test organisms are separated into "relevant organisms" and those organisms associated with settlement plate testing (Section 3.5.8). Relevant organisms should be identified to species level. The type of biofouling on settlement plates can be described in broad taxonomic and morphological categories (such as erect bryozoans, barnacles, and encrusting coralline algae) by a suitably qualified independent scientist.

[2] The description of position in test area is to be accompanied by a schematic of the location of the test areas on the vessel, and the locations of the samples and assessments within these areas.

[3] Biofouling condition and assessment of viability for each taxonomic group should be assessed and described from image and video analysis using the methods described in Sections 3.5.11.1, 3.5.2, 3.6.4, 3.7.4, 3.8.3 and Appendix 8.2.

[4] All organisms that survive the treatment should be identified to *species* level by a suitably qualified independent scientist.

8.3.7.8 Assessment of structural integrity of internal niche areas[1].

Assessor:_____	Company:_____		Qualifications:_____	
	Pre-treatment		**Post-treatment**	
Internal niche area[2]	**Image identifier(s)**	**Observations**	**Image identifier(s)**	**Observations**
1 [3]				
2				
3				

[1] Section 2.4; 3.5.5

[2] Sea chest, internal pipework or both, including dimensions, in accordance with Section 3.5.6. The sample identifier is to be accompanied by a schematic of the location of the test areas on the vessel, and the locations of the samples and assessments within these areas, as applicable.

[3] Replication as applicable to system tested (Section 3.5.6; 3.5.7).

Table 8-4. Douglas Sea Scale (adapted from World Meteorological Organization).

State of the sea (wind sea)		
Degree	**Wave height (m)**	**Description**
0	0 (no wave)	Calm (glassy)
1	0–0.1	Calm (rippled)
2	0.1–0.5	Smooth (wavelets)
3	0.5–1.25	Slight
4	1.25–2.5	Moderate
5	2.5–4	Rough
6	4–6	Very rough
7	6–9	High
8	9–14	Very high
9	> 14	Phenomenal

Swell		
Degree	**Swell wave length (m)/height (m)**	**Description**
0	0/0	No swell
1	< 100/< 2	Very low (short and low wave)
2	> 200/< 2	Low (long and low wave)
3	< 100/2–4	Light (short and moderate wave)
4	100–200/2–4	Moderate (average and moderate wave)
5	> 200/2–4	Moderate rough (long and moderate wave)
6	< 100/> 4	Rough (short and heavy wave)
7	100–200/> 4	High (average and heavy wave)
8	> 200/> 4	Very high (long and heavy wave)
9		Confused (wavelength and height indefinable)

8.4 CONTACT DETAILS OF RELEVANT NEW ZEALAND AUTHORITIES FOR RESOURCE CONSENTING FOR IN-WATER SYSTEM USE

National authority
Environmental Protection Authority
http://www.epa.govt.nz/Pages/default.aspx
0800 HAZSUBS (0800 429 7827)

Regional authorities
Auckland Council
http://www.aucklandcouncil.govt.nz/EN/Pages/default.aspx
09 301 0101

Bay of Plenty Regional Council
http://www.boprc.govt.nz/
0800 884 880

Environment Canterbury
https://www.ecan.govt.nz/
0800 324 636

Environment Southland
http://www.es.govt.nz/Pages/default.aspx
0800 76 88 45

Gisborne District Council
http://www.gdc.govt.nz/
06 867 2049

Greater Wellington Regional Council
http://www.gw.govt.nz/
0800 496734

Hawkes Bay Regional Council
http://www.hbrc.govt.nz/Pages/default.aspx
+646 833 8090

Horizons Regional Council
http://www.horizons.govt.nz/
0508 800 800

Marlborough District Council
http://www.marlborough.govt.nz/
03 520 7400

Nelson City Council
http://nelson.govt.nz/
03 546 0200

Northland Regional Council

http://www.nrc.govt.nz/
0800 002 004

Otago Regional Council
http://www.orc.govt.nz/
0800 474 082

Taranaki Regional Council
https://www.trc.govt.nz/
0800 736 222

Tasman District Council
http://www.tasman.govt.nz/
03 543 8400

Waikato Regional Council
https://www.waikatoregion.govt.nz/
0800 800 402

West Coast Regional Council
http://www.wcrc.govt.nz/Pages/default.aspx
0508 800 118