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Manatū Ahu Matua

Tales from the CRYPT

A prototype heat treatment system for biofouling in internal pipework of recreational vessels

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Executive Summary

The aim of MPI Project 405135 – Reactive Pipework Treatment for Recreational Vessels – was to develop and validate a reactive biosecurity treatment tool for biofouling in internal pipework systems of recreational vessels. Vessel internal pipework 'niche' areas are difficult to inspect and quantify biosecurity risk but there are documented examples of invasive species introductions via fouled pipework systems. A research gap was identified with respect to managing fouled pipework on high risk recreational vessels.

A variety of treatment approaches have been assessed previously for reactive treatment of vessel pipework, and related scenarios. The current project reviewed the literature and consulted industry experts to summarise existing information pertaining to: vessel pipework configurations; relative biosecurity risks; and candidate treatment approaches. A decision framework was formulated to enable the selection of a suitable treatment approach and the development of a prototype treatment system. The outcomes of the review and decision analysis are published in a separate report.

Thermal stress (i.e., heat treatment) was selected as the 'best' candidate treatment approach based on the following considerations:

- demonstrated efficacy against a range of relevant marine biofouling organisms, with 60 ± 2 °C for 60 minutes a recognised benchmark for biosecurity treatment purposes;
- low risk to operators and the environment;
- low likelihood to exacerbate biosecurity risk if pipework is properly isolated;
- existing precedence for consented discharge of heated water to the New Zealand marine environment;
- compatibility with pipework and other vessel components at or below 65 °C;
- availability of a range of heating systems that could form the basis for a tailor-made treatment system; and
- ability to monitor treatment parameters in the field using readily available equipment (i.e., temperature probes).

Because lethal thermal parameters for biofouling organisms had already been established in prior studies, the primary consideration for the current project was to design a functioning heat treatment system and develop procedures for its use. Several alternate heat treatment approaches were considered but recirculation of seawater through an external fluid-califont was ultimately prioritised. The **C**alifont **R**ecirculator for **Y**acht **P**ipework **T**reatment (CRYPT) was designed and built. The CRYPT is based around a marine hot water cylinder fitted with a heating element and adjustable thermostat. The main body of the cylinder is designed to be filled with freshwater and contains a heat exchange coil. Seawater is driven through the heat exchange coil via a centrifugal pump. Heat then diffuses from the body of freshwater inside the cylinder to the seawater being pumped through the heat-exchange coil. A custom-made counter-current manifold with interchangeable sealing devices creates a 'closed system' and allows delivery and recirculation of seawater through a single pipework inlet or outlet.

A series of laboratory experiments was performed using 'mock pipework systems' (MPS) to optimise treatment parameters in a controlled setting. Three individual MPS were constructed to represent engine-cooling, ancillary seawater supply, and below-water discharge systems that are common to recreational vessels. For all experiments, temperatures were monitored in real time throughout the MPS, and sentinel biofouling organisms were stationed at key locations within the MPS to validate treatment efficacy. The Pacific oyster *Magallana gigas* was used as

the sentinel organism because it is recognised to be the most heat resilient biofouling organism available in New Zealand.

The preliminary set of experiments used the engine-cooling MPS to optimise variations of delivery tube insertion depth, cylinder temperature setting, recirculation flow rate through the heat exchange coil, and rate of cooling once the CRYPT is deactivated. Temperature profiles at ‘high risk’ locations in the engine-cooling MPS met the target treatment conditions of 58 to 65 °C for 60 minutes in all instances, and *M. gigas* mortality was always 100%. However, the optimisation process decreased the initial time required to heat pipework to the target zone by almost threefold (from 87 to 27 minutes), and reduced subsequent temperature fluctuation during the treatment phase. The optimal combination of treatment parameters were:

- insertion to the first bend in the system;
- cylinder thermostat set to 72.5 °C;
- main pump flow rate set to 20 L minutes^{-1}; and
- no cooling step.

These optimal treatment parameters were then assessed for suitability with the ancillary seawater supply and below-water discharge MPS. The parameters optimised for the engine-cooling MPS transferred easily to the below-water discharge MPS; however, preliminary attempts to treat the ancillary seawater supply MPS were only partially effective. An additional flow-through step for small-diameter pipework systems was developed, which resulted in efficient heating of ‘high risk’ features in the ancillary seawater supply MPS.

A draft treatment protocol was developed based on the outcomes of the laboratory experiments, and was then validated in the field. Six independent pipework systems on board domiciled recreational vessels (7 to 23 m) berthed in Nelson Marina, Nelson, New Zealand were treated using the CRYPT. Engine-cooling, ancillary seawater supply, and below-water discharge systems with nominal diameters from 12 to 125 mm and total lengths from 0.75 to 3 m were included in the validation. Qualitative assessments of biofouling ‘load’ within pipework systems were made pre- and post-CRYPT treatment. Pipework temperatures were monitored indirectly by affixing temperature probes to the outside of pipework features on board the vessels.

The CRYPT effectively heated pipework systems of recreational vessels in the field when an effective seal was achieved at the pipework inlet or outlet and ambient heat loss was minimised. These conditions were achieved for three of the six vessels assessed. The temperature profiles for these effectively heated pipework systems closely matched the results observed during the laboratory testing phase. Engineering challenges prevented effective treatment of the other three vessels. Even though pipework configurations were similar to systems treated effectively on other vessels, vessel layout (e.g., position of the keel and presence of inlet grates), condition (e.g., extent of hull fouling present around inlets) and orientation (e.g., distance of inlet from adjacent marine pontoon) interfered with the sealing apparatus or increased ambient heat loss to above an acceptable threshold. Difficulty forming an effective seal at some pipework inlets or outlets was a noteworthy limitation to the CRYPT system in its current form. When the seal is not effective, the CRYPT is unable to heat pipework to required conditions due to ingress of ambient seawater.

Based on the outcomes of the field validation, an operational treatment protocol was finalised. The protocol details criteria and procedures for effective biosecurity treatment of a defined subset of vessel pipework systems. Included in the protocol are provisions to validate additional apparatus and guidance for expanding the applicability of the system. Modifications and validations could include:

- a wider range of pipework inlet or outlet sealing devices;
- more user-friendly quality control measures;
- applicability under a wider range of ambient environmental conditions; and
- enhanced heating capacity of the CRYPT.

The end goal of further validation should be a treatment protocol and associated apparatus that accounts for the full diversity of pipework systems commonly encountered on board recreational vessels.

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1 General introduction

The Ministry for Primary Industries (MPI) commissioned Cawthron Institute, in partnership with National Institute of Water and Atmospheric Research (NIWA) and Biofouling Solutions Pty Ltd (BFS), to develop a reactive in-water treatment for the internal pipework systems of recreational vessels. A research gap was identified with respect to managing fouled pipework on high risk recreational vessels. A treatment for these internal niche areas would enable high risk recreational vessels to comply with the Craft Risk Management Standard, and thus minimise the likelihood of introduction and establishment of non-indigenous species in New Zealand (Ministry for Primary Industries, 2014).

The project commissioned by MPI was divided into three stages:

1. industry expert consultation and literature review;
2. laboratory trials to establish treatment and operational protocols; and
3. *in situ* trials on domiciled recreational vessels.

1.1 INDUSTRY EXPERT CONSULTATION AND LITERATURE REVIEW

This first stage of the project aimed to categorise the types of pipework systems found on board recreational vessels, define relative biosecurity 'risks' posed by pipework biofouling, and assess the suitability of a selection of candidate treatment approaches. The findings of the consultation and review have been published separately (Cahill et al., 2018), with a summary of that report presented below.

Three main types of pipework systems that come into direct contact with seawater were identified:

1. engine-cooling systems to supply a flow of seawater when a vessel's engine is in operation;
2. ancillary seawater supply systems to service a range of on-board features, including deck wash down, toilets, refrigeration condensers, and desalination plants; and
3. below-water discharge systems primarily limited to blackwater discharge and deck-draining scuppers.

Engine-cooling, ancillary seawater supply, and below-water discharge pipework systems can all develop biofouling, but systems with open exchange of seawater with the ocean pose the highest risk for biofouling organisms to colonise, grow, and reproduce. As such, biofouling is typically encountered near pipework inlets or outlets, or further into systems that actively pump seawater. It is difficult to accurately quantify biosecurity 'risks' posed by pipework biofouling but anecdotal insights of the composition, intensity, and location of biofouling were gained. When biofouling is present within pipework, a range of taxonomic groups can be encountered and the most commonly reported examples were barnacles, hydroids, mussels, oysters, and tubeworms. Available accounts suggest that pipework systems feature low to moderate biofouling loads because high fouling loads would impair or prevent the operation of a vessel. However, exceptions do occur and even low biofouling loads pose biosecurity risks if biofouling is able to be dislodged or if reproductively-mature individuals are present. These risks are particularly apparent for international yachts, whose biofouling communities are unlikely to be native to New Zealand.

Using the knowledge gathered relating to pipework systems and associated biofouling, a decision framework was defined to assess the operational feasibility of prospective treatment agents. Suitable treatment agents for vessel internal pipework should meet the following criteria:

- **effective** against adults and juveniles of relevant biofouling organisms at realistic working concentrations or intensities and within 48 h;
- **safe** for the environment and operators at relevant concentrations or intensities;
- **biosecure** and will not exacerbate risk of release and establishment of marine non-indigenous species;
- **consented** for use, or some existing precedent for use, in the New Zealand marine environment;
- **compatible** with pipework and other vessel components;
- **feasible** to apply, with realistic resource, cost, and infrastructure requirements; and
- **quality control** methods are available to confirm that treatment thresholds (e.g., biocide concentration or temperature) have been met.

1.2 THERMAL TREATMENT OF INTERNAL PIPEWORK

An array of chemical and physical treatment agents identified by Growcott et al. (2016) were assessed against the decision framework. **Thermal stress** (i.e., the application of heat) was selected as the preferred treatment agent for biofouling in internal pipework of recreational vessels, based on:

- demonstrated efficacy against a range of relevant marine biofouling organisms;
- low risk to operators and the environment;
- low likelihood to exacerbate biosecurity risk;
- existing precedent for consented discharge of heated water to the New Zealand marine environment;
- compatibility with pipework and other vessel components;
- availability of heating systems that could form the basis for a tailor-made treatment system; and
- ease of monitoring treatment temperatures in the field.

Thermal stress involves increasing the temperature inside vessel pipework to above the thermal limits of biofouling organisms. This approach is known to be effective against a range of marine biofouling organisms at realistic working temperatures (≤ 60 °C) and within short timeframes (< 1 hour). Given that treatments are likely to be applied to vessels in water with effluent potentially discharged to the sea, gaining consent for wide-scale application is a major consideration for any treatment approach. Most, if not all, regional bodies in New Zealand have existing precedent for discharge of heated water. While heated water potentially poses some risks to operators and the environment, these risks are easily mitigated. Commonsense handling procedures and personal protective equipment adequately account for managing the risk of burns to operators. Cooling water before discharge or ensuring adequate mixing in the receiving environment will prevent harm to non-target organisms. Temperatures required to render biofouling non-viable (≤ 60 °C) are also compatible with common pipework materials, which typically include plastics (e.g., HDPE and PVC) and metal alloys (e.g., stainless steel, brass, and bronze). A robust quality control measure is required to ensure treatment parameters are met but not exceeded. In the case of thermal treatment, temperature profiles can be easily monitored in the field via in-pipe or externally-mounted (i.e., on outside of pipework) thermistors.

The tolerance of biofouling organisms to thermal stress has been investigated in prior studies (e.g., Rajagopal et al., 2012). Two studies present in-depth evaluations of the thermal limits of a range of common biofouling organisms in laboratory settings and model sea chest systems (Leach, 2011; Piola and Hopkins, 2012). Previously tested organisms include ascidians, barnacles, bivalves, sponges, anemones, gastropods, crustaceans, and echinoderms. Although temperature tolerance varies within and between species, upper lethal limits have been shown to be consistently at or below 60 °C (Table 1). Required exposure periods at 60 °C range from less than 5 minutes for soft bodied biofouling organisms to 30 minutes for resilient bivalves.

A precautionary approach is advisable when developing a biosecurity treatment protocol, whereby the treatment parameters effective against the most resilient organisms are targeted. Such a treatment will likely be effective against all other organisms present in pipework, including any thermally tolerant organisms that may be found on board vessels arriving from overseas. Bivalves are highly resilient to many treatment types because they can close their valves and exclude the external environment (e.g., Forrest et al., 2007; Piola and Hopkins, 2012; Atalah et al., 2016; Hopkins et al., 2016). Of the species tested in New Zealand, the oysters *Magallana gigas*[1] is the most resilient organism to thermal stress, with 100% mortality following exposure to 60 °C for 30 minutes or 57.5 °C for 60 minutes (Piola and Hopkins, 2012). In accordance with these experimentally validated thermal limits, a treatment threshold of 60 ± 2 °C for 60 minutes has been proposed for biosecurity treatment purposes (Growcott et al., 2017).

1.3 PROSPECTIVE HEAT TREATMENT SYSTEMS

Due to the thermal limits of biofouling organisms already being sufficiently well described, the primary consideration for treating vessel pipework was to develop an automated system capable of raising and maintaining temperatures inside pipework to 60 ± 2 °C. Adequate control of temperature is essential to ensure lethal conditions are met, whilst avoiding harming pipework components (~ 65 °C as a precautionary limit, although pipework components are typically rated for higher temperatures). Most pipework systems on board recreational vessels are not amenable to flow-through recirculation because they only have a single inlet or outlet, or barriers to water flow are present (e.g., positive displacement impeller pumps). For this reason, any universally applicable heat delivery system needs to operate using a single inlet or outlet.

Two alternative approaches to heat vessel pipework were considered:

- passive heating using heating elements inserted into pipework; and
- active heating via recirculation of seawater through an external heating system.

Passive heating could utilise flexible heating elements inserted into pipework inlets or outlets. Low temperature self-limiting heating cables that automatically regulate heat outputs to a set temperature endpoint of 65 °C appear suitable for this application. These cables, which are widely used in industrial and domestic reticulation systems, use a polymer-carbon matrix conductor that alters its resistance in response to temperature, allowing heat output to be infinitely regulated along the length of the cable.[2] The self-limiting nature of these heating cables would avoid 'hot spots' damaging pipework, and a digital thermostat could be included to provided fine scale control of the system and to log treatment conditions. The effectiveness of this passive approach is reliant upon diffusion of heat throughout the entire pipework system via convection and conduction. The rate at which heat will diffuse throughout vessel pipework systems is unknown and is likely to vary with pipework configuration, construction materials, and ambient conditions. An expert in the design of industrial heating systems (Rory Comer, New Zealand Electrical Solutions) indicated that even heating may be difficult to achieve throughout complex pipework systems.

Active heating requires water within pipework systems to be recirculated through an external heating system. A fluid califont principle, whereby seawater is recirculated through a heat-

[1] Formerly known as *Crassostrea gigas* (http://www.marinespecies.org/aphia.php?p=taxdetails&id=140656)
[2] http://www.advancedthermaldesigns.com/blog/self-regulating-heating-cables.html

exchange coil inside a hot water cylinder filled with freshwater, is one potentially suitable approach. Indirect heating via a hot water cylinder is an inherently ‘safe’ way to apply heat with minimal risk of overheating pipework because of the associated buffering effect of the volume of freshwater inside the cylinder. Constraining the seawater within a heat-exchange coil protects heating elements and other sensitive components inside the hot water cylinder from corrosion. Integrating a counter-current fitting to a sealing device is one approach to recirculate treatment water through a single pipework inlet or outlet. Water is delivered to, and drawn back from, the counter-current fitting via separate lines attached to the inlet and outlet of the fluid califont.

Both passive and active heat delivery are likely to have advantages and disadvantages that would only be fully understood if each system is trialled in a controlled setting. Passive heat delivery using self-limiting heating cables is an elegant solution without the need for bulky infrastructure; however, actively recirculating heated water is less likely to result in uneven heating throughout pipework systems. Active recirculation of heated water using a fluid califont principle was prioritised because it was considered a less risky avenue for rapidly developing a biosecure treatment protocol within the temporal and financial constraints of this project.

1.4 OPTIMISING AND VALIDATING A TREATMENT APPARATUS AND PROTOCOL

A tiered approach was used to develop a treatment protocol based on the active recirculation of heated water. A prototype heat-treatment unit was designed and optimised in a laboratory setting using a set of ‘mock pipework systems’ (MPS). Detailed laboratory testing was prioritised for the project overall because, by their very nature, vessel pipework systems are highly confined areas with poor accessibility. Detailed laboratory testing allowed accurate quantification of treatment conditions and biofouling viability, which is not realistically achievable in a field setting. A draft treatment protocol developed from the outcomes of the laboratory component of the project was subsequently field validated on a subset of actual vessels. The field validation served to confirm the operational suitability of the systems, whilst uncovering additional practical considerations that may not have been apparent in the laboratory.

The current document is divided into a series of chapters developed from successive project Milestone Reports:

- **Chapter 2** – Design and laboratory optimisation of a prototype heat-treatment system to inform drafting of an operational treatment protocol;
- **Chapter 3** – Field validation to assess the operational suitability of the prototype and refine the operational protocol; and
- **Chapter 4** – Discussion of the project, including outcomes, limitations, and additional research and development requirements.

Table 1. Thermal parameters effective against marine biofouling organisms in laboratory and mock sea chest experiments.

Description	Experimental design	Test organisms	Thermal tolerance	Reference
Laboratory and mock sea chest experiments using heated seawater to kill a range of temperate biofouling organisms.	Small (10 – 30 mm) and large (55 – 80 mm) mussels (*Perna canaliculus* and *Mytilus galloprovincialis*) exposed to 35, 37.5, 40, 42.5, 45, or 50 °C for 5, 10, 20, 30, 45 or 60 min (*n* = 5).	Small *P. canaliculus* Large *P. canaliculus*	100% mortality at 40 °C for ≥ 5 min, or 37.5 °C for ≥ 20 min.	Piola and Hopkins (2012)
		Small *M. galloprovincialis* Large *M. galloprovincialis*	100% mortality at 45 °C for ≥ 5 min, or 40 °C for ≥ 20 min.	
	Hydroids, solitary and colonial ascidians, bryozoans, anemones, shrimp, oysters, gastropods, barnacles, isopods, and sea stars exposed to 37.5, 40, 42.5, or 60 °C for 60, 30, 20, or 30 min, respectively (*n* = 5).	*Magallana gigas*	100% mortality at 60 °C for 30 min, or 57.5 °C for 60 min.	
		Austrominius modestus	100% mortality at 42.5 °C for 20 min.	
		Bougainvillea muscus *Ciona* spp. *Botrylloides leachi* *Didemnum vexillum* *Bugula neritina* *Anthothoe albocincta* *Palaemon affinis* *Melagraphia aethiops* *Austrominius modestus* *Epopella plicata* *Natatolana pellucida* *Stichaster australis* *Patiriella regularis* *Coscinasterias calamaria*	100% mortality in all treatments.	
Field trials using a patented heat application system to kill intact biofouling communities on fouled panels and in a mock sea chest.	Fouled plates submerged in seawater heated to 30, 40, 60, or 70 °C for 15 or 30 min (*n* = 4) – survivorship assessed after 12 h.	Bryozoa spp. Polychaeta spp. Cirripedia spp. Bivalvia spp. Ascidiacea spp. Porifera spp.	100% mortality at ≥ 40 °C for 15 or 30 min.	Leach (2011)
	Mock sea chest treated at: 70 °C for 10 min; 60 °C for 10 min; 40 °C for 15 min; and 40 °C for 30 min (*n* = 1).	*Mytilus edulis* *Trichomya hirsuta*	100% mortality at 60 °C or 70 °C for 10 min.	
		Bryozoa spp. Polychaeta spp. Cirripedia spp. Bivalvia spp. Ascidiacea spp. Porifera spp.	100% mortality in all treatments.	

2 Prototype development and laboratory optimisation

2.1 SCOPE AND OBJECTIVES

This chapter describes the design and construction of an active heat treatment system, and outcomes of laboratory experiments to optimise system performance. The heat treatment system recirculates seawater from vessel pipework through a temperature-regulated fluid califont. The prototype unit designed for this project has been named the 'CRYPT' – **C**alifont **R**ecirculator for **Y**acht **P**ipework **T**reatment. The Latin and Greek roots of the word crypt mean 'hidden place', a fitting association for internal pipework biofouling.

To optimise key operational parameters of the CRYPT, a series of experiments were performed using MPS representative of engine-cooling, ancillary seawater supply, and below-water discharge systems commonly encountered on board recreational vessels. Both temperature profiles and sentinel organism mortality are used as proxies for treatment efficacy. The outcomes of these experiments informed drafting of an operational treatment protocol for field validation on actual vessels (Chapter 3).

2.2 METHODS

2.2.1 Mock pipework systems

A modular tank system, constructed by Nelson Plastics, Nelson, New Zealand, housed three MPS that replicate the three most common types of pipework systems encountered on board recreational vessels:

- engine-cooling systems;
- ancillary seawater supply systems; and
- below-water discharge systems.

The modular tank system consisted of an inner (1 m wide, 1 m deep, 1 m high) and an outer (1.4 m wide, 1.4 m deep, 1.2 m high) polyethylene tank to replicate the hull of a vessel and the surrounding ocean, respectively (Figure 1). When in use, the outer tank was partially filled with seawater and heavy-duty chains and steel angle irons were used to hold the top of the two tanks level.

Figure 1. Mock pipework systems housed in a set of tanks designed to replicate the hull and waterline of a recreational vessel. Steel bars and chains hold the two tanks level when filled with water. Image: P. Cahill.

The designs of the MPS described below are based on a literature review and industry expert opinions of pipework configurations on board recreational vessels (Cahill et al., 2018). The MPS were designed to a complexity exceeding most systems likely to be encountered.

Chlorinated polyvinyl chloride (PVC) and high-density polyethylene (HDPE) pipework and fittings were used to construct the three MPS. Pipework internal diameters (IDs) were 100 mm for the engine-cooling MPS, 25 mm for the ancillary seawater supply MPS, and 75 mm for the below-water discharge MPS. Each MPS commenced at an appropriately sized, threaded fitting in the bottom of the inner tank (i.e., the external hull surface of a vessel), followed immediately by a ball or gate valve for isolation of the system from the surrounding seawater.

The engine-cooling (76 mm suction filter, ARAG, Rubiera, Italy) and ancillary seawater supply (25 mm raw-water strainer, unbranded, purchased from Burnsco, Nelson, New Zealand) MPS included raw-water strainers as used on actual vessels. Strainer baskets were removed from the raw-water strainers for experiments because it is anticipated that strainer baskets will be removed prior to treating actual vessels. A mock heat exchanger was constructed for the engine-cooling MPS using a series of five parallel lengths of 10 mm ID PVC pipe connected via 90-degree bends and tees. The engine-cooling MPS terminated above the waterline, mimicking discharge of cooling water into the engine exhaust (Figure 2). A three-way manifold system was fitted to the ancillary seawater supply MPS, constructed using 90-degree bends and tees (Figure 3). A 1 m long vertical section of pipe followed by a vented loop was the primary feature of the below-water discharge MPS (Figure 2).

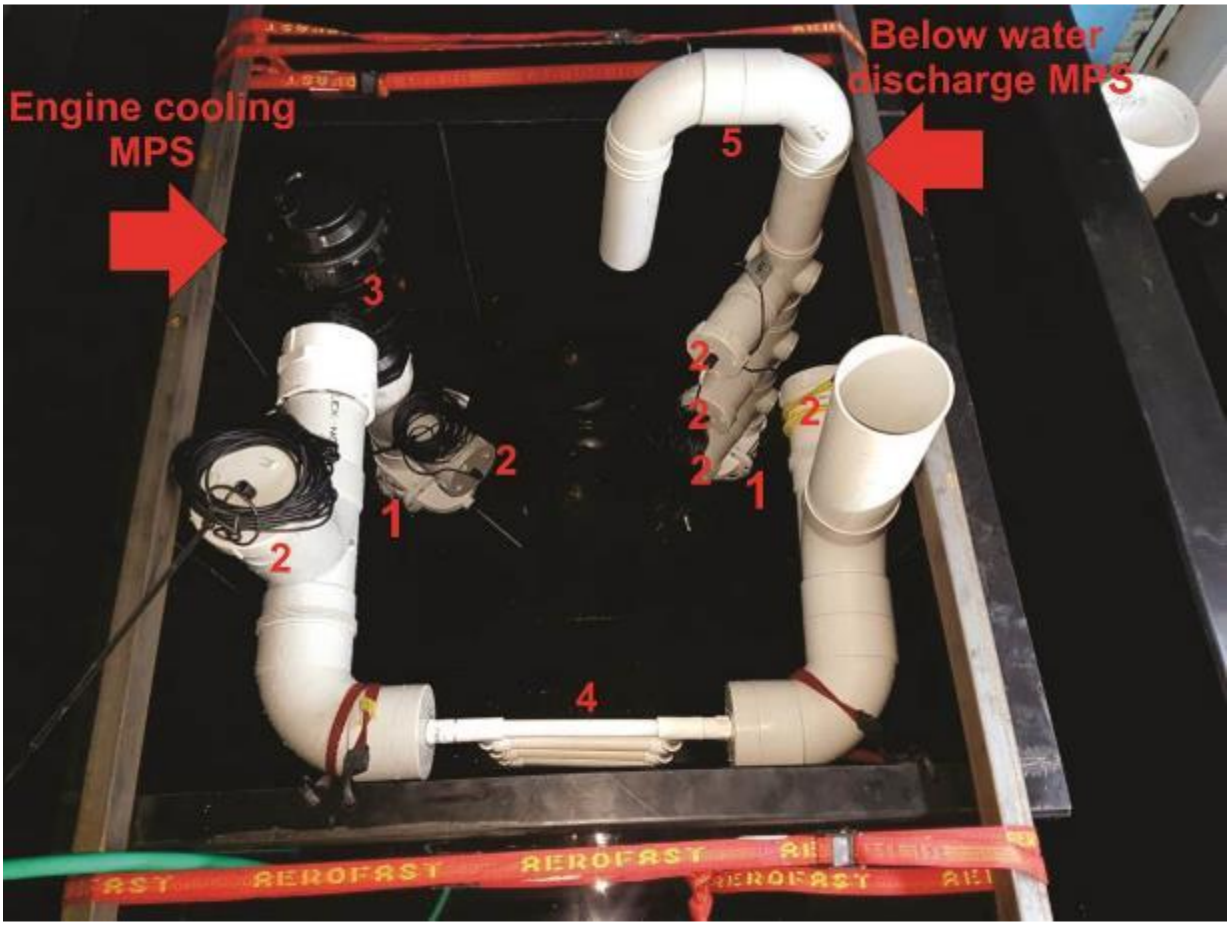

Figure 2. Mock pipework systems (MPS) replicating engine-cooling and below-water discharge systems commonly encountered on board recreational vessels. 1: gate valve; 2: sampling port; 3: raw-water strainer; 4: mock engine heat exchanger; 5: vented loop. Image: P. Cahill.

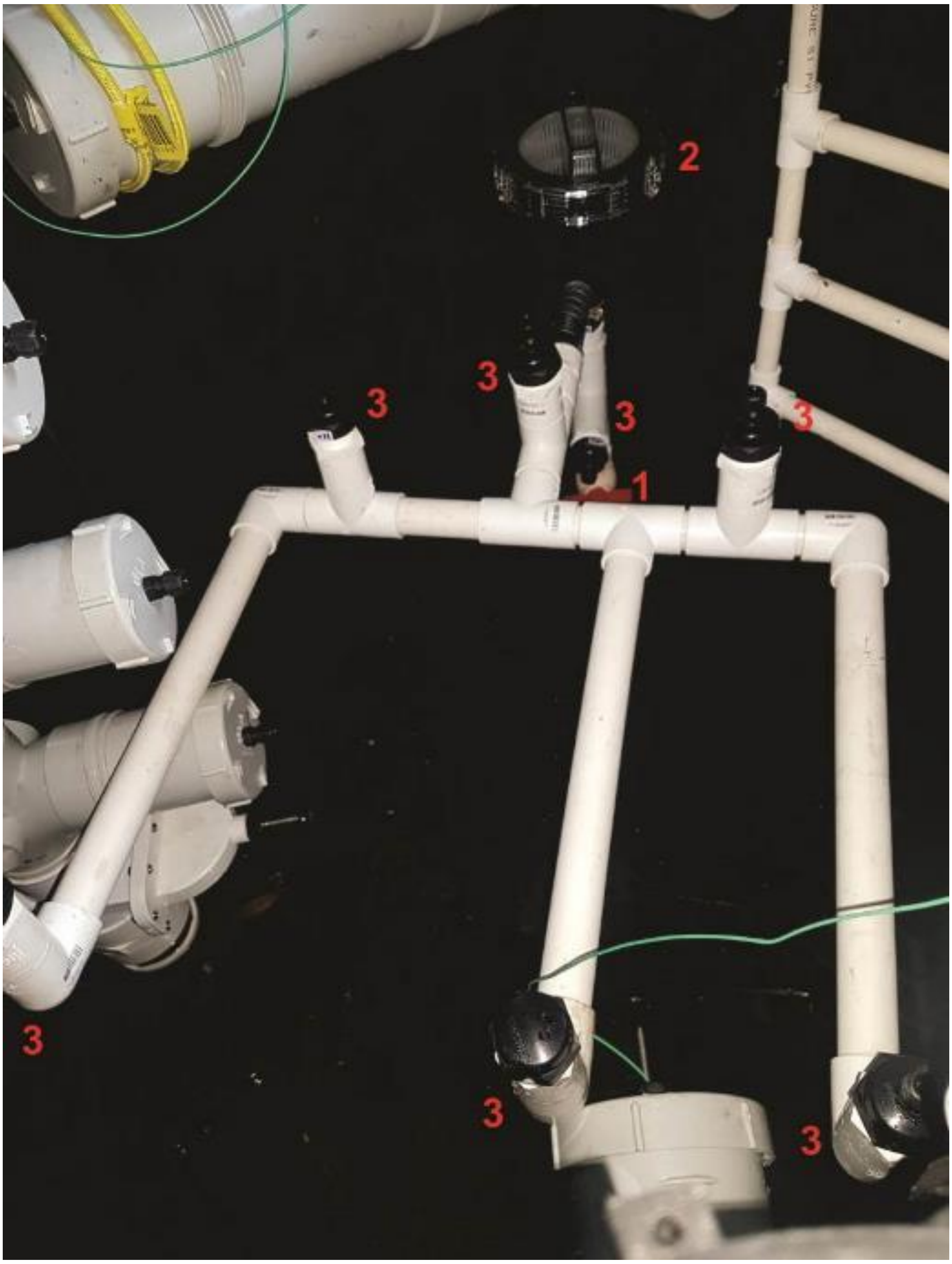

Figure 3. Mock pipework systems replicating ancillary seawater supply systems commonly encountered on board recreational vessels. 1: ball valve; 2: raw-water strainer; 3: sampling port. Image: P. Cahill.

The relative biofouling 'risk' of pipework features is of high importance to defining effective treatment protocols. The MPS incorporated sampling ports in locations considered both 'high risk' and 'low risk' (Figure 5). 'High risk' locations are those that under normal vessel operation conceivably support physical parameters, particularly seawater exchange, conducive to the establishment and survival of biofouling organisms (Cahill et al., 2018). 'Low risk' locations are characterised by physical parameters unlikely to support the establishment and survival of biofouling. Many locations that are considered 'low risk' have low volume or no seawater exchange with the external environment. Although passive diffusion is likely sufficient to support biofouling close to all pipework inlets (i.e., first 0.5 m), biofouling is unlikely to occur further into sporadically operated seawater systems with restricted seawater exchange, particularly those with smaller pipework diameters. The terminal sampling ports incorporated to the ancillary seawater supply MPS are an example of 'low risk' locations due to restricted seawater exchange. Additionally, the operation of given seawater systems can preclude biofouling in given locations. For example, areas downstream of engine-cooling heat exchangers present ‘low risk’ because heat exchangers raise seawater temperatures when in operation. When not in operation, positive displacement impellers incorporated in engine-cooling system completely prevent seawater exchange from the heat exchanger onwards. Although it is useful to understand the capacity of the CRYPT to heat beyond restrictive pipework features, such as engine heat exchangers, focussing on pipework locations posing ‘high risk’ is of most relevance to defining practical treatment protocols.

Sampling ports were distributed throughout each MPS: 3 for engine-cooling; 6 for ancillary seawater supply; and 3 for below-water discharge (Figure 5). The sampling ports consisted of appropriately sized tees with one port blanked off with a removable screw cap, and coarse mesh screens to allow sentinel organisms to be retained at the sampling port (Figure 4). Thermocouple temperature sensors, connected to a TC-08 multi-channel data logger (Pico Technology, St Neots, United Kingdom), were stationed at each sampling port using gland fittings (McCabe Industries, Upper Hutt, New Zealand). In addition to the dedicated sampling ports, temperature sensors were stationed at the raw-water strainer in the engine-cooling MPS and on the return line of the CRYPT system.

Figure 4. Sampling port design with mesh screen to retain sentinel organisms, thermocouple temperature probe, and screw cap. Image: P. Cahill.

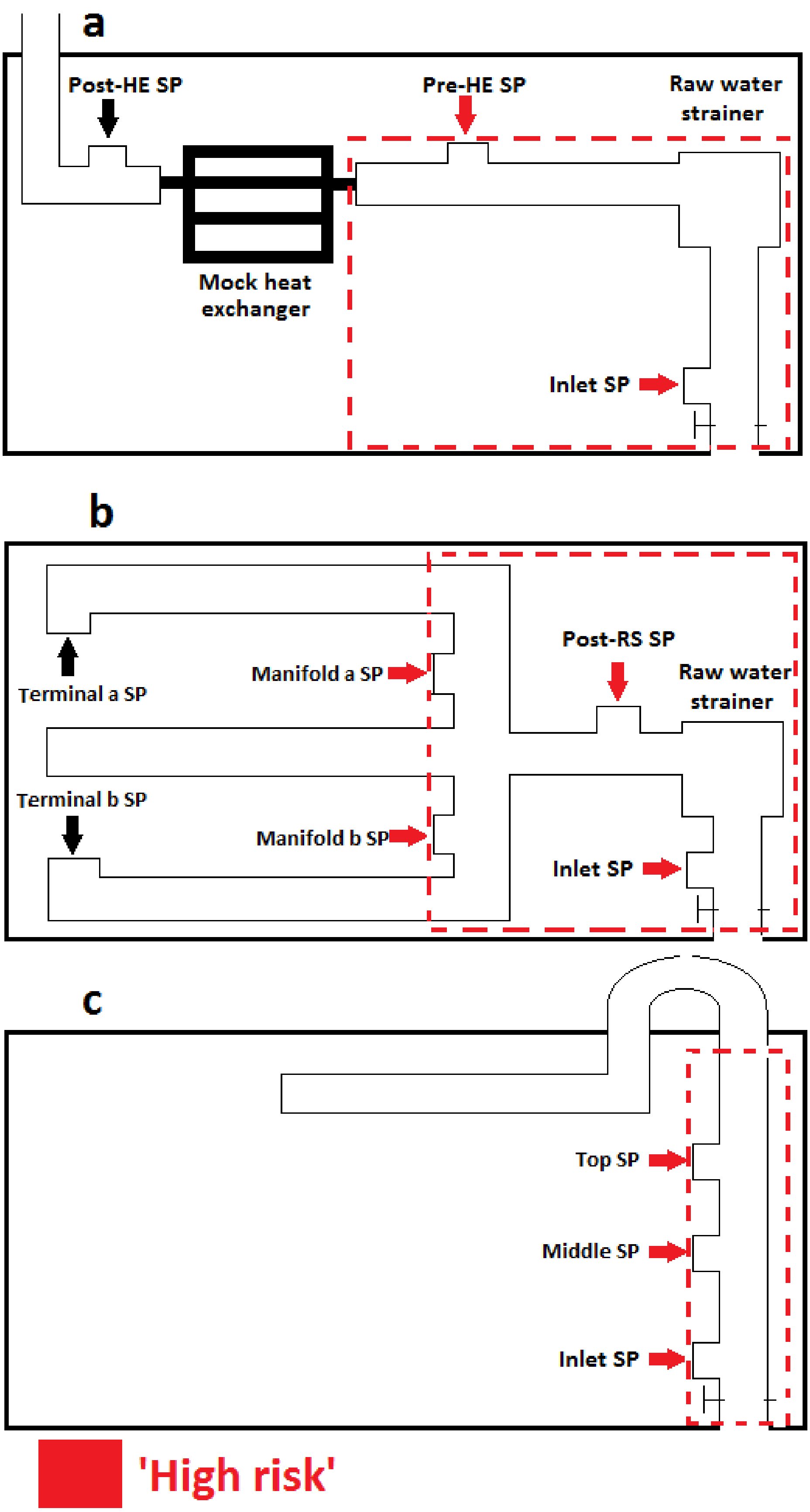

Figure 5. Location and nomenclature of sampling ports (SP) throughout engine-cooling (a), ancillary seawater supply (b), and below-water discharge (c) mock pipework systems. Locations considered to pose 'high risk' for biofouling to accumulate are shown. HE: heat exchanger; RS: raw water strainer. Not drawn to scale.

2.2.2 Sentinel organisms

The oyster *M. gigas* was used as a sentinel organism because it has previously been shown to be more resilient to heat than any other temperate macrofouling organism examined in New Zealand (Piola and Hopkins, 2012). A 15-kg bin of 50 ± 5 mm shell length *M. gigas* were sourced from Marlborough Oysters Ltd, Croisilles Harbour, Marlborough, and a 1-kg bag of 4 ± 0.5 mm shell length *M. gigas* were sourced from the MOANA Hatchery at Cawthron Aquaculture Park, Nelson, New Zealand. The oysters were acclimated to Cawthron Institute's recirculating seawater system (20 ± 1 °C, 33 ± 1 PSU) and fed an appropriate mix of bulk-cultured microalgae for approximately 1 month prior to experimentation.

Croisilles Harbour and Cawthron Aquaculture Park fall within the *Bonamia ostreae* Controlled Movement Area and ostereid herpesvirus type-1 µvar has been previously detected in Croisilles Harbour. An enquiry with MPI's Spatial Allocations Team confirmed that no permits were required for these transfers because the oysters were transferred within the Controlled Movement Area and the destination was not a marine farm. Regardless, best practice biosecurity procedures were implemented to minimise the risk of disease transfer (Appendix 1).

2.2.3 The CRYPT

The CRYPT was based around a 240-V, 120-L marine hot water cylinder constructed by Sigma Sheet Metal Products Ltd, Onehunga, New Zealand (Figure 6). The hot water cylinder was fitted with a 2-kW heating element with adjustable thermostat. The main body of the cylinder was designed to be filled with freshwater only, and a heat-exchange coil consisting of 20 m of 20 mm ID 316 stainless steel tube was installed inside the main body of the cylinder (Figure 7). Seawater was driven through the heat exchange coil via a marine grade centrifugal pump with nickel aluminium bronze impeller and housing (AZCUE CP 25-130, Bombas Azcue S.A, Gipuzkoa, Spain).

Heat diffuses from the body of freshwater inside the cylinder to the seawater being pumped through the heat-exchange coil. The pump was placed on the downstream side of the heat-exchange coil to minimise the suction head required by the system. The pump was selected to overcome the considerable hydrodynamic friction associated with the seawater recirculation system, which was calculated at around 20 m of hydraulic head loss[3] (calculated by Mike Bollard, Pump Engineer, Pumps and Filters, Nelson, New Zealand). As the main pump has a three-phase motor, a single-phase to three-phase variable frequency drive unit (CFW10, WEG Industries, Matamata, New Zealand) was fitted to allow flow rate to be altered and tuned to the duty cycle of the system.

Water was delivered from, and returned to, the heat-exchange coil and pump using 16 mm ID wire reinforced plastic tubing (Plutone Bio, IPL, Italy) with appropriate 316 stainless steel and brass 25 mm threaded (BSPT[4]) fittings and valves. A brass sight glass flow indicator (SFI-100-3/4, W. E. Anderson, Indiana, United States) fitted to the outlet of the pump allowed for easy confirmation of water flow. A diaphragm pump (FL-22, Flomaster Pumps, Auckland, New Zealand) was used to prime the return seawater pipework prior to activating the main pump. A standard garden hose supply can be used for this function where available.

Power was supplied from a 240-V single-phase lead with interchangeable standard 'domestic' and 'caravan' three-pin plug fittings to account for the varied power supplies available at ports and marinas around New Zealand. A water-resistant fuse box, with individual fuses and residual cut-off protection distributed power to the various electrical components. Each electrical

[3] Hydraulic head or piezometric head is a specific measurement of liquid pressure above a geodetic datum.
[4] British Standard Pipe Taper

component was then wired through additional switches to provide failsafe protection. All electrical components were assembled and certified by registered electricians. The entire system was mounted on a custom-made sack trolley, enabling the system to be manoeuvred by a single operator when empty[5] or by two operators when full of water.[6]

Figure 6. The CRYPT heat delivery system 1: priming pump switch; 2: main pump switch; 3: priming and main pump over-ride switches; 4: fuse box with residual cut-out device; 5: cylinder element switch; 6: seawater delivery line with ball valve; 7: priming pump; 8: single- to three-phase variable drive unit; 9: cylinder filling valve; 10: main pump; 11: seawater return line; 12: heat-exchange coil outlet; 13: heat-exchange coil inlet; 14: adjustable thermostat; 15: sight glass flow indicator; 16: bypass valves for priming seawater return. Image: P. Cahill.

[5] Approximately 100 kg total weight
[6] Approximately 220 kg total weight

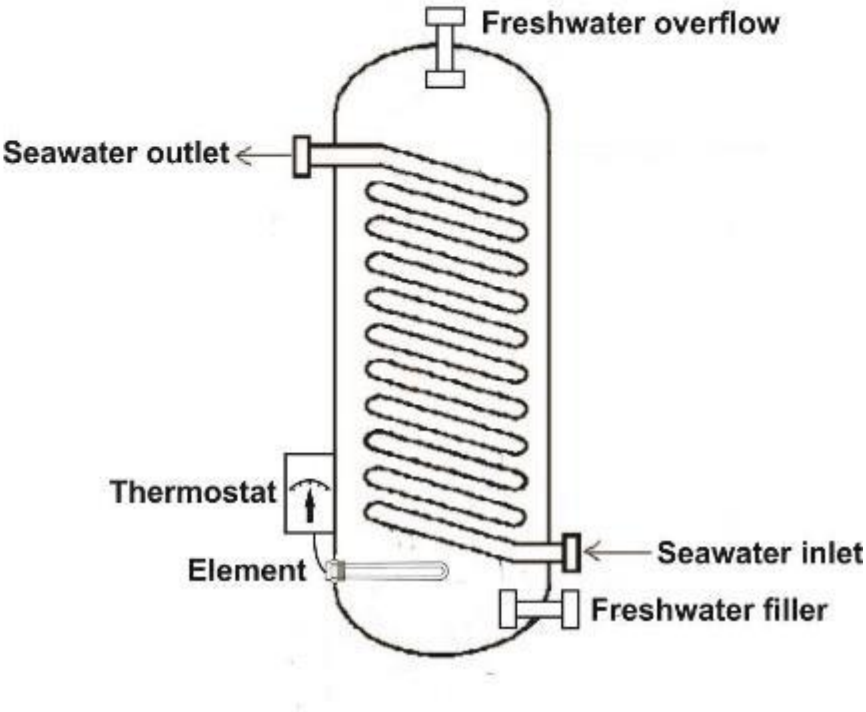

Figure 7. Fluid califont forming the basis of the CRYPT heat treatment system. Not drawn to scale.

'Expandable test plugs' were used to seal pipework systems being treated (Haron Plumbing, Haron International Pty. Ltd; 94 to 113 mm, 70 to 89 mm, and 37 to 53 mm). The test plugs were retrofitted with 15-mm BSPT threaded brass tubing central stems (the mild steel threaded tubing supplied with the plug rapidly corrodes in heated seawater), and the input on the end plate of each plug was drilled to the maximum allowable diameter of 16 mm to reduce restriction of water flow.

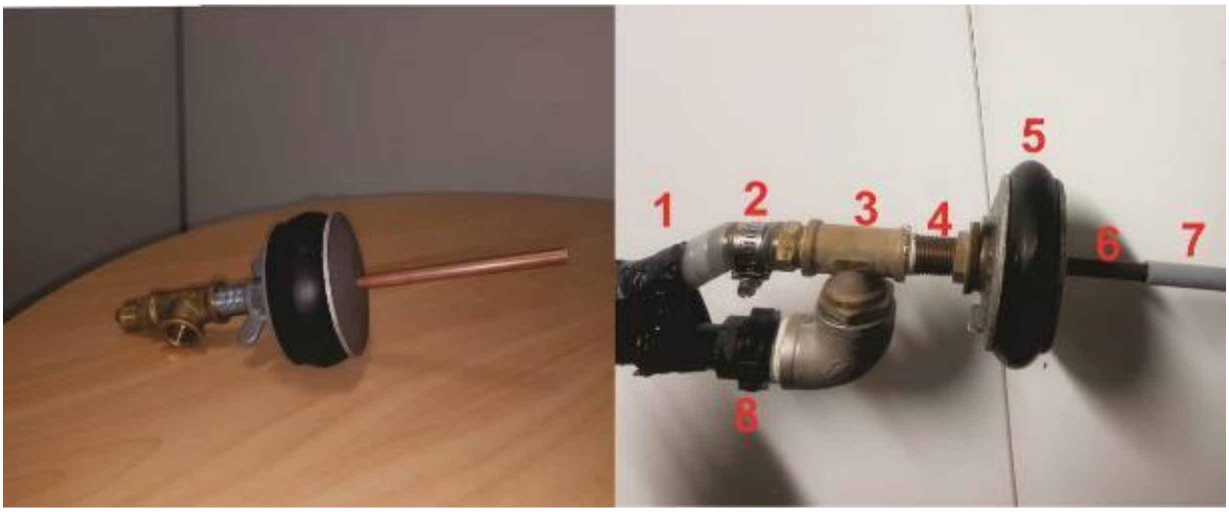

Figure 8. Counter-current plug to seal pipework inlets for recirculation of seawater via the CRYPT. 1: seawater delivery line; 2: 10 mm hose tail fitting; 3: 15 mm tee fitting; 4: brass nippling tube; 5: expandable test plug; 6: brass delivery tube; 7: Buteline™ delivery tube; 8: seawater return line. Image: P. Cahill.

The various sizes of expandable test plug are interchangeable to a custom-made counter-current hot water delivery and return fitting (Figure 8). The counter-current fitting was made from a 15-mm threaded BSPT brass tee with a 10-mm hose tail fitted centrally (interference fit) with a 9.7-mm brass tube that extends beyond the outlet of the test plug. Steel epoxy was packed in the back of the hose tail fitting to provide additional support to the brass tube. Different lengths of 12-mm Buteline™ tube (Buteline NZ Ltd, East Tamaki, New Zealand) were attached to the end of the brass tube to deliver heated water at specified distances into pipework systems. Water returned to the side tee of the counter-current fitting around the outside of the brass tube.

2.2.4 Experimental conditions

All experiments were performed in a controlled temperature laboratory (20 ± 1 °C), using filtered (0.15 µm) natural seawater to fill pipework and the outer tank of the MPS (Table 2). All treatments were independently replicated three times. Water temperatures inside the MPS were monitored continuously at all sampling ports for each experiment. For experimental runs involving sentinel organisms, oysters were placed inside sampling ports prior to initiating treatments. For the engine-cooling and below-water discharge MPS, each sampling port housed 10 individual 50-mm oysters. Because of the small diameter of pipework used in the ancillary seawater supply MPS, 10 individual 4-mm oysters were housed in each sampling port of this system. Experimental controls consisted of sampling ports identical to those in corresponding MPS that were filled with ambient temperature (20 ± 1 °C) seawater and 10 appropriately sized oysters, capped at all ends, and immersed in a water bath held at 20 ± 1 °C for exposure periods corresponding to the total treatment times (heating time + exposure time) being evaluated in the MPS. The ambient conditions used in the controls, and acclimation of experimental organisms (Section 2.2), correspond to average summertime seawater temperatures at relevant ports in northern New Zealand that receive recreational vessels[7]. Organisms were acclimated to these temperatures to simulate increased thermal tolerance due to prior acclimation to elevated temperatures (McMahon and Ussary, 1995).

Viability of sentinel organisms was validated: (a) immediately prior to heat treatment experiments; and (b) after being held in a recirculating seawater system for 24 h. Individuals were considered moribund if the shell was gaping and the organism did not respond to tapping of the shell or gentle prodding of the soft tissue. At the 24-h revaluation, decaying internal tissues provided additional evidence of mortality (Hopkins et al., 2016).

Table 2. Experimental conditions to establish heat treatment parameters using three mock pipework systems (MPS).

	Engine-cooling MPS	Ancillary seawater supply MPS	Below-water discharge MPS
Replicates	*n* = 3	*n* = 3	*n* = 3
Temperature monitoring	30 s intervals	30 s intervals	30 s intervals
Target temperature	60 ± 2 °C	60 ± 2 °C	60 ± 2 °C
Max. allowable temperature	65 °C	65 °C	65 °C
Exposure duration	60 min	60 min	60 min
Sampling ports (SP)	3	6	3
Sentinel organisms/SP (size)	10 (50 mm)	10 (4 mm)	10 (50 mm)

2.2.5 Experimental design

A tiered experimental approach was used to derive 'optimal' treatment parameters to be used for field testing (Chapter 3). A first round of experiments using the engine-cooling MPS defined effective treatment parameters, which were subsequently validated using the ancillary seawater supply and below-water discharge MPS (Figure 9). The engine-cooling MPS was selected to start the tiered experimental process because, of the three MPS, it accommodated the largest volume of seawater to be heated by the CRYPT. It also incorporated both unrestrictive (e.g., 100-mm straight pipework) and restrictive (e.g., mock heat exchange manifold) pipework features. It was deemed that a protocol developed with the engine-cooling MPS was more likely

[7] www.seatemperature.org/australia-pacific/new-zealand/

to transfer effectively to the smaller ancillary seawater supply and below-water discharge MPS than vice versa.

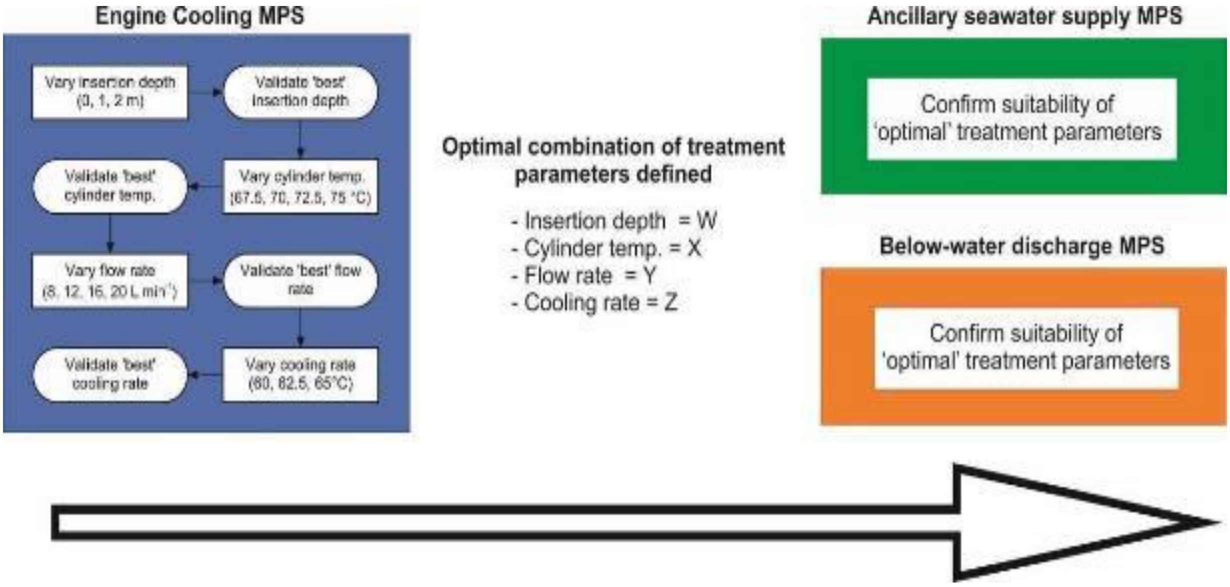

Figure 9. Tiered experimental approach to define, validate, and confirm a heat treatment protocol for three vessel mock pipework systems (MPS): engine-cooling, ancillary seawater supply, and below-water discharge. Initial 'variations' of treatment parameters monitored temperature only, while 'validation' and 'confirmation' treatments also assessed mortality of sentinel biofouling organisms after 1 h of exposure. All 'variations', 'validations', and 'confirmations' were replicated 3 times.

2.2.5.1 Defining effective treatment parameters

An effective treatment protocol must ensure that the target temperature is achieved and maintained at the 'high risk' locations within an MPS for a defined period. Different levels of each of four factors were evaluated using the engine-cooling MPS without the presence of sentinel organisms in the sampling ports (*n* = 3; Table 2). The optimal level of each factor was subsequently validated with sentinel organisms (Figure 9). This approach allowed for finetuning of treatment parameters without unnecessary use of sentinel organisms. Treatment variables were evaluated in the following order:

- delivery tube insertion depth;
- cylinder temperature setting;
- recirculation flow rate through the heat exchange coil; and
- rate of cooling once the CRYPT had been deactivated.

To evaluate insertion depths, the terminus of the delivery tube was placed at: (a) the expandable test plug; (b) the raw-water strainer (first bend in the system); or (c) the start of the engine heat exchanger. The cylinder temperature settings centred on the target temperature zone in the pipework of 58 to 65 °C, while differentially accounting for loss of heat along the delivery and return lines. The flow rates evaluated spanned the capacity of the pump and drive unit used. Cooling rates for various preheat temperatures were assessed to potentially allow pipework systems to be brought to target temperature, capped, and left in a static condition for allotted exposure periods.

At the commencement of each treatment, the CRYPT was activated and the temperature at sampling ports in the MPS left to reach 60 ± 2 °C. Once target temperatures had been achieved, the system was left to maintain this temperature for the allotted exposure period of 60 min. This exposure period provides a large treatment buffer to ensure lethal exposure to all macrofouling organisms based on the knowledge that *M. gigas* is rendered non-viable within 30 min of exposure to 60 °C (Piola and Hopkins, 2012). Because temperatures stabilised (≤ 1 °C change over 5 min) after the first 30 to 60 min of seawater recirculation in the first round of experiments assessing insertion depth, subsequent cylinder temperature setting and flow rate experiments focused on the initial heating phase of treatments. Specifically, temperatures were stabilised at ‘high risk’ sampling locations and then monitored for ~ 15 min, rather than the full 60-min treatment period. The cooling rate experiments monitored the temperature of the preheated pipework until values dropped below 58 °C at ‘high risk’ sampling locations, or for a maximum of 60 min. Default parameters (i.e., parameters of the factors not being tested at the time) were based on the outcomes of preceding experiments where possible (Table 3).

2.2.5.2 Validating effective treatment parameters

The optimal combination of treatment parameters determined from the experiments detailed in Section 2.2.5.1 were subsequently confirmed for the ancillary seawater supply and below-water discharge MPS. In both instances, sampling ports contained sentinel organisms for three replicate iterations of an optimal combination of treatment parameters (n = 3). The treatment parameters were:

- insertion to the first bend in the system (0.5 and 1 m, respectively);
- cylinder thermostat set to 72.5 °C;
- main pump flow rate set to 20 L min^{-1}; and
- no cooling step.

An additional flow-through step was also trialled for the ancillary seawater supply system to heat further into these smaller diameter pipework systems. With the main pump activated (i.e., system proceeding under recirculation), the CRYPT’s priming mechanism was used to introduce additional seawater to the recirculation system by opening the priming tap and activating the priming pump[8]. Each terminal end of the ancillary seawater supply MPS was opened in succession, resulting in heated water being rapidly driven to terminal sampling ports. Once temperatures had stabilised, the terminal sampling ports were recapped and the CRYPT priming mechanism deactivated. Temperatures were monitored for the subsequent 60 min.

2.2.6 Data analysis

Temperatures at the sampling port were recorded using PicoLog software (Pico Technology, St Neots, United Kingdom). Temperature data for each treatment variation were averaged across the three replicates and plotted against time for each sampling port. The following variables with 95% confidence intervals (CI) were also calculated for each sampling port:

- mean time to reach 58 °C (i.e., minimum acceptable target temperature);
- mean time to reach 60 °C (i.e., ideal target temperature);
- minimum temperature observed across all three of the replicate treatment runs once 60 °C had first been achieved (i.e., to ensure temperatures remain in target zone);
- maximum temperature observed across all three of the replicate treatment runs once 60 °C had first been achieved (i.e., to ensure 65 °C is not exceeded); and
- mean ± 95% confidence interval of temperature once 60 °C had first been achieved (i.e., a measure of variation in treatment temperature at each sampling port).

For validation and confirmation experiments, treatments were considered effective if sentinel organism mortality was 100% at all ‘high risk’ sampling ports. Sentinel oyster mortality at each MPS and control sampling port was calculated as a percentage:

Mortality = (number of dead oysters / 10) × 100

[8] Alternatively, freshwater from reticulated supply can be used when available

Table 3. Experimental factors evaluated for the CRYPT heat delivery system applied to a mock engine-cooling pipework system.

Factor	Levels	Rationale	Default parameters
Delivery tube insertion depth (m)	0, 1, 2	Depth that delivery tube can be inserted will vary among and within vessels. *Define minimum insertion depth required to treat all 'high risk' portions of pipework.*	Cylinder temperature setting: 70 °C Flow rate: 12 L min^{-1} Cooling post-recirculation: none
Cylinder temperature setting (°C)	67.5, 70, 72.5, 75	Higher cylinder temperatures should increase the rate of heating but exceeding thresholds could result in overheating pipework. *Define efficient setting that will not result in overheating (> 65 °C).*	Delivery tube insertion depth: 1 m Flow rate: 12 L min^{-1} Cooling post-recirculation: none
Recirculation flow rate (L min^{-1})	8, 12, 16, 20	Flow rate of seawater through the system should affect heat distribution in vessel pipework and rate of cooling of the cylinder. Excessive flow (i.e., exceeding duty cycle of system) can damage pumps. *Define flow rate that efficiently heats vessel pipework but will not overwhelm heating capacity of cylinder or damage the pump.*	Delivery tube insertion depth: 1 m Cylinder temperature setting: 72.5 °C Cooling post-recirculation: none
Cooling post-recirculation (°C)	Preheat to 60, 62.5, 65	If preheated pipework maintains effective treatment temperatures (60 ± 2 °C) for extended periods, operational efficiencies would improve because operators could 'cap' the system prior to 60 min of recirculation. *Define rate of cooling from set preheat temperatures.*	Delivery tube insertion depth: 1 m Cylinder temperature setting: 72.5 °C Flow rate: 20 L min^{-1}

2.3 RESULTS

2.3.1 Engine-cooling MPS

2.3.1.1 Delivery tube insertion depth

All three of the delivery tube insertion depths examined resulted in effective treatment temperatures at ‘high risk’ sampling ports, with tight temperature control after the initial heating phase (Figure 10). The rate of heating did differ with delivery tube insertion depth for the various sampling ports. Sampling ports nearest to the terminus of the delivery tube recorded quicker rates of heating and higher maximum temperatures. For example, the time to reach 60 °C for the pre-heat exchanger (pre-HE) sampling port decreased from 88 min using the minimum delivery tube insertion depth (0 m) to 48 min when the delivery tube was fully inserted to the start of the mock heat exchanger (2 m). This relationship was reversed (35.5 min versus 63.7 min) for the input sampling port because the minimum insertion depth placed the terminus of the delivery tube at the input sampling port. Although the ‘low risk’ sampling port following the mock heat exchanger did not reach the target treatment zone, temperatures increased steadily over time. The 1 m and 2 m insertion depths raised temperatures after the heat exchanger to maximum observed values of 35.6 °C and 39.3 ° C, respectively (Table 5).

Treatment temperatures increased more slowly after the first ~ 10 to 20 min, with up to 30 min required to raise temperatures at the pre-HE sampling port from 58 to 60 °C (Figure 10). This slower rate of increase once temperatures approached the target zone was mirrored by a drop in the temperature of the cylinder (Figure 11a). The temperature in the cylinder drops once the thermal mass of the freshwater inside the cylinder has been overcome by cooling of the heat-exchange coil. Temperatures drop to the lower limit of the thermostat, which activates the element and regulates the temperature back towards the set point. The result is tight control of temperature in the pipework being treated. Once 60 °C had been achieved in the pipework, the 95% CI for temperature fluctuation was 0.1 to 0.2 °C in all instances for the remainder of the treatment application (Table 5). Importantly, temperatures never exceeded 65 °C at any of the sampling ports or at the return line (Figure 11b).

There was an unexpected drop in temperature around 55 min for the 2 m insertion depth experiments due to an airlock of the system during one replicate run. This airlock was subsequently cleared and the temperature returned quickly to the target zone (Figure 10). These data are presented graphically (Figure 10) to highlight this potential problem but have been removed from subsequent analysis.

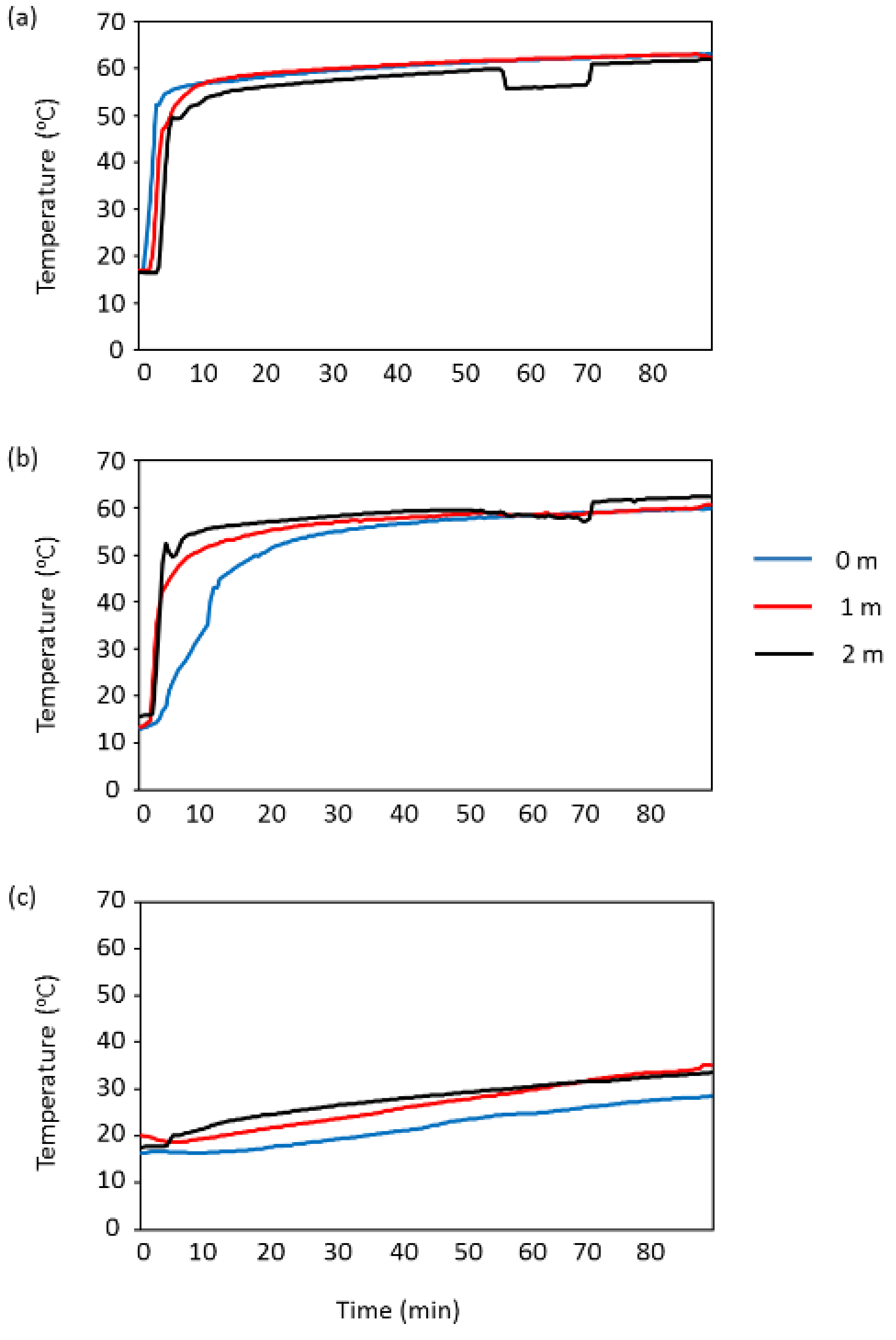

Figure 10. (a) Input, (b) pre-heat exchanger (HE) and (c) post-HE temperatures for variations of delivery tube insertion depth. Values are means (*n* = 3).

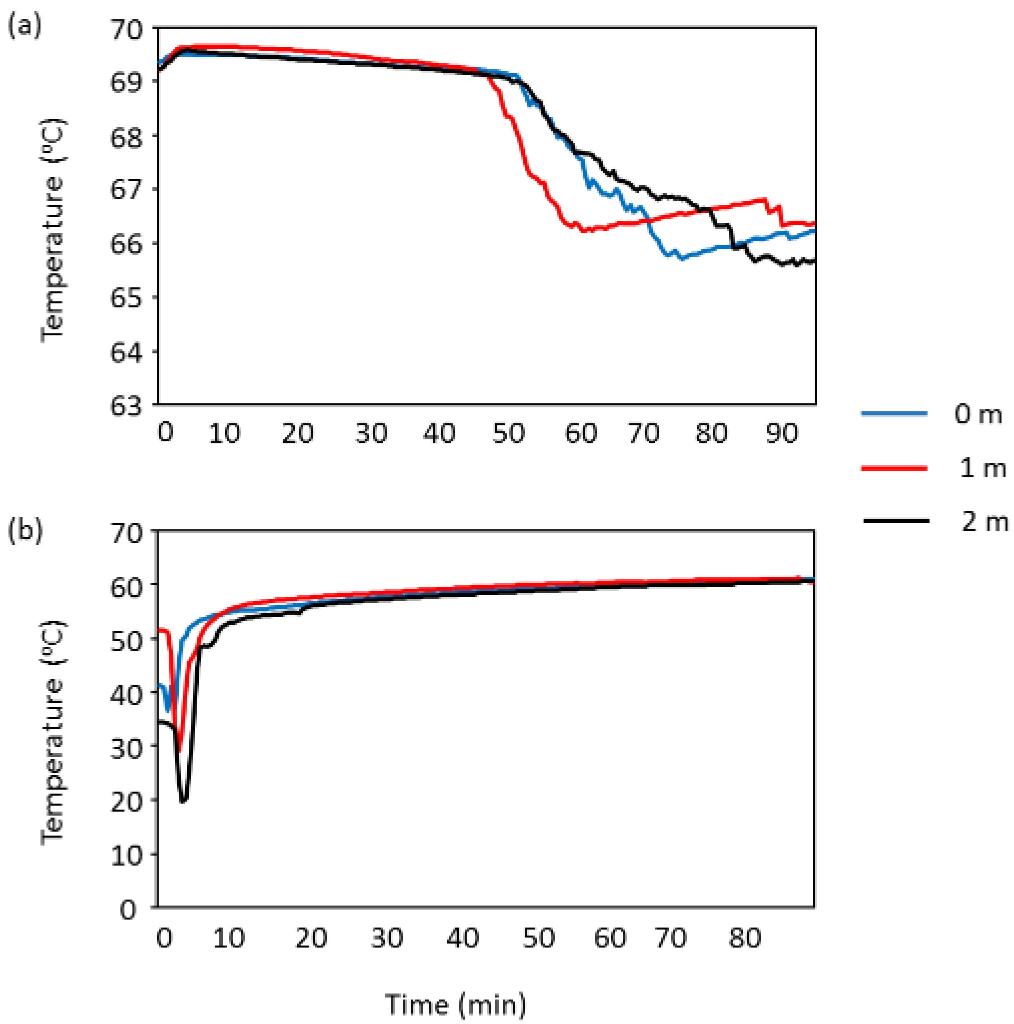

Figure 11. (a) Hot water cylinder and (b) seawater return line water temperatures for variations of delivery tube insertion depth. Values are means (*n* = 3).

The 1 m insertion depth was progressed to the validation experiment using sentinel oysters. An additional temperature sensor was included at the raw-water strainer (RS) sampling port to measure temperatures directly at the outlet of the delivery tube for the experiments going forward. As with the preliminary experiments, temperatures increased rapidly to around 58 °C, and then increased at a slower rate before reaching the optimal target temperature of 60 °C across the 'high risk' sampling ports (Figure 12). However, relative rates of heating were slower than observed for the initial 1 m delivery tube insertion depth experiments (Table 5). For example, time to reach 60 °C increased from 30.8 min in the initial 1 m insertion depth experiment to 62.5 min in the validation experiment.

Temperatures at all 'high risk' sampling ports were stable after 60 °C had been met and at no point exceeded 65 °C (Table 2). Sentinel oyster mortality was 100% at all 'high risk' sampling ports. Some mortality was observed for oysters from the 'low risk' post-HE sampling port after 24 h of recovery (37 ± 34%). These results reflect mortality in one replicate only, which had a maximum observed temperature of 39.3 °C. The average maximum observed temperature across all three replicates was 32.5 °C (Table 5).

The temperature profile of the seawater return line is worth noting. Once temperatures had stabilised, the average temperature of the return line (60.7 ± 0.1 °C) was within ± 2 °C of temperatures recorded at the 'high risk' sampling ports (Table 5). The average return line temperature was 0.5 °C higher than that of the pre-HE sampling port (60.2 ± 0.1 °C) and 1.5 °C lower than that of the RS sampling port, which were consistently the coolest and hottest 'high risk' sampling ports, respectively.

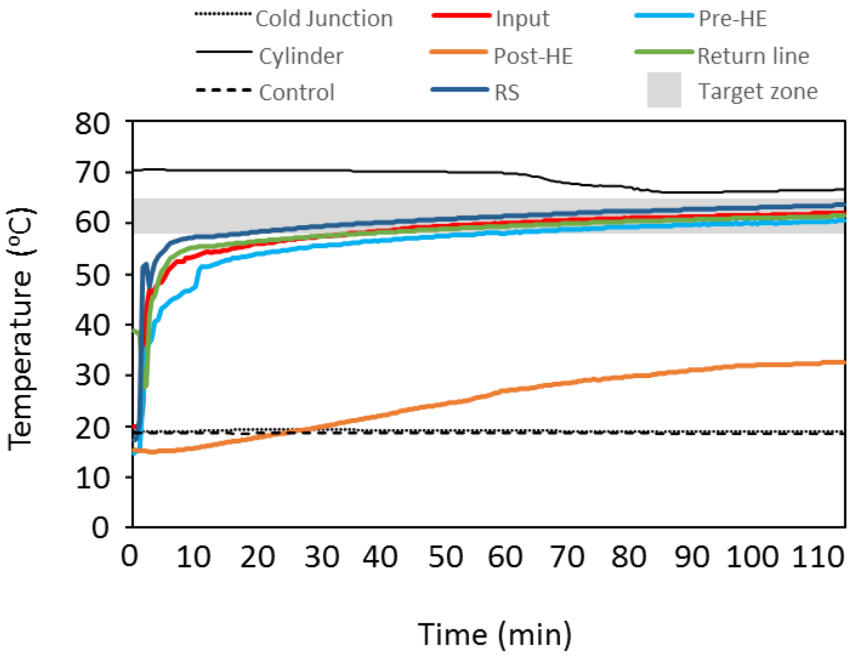

Figure 12. Temperatures recorded at sampling ports throughout an engine-cooling pipework system during validation experiments using an optimal delivery tube insertion depth of 1m. Values are means (*n* = 3).

2.3.1.2 Cylinder temperature setting

The relative rate of heating increased with cylinder temperature setting for all sampling ports (Figure 13). Setting the cylinder thermostat to 67.5 °C was insufficient to achieve target treatment temperatures throughout all sampling ports within the MPS, with 58 °C met at the input and RS sampling ports only (Table 5). In contrast, the highest temperature setting of 75 °C resulted in rapid overheating of pipework; temperatures exceeding 65 °C were observed at the RS sampling port within 1 min of activating the system. Experiments using the 75 °C temperature setting were aborted shortly after initiation to prevent harm to the experimental apparatus.

Temperature settings of 70 and 72.5 °C both resulted in temperatures within the targeted treatment range at all 'high risk' sampling ports. However, the higher temperature setting of 72.5 °C achieved the target temperatures within shorter periods of time (Figure 13). At the pre-HE sampling port, 60 °C was recorded after 51 min with the 72.5 °C temperature setting versus 87 min with the 70 °C temperature setting (Table 5). The actual temperature of the cylinder varied up to almost 2 °C from the value set at the thermostat (Figure 14), reflecting the ± 2 °C trigger limits of the thermostat.

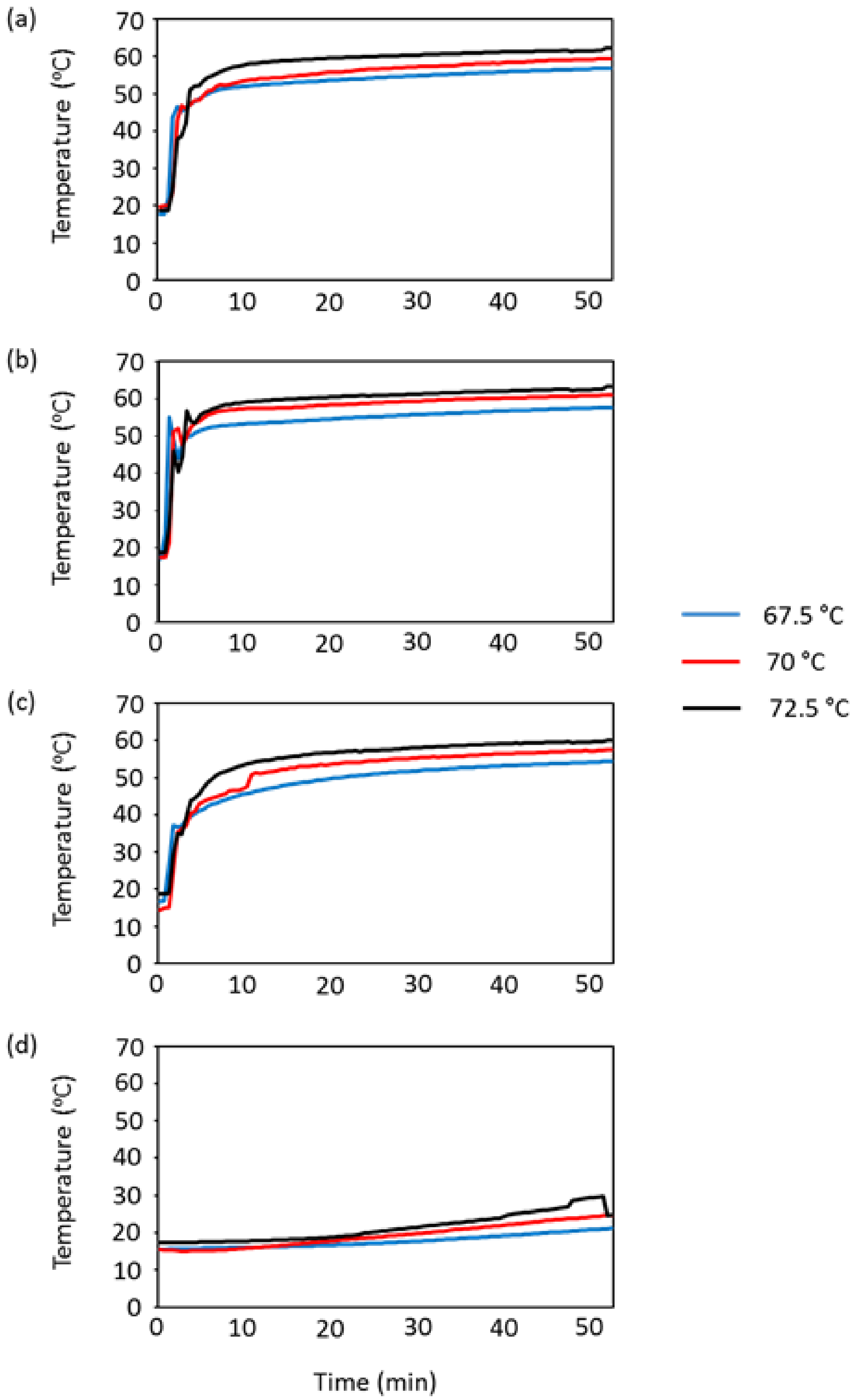

Figure 13. (a) Input, (b) raw-water strainer, (c) pre-heat exchange (HE) and (d) post-HE temperatures following variations of cylinder temperature setting. Values are means (*n* = 3).

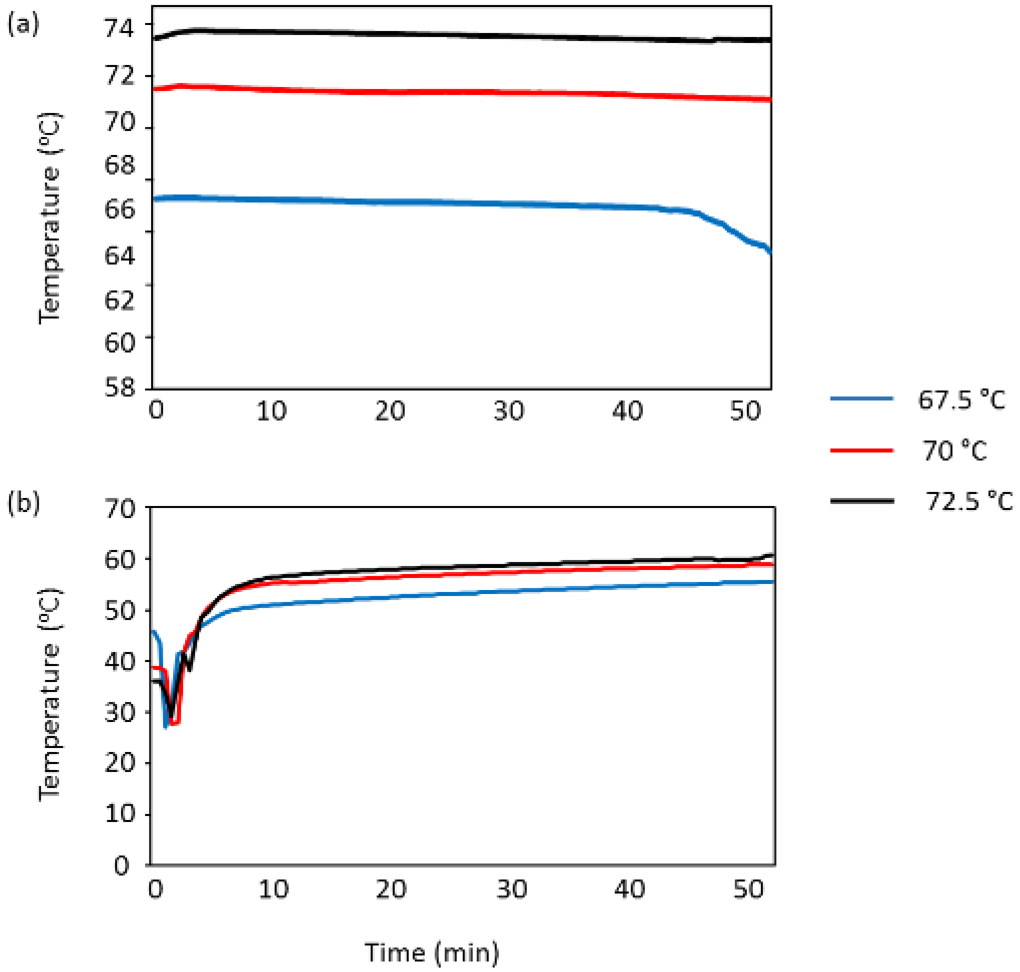

Figure 14. (a) Hot water cylinder and (b) seawater return line water temperatures following variations of cylinder temperature setting. Values are means (*n* = 3).

When validated for the full 60 min treatment period, the 72.5 °C temperature setting achieved 60 °C at all 'high risk' sampling ports for the prescribed treatment time of 60 min (Table 5, Figure 15). Although the addition of oysters to sampling ports slowed heating rates relative to the initial experimentation (Table 5), the 72.5 °C temperature setting resulted in a higher maximum observed temperature at the post-HE sampling port of 39.3 °C (Figure 15) compared to 32.6 °C for the 70 °C temperature setting (Figure 12). The average return line temperatures were within ± 2 °C of the temperatures recorded at 'high risk' sampling ports (Figure 14b, Table 5).

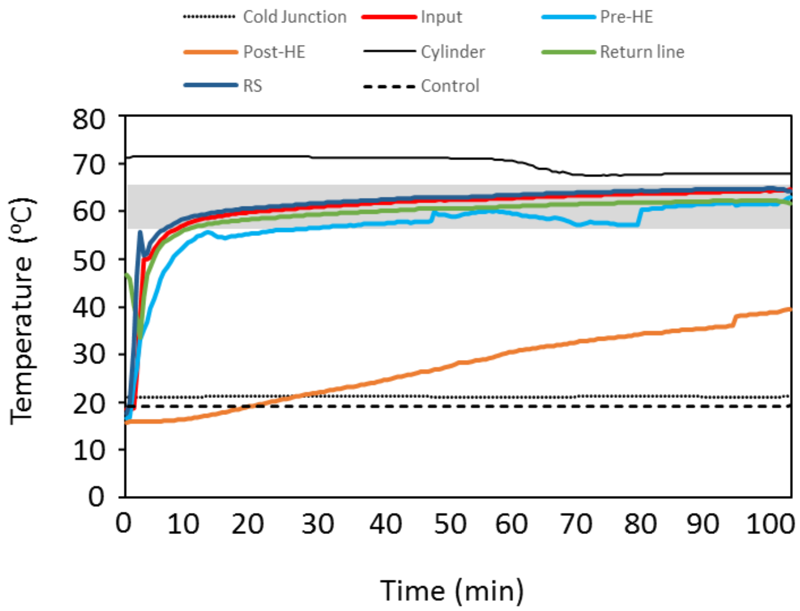

Figure 15. Temperatures recorded at sampling ports throughout an engine-cooling pipework system during validation experiments using an optimal cylinder temperature setting of 72.5 °C. Values are means (*n* = 3).

2.3.1.3 Recirculation flow rate

Higher flow rates increased the rates of heating observed throughout the MPS (Figure 16). Times to achieve 60 °C was more than halved for the highest flow rate of 20 L min^{-1} versus the lowest flow rate of 8 L min^{-1} (Table 5). Although the initial heating phase was shortened, temperatures stabilised at similar values for the four flow rates and maximum observed temperatures were consistent across flow rates (Figure 16, Table 5). Higher flow rates more evenly distributed heat throughout the MPS, with time to reach target temperatures, and the variation once temperatures had stabilised, differing less among sampling ports as flow rate increased. Again, temperatures observed at the return line lagged those observed within the MPS by approximately 2 °C (Figure 17, Table 5).

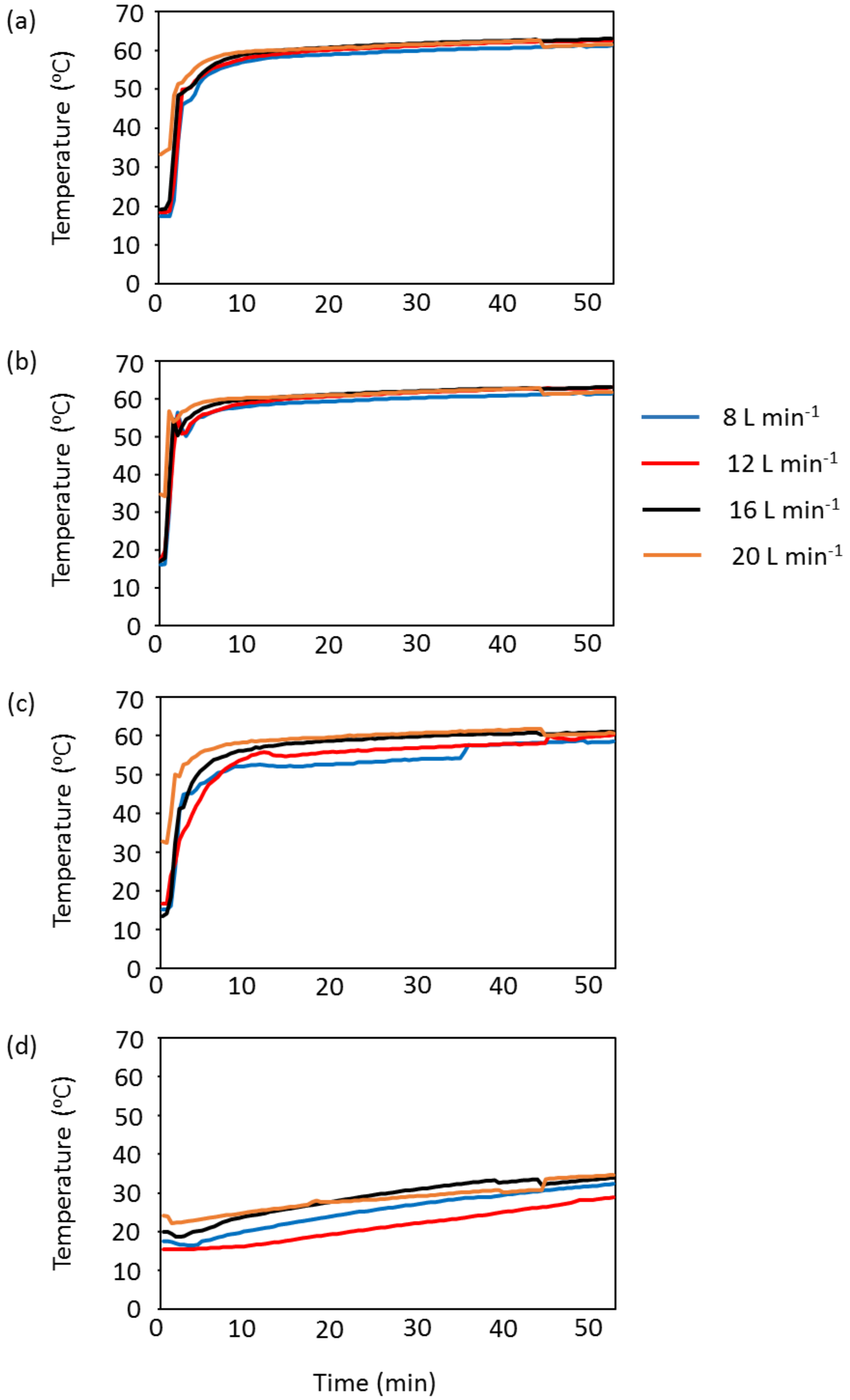

Figure 16. (a) Input, (b) raw-water strainer, (c) pre-heat exchange (HE) and (d) post-HE temperatures following variations of recirculation flow rate. Values are means (*n* = 3).

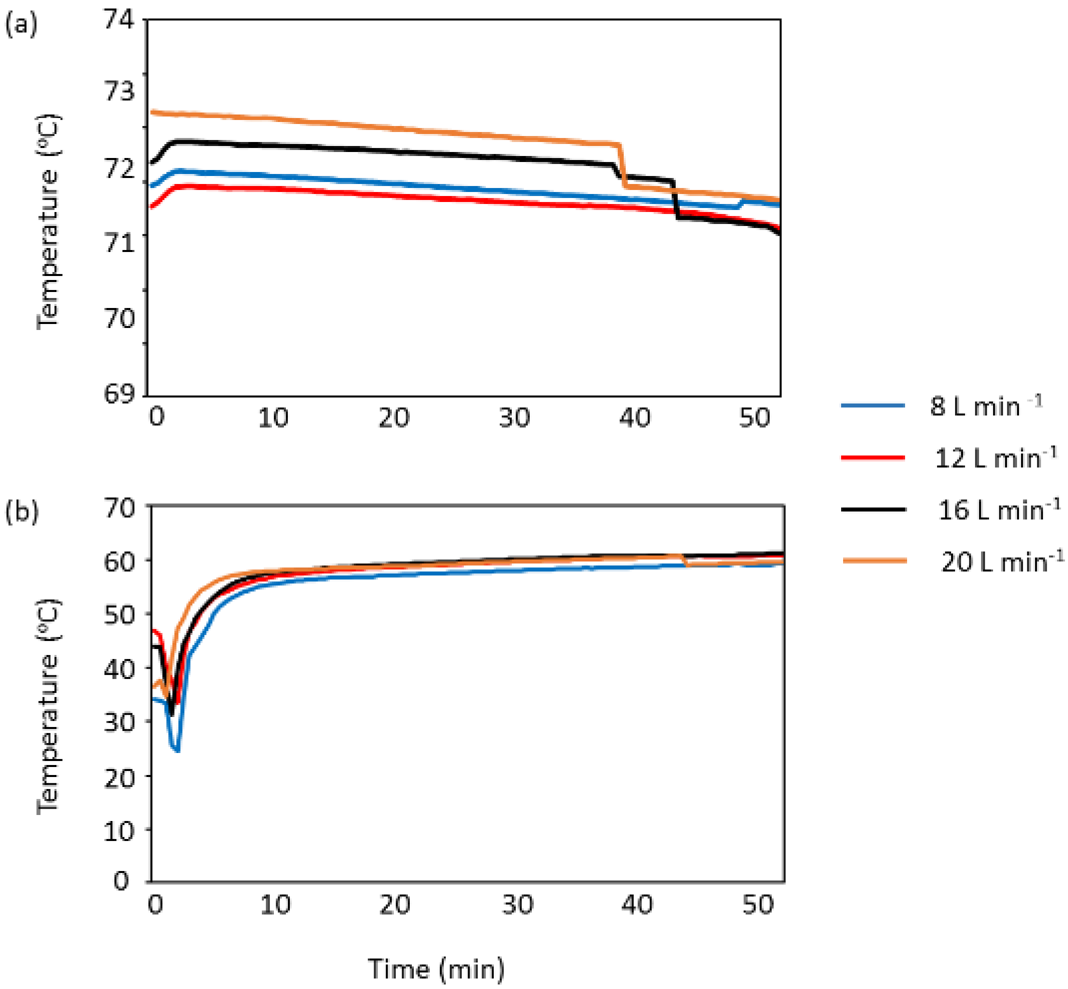

Figure 17. (a) Hot water cylinder and (b) seawater return line water temperatures following variations of recirculation flow rate. Values are means (*n* = 3).

When the highest flow rate of 20 L min^{-1} was validated for the full treatment period of 60 min, temperatures at all 'high risk' sampling ports stabilised within the prescribed treatment zone after a maximum of 26.5 min (Table 5, Figure 18). The increased flow rate reduced the disparities in heating rates observed between the initial experiments and the validation experiment. For example, time to reach 60 °C at the input sampling point increased by only 4.5 min with the 20 L min^{-1} flow rate, compared to a more than 30 min increase seen for the insertion depth validation (Table 5). As seen in the initial flow rate experiments, temperature variation among sampling ports was also reduced with the higher recirculation flow rate (Figure 18) when compared to the validation experiment performed for cylinder temperature setting (Figure 15).

Temperatures were confined within the target range of 58 to 65 °C, and the maximum temperature observed at the post-HE sampling port was 38.7 °C (Table 5). These exposure conditions led to 100% mortality of sentinel oysters at all 'high risk' sampling ports. Temperatures at the post-HE sampling port had increased to close to 40 °C after the treatment period of 60 min (Figure 18), with corresponding partial mortality of the sentinel oysters (7 ± 5% at 0 h; 37 ± 30% at 24 h).

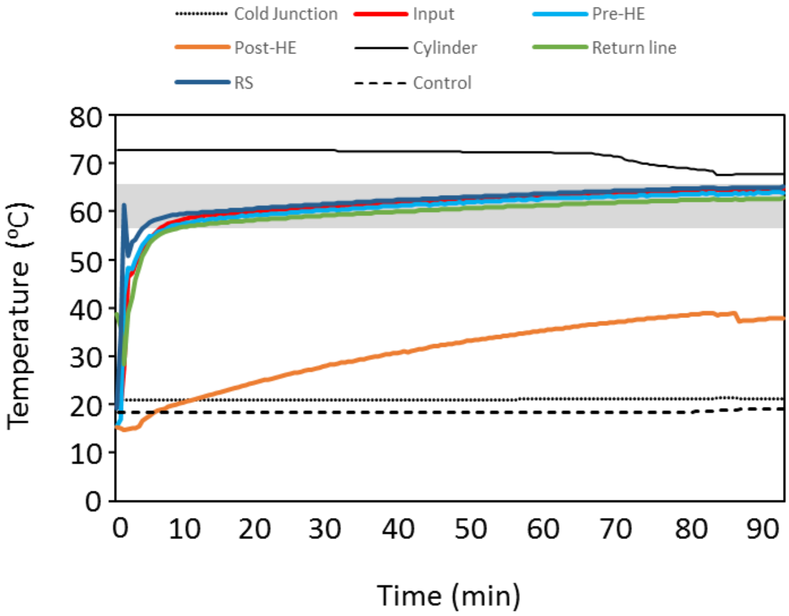

Figure 18. Temperatures recorded at sampling ports throughout an engine-cooling pipework system during a validation experiment using a recirculation flow rate of 20 L min^{-1}. Values are means (n = 3).

2.3.1.4 Cooling rate post-recirculation

Deactivating the CRYPT once 65 °C had been achieved resulted in a maximum time to cool to 58 °C (lower treatment limit) of 27.5 ± 1.1 min (Table 4). Deactivating the CRYPT once 62.5 °C has been achieved afforded up to 20 min of leeway until temperatures fell below the target zone (Table 4). Decreasing the preheat temperature to 60 °C further reduced leeway to approximately 10 min (Table 4).

Table 4. Cooling dynamics for a mock engine-cooling system preheated to specified levels using the CRYPT system.

Preheat temp.	Sampling port	Time to 60 °C (min ± 95%)	Time to 58 °C (min ± 95%)
60 °C	Input	3.5 ± 1.5	7.8 ± 0.9
	Pre-HE	3.2 ± 2.0	11.3 ± 1.4
	Return line	< 0.5	2.1 ± 1.4
	RS	2.3 ± 1.3	8.8 ± 1.7
62.5 °C	Input	7.0 ± 2.0	13.5 ± 2.5
	Pre-HE	9.8 ± 2.1	17.0 ± 0.6
	Return line	2.3 ± 1.2	4.8 ± 1.2
	RS	7.8 ± 2.6	15.2 ± 2.8
65 °C	Input	15.0 ± 0.6	22.3 ± 1.2
	Pre-HE	18.8 ± 0.9	27.5 ± 1.1
	Return line	5.0 ± 0.6	7.0 ± 0.6
	RS	18.0 ± 1.1	26.7 ± 1.4

HE: heat exchanger, RS: raw-water strainer

The validation experiment deactivated the CRYPT once 62.5 °C had been achieved at all 'high risk' sampling ports, with a subsequent cooling step of approximately 20 min. Previous experiments demonstrated that the CRYPT requires approximately 40 min to heat the engine-cooling MPS from 60 to 62.5 °C (Figure 18), which corresponds well with a cooling step of 20 min. Once the CRYPT had been deactivated, temperatures decreased steadily to reach a mean of 53.1 °C at the input, 56.8 °C at the pre-HE, and 58.6 °C at the RS sampling port after 20 min (Table 5).

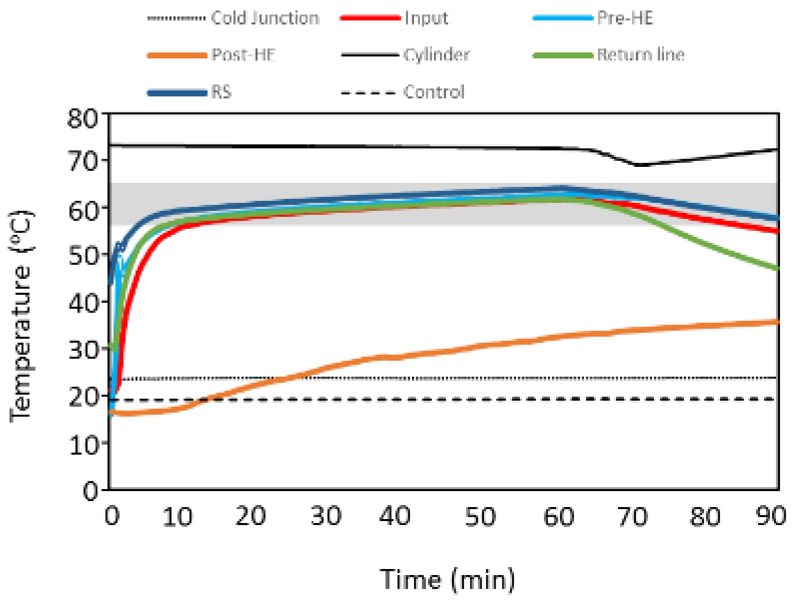

Figure 19. Temperatures recorded at sampling ports throughout an engine-cooling pipework system during a validation experiment with a cooling phase of 20 min post-heating to 62.5 °C. Values are means (*n* = 3).

Table 5. Heating dynamics and oyster mortality within a mock engine-cooling pipework system for select parameter variations of the CRYPT heat treatment system.

			Mean time to reach		After 60 °C was reached			Percent oyster mortality	
			58 °C (min)	60 °C (min)	Minimum (°C)	Maximum (°C)	Mean (°C ± 95%)	0 h (± SD)	24 h (± SD)
Delivery tube insertion depth	0 m	Input	17.2	35.5	60	64.9	62.3 ± 0.1	-	-
		Pre-HE	58.0	88.2	60.1	62.5	61.4 ± 0.1	-	-
		Return line	34.7	63.8	60	62.6	61.1 ± 0.1	-	-
		Post-HE	-	-	-	31.6*	-	-	-
	1 m	Input	13.8	30.8	60	63.6	61.9 ± 0.1	-	-
		Pre-HE	41.7	86.2	60	61.6	60.4 ± 0.2	-	-
		Return line	25.5	58.5	59.7	61.9	60.8 ± 0.1	-	-
		Post-HE	-	-	-	35.6*	-	-	-
	2 m	Input	34.8	63.7	60.0	63.3	61.5 ± 0.1	-	-
		Pre-HE	27.7	48.0	60.0	63.7	61.9 ± 0.1	-	-
		Return line	38.5	72.3	59.8	61.1	60.6 ± 0.1	-	-
		Post-HE	-	-	-	39.3*	-	-	-
	Validation 1 m	Input	26.5	62.5	60.0	61.9	61.1 ± 0.1	100 ± 0	100 ± 0
		Pre-HE	61.5	87	60.0	60.3	60.2 ± 0.1	100 ± 0	100 ± 0
		Return line	38.5	73.5	60.0	61.4	60.7 ± 0.1	-	-
		Post-HE	-	-	-	32.6*	-	0 ± 0	37 ± 34
		RS	18	38.5	60.0	63.5	62.0 ± 0.1	-	-
		Control	-	-	-	-	18.8 ± 0.01	0 ± 0	0 ± 0
Cylinder temperature setting	67.5 °C	Input	66	-	-	-	-	-	-
		Pre-HE	-	-	-	-	-	-	-
		Return line	-	-	-	-	-	-	-
		Post-HE	-	-	-	26*	-	-	-
		RS	58.5	-	-	-	-	-	-
	70 °C	Input	36.5	62.5	60.0	61.9	61.1 ± 0.1	-	-
		Pre-HE	61.5	87	60.0	60.3	60.2 ± 0.1	-	-
		Return line	38.5	73.5	60.0	61.4	60.7 ± 0.1	-	-
		Post-HE	-	-	-	32.6*	-	-	-
		RS	18	38.5	60.0	63.5	62.0 ± 0.1	-	-
	72.5 °C	Input	21	23	60.0	62.4	61.1 ± 0.2	-	-
		Pre-HE	29	51	60.1	60.2	60 ± 0.1	-	-

			Time to reach		After 60 °C was reached			Percent oyster mortality	
			58 °C (min)	60 °C (min)	Minimum (°C)	Maximum (°C)	Mean (°C ± 95%)	0 h (± SD)	24 h (± SD)
Cylinder temperature setting	72.5 °C	Return line	21	51	60.6	60.7	60.6 ± 0.1	-	-
		Post-HE	-	-	-	24.7*	-	-	-
		RS	7.5	17	60.1	63.2	61.5 ± 0.2	-	-
	75 °C	Input	Exceeded 65 °C within 1 minute					-	-
		Pre-HE						-	-
		Return line						-	-
		Post-HE						-	-
		RS						-	-
	Validation 72.5 °C	Input	10.5	20.5	60.05	64.4	62.6 ± 0.2	100 ± 0	100 ± 0
		Pre-HE	45	53	58.4	63..0	59.9 ± 0.4	100 ± 0	100 ± 0
		Return line	15.5	35.5	60.0	62.4	62.4 ± 0.1	-	-
		Post-HE	-	-	-	39.3*	-	0 ± 0	20 ± 17
		RS	8	14	60.0	64.9	63.2 ± 0.2	-	-
		Control	-	-	-	-	19.3 ± 0.1	0 ± 0	0 ± 0
Recirculation flow rate	8 L min^{-1}	Input	12.5	30.5	60.0	62.9	61.2 ± 0.2	-	-
		Pre-HE	43	-	-	-	-	-	-
		Return line	31.5	59	60.5	60.7	60.6 ± 0.1	-	-
		Post-HE	-	-	-	36.5*	-	-	-
		RS	9.5	24	60.0	63.4	61.5 ± 0.2	-	-
	12 L min^{-1}	Input	10.5	20.5	60.05	64.4	62.6 ± 0.2	-	-
		Pre-HE	45	53	58.4	63.0	59.9 ± 0.4	-	-
		Return line	15.5	35.5	60.0	62.4	62.4 ± 0.1	-	-
		Post-HE	-	-	-	39.3	-	-	-
		RS	8	14	60.0	64.9	63.2 ± 0.2	-	-
	16 L min^{-1}	Input	8.5	15	60.0	63.6	62.2 ± 0.2	-	-
		Pre-HE	15	31.5	60.0	61.8	60.9 ± 0.1	-	-
		Return line	12.5	30.5	60.0	61.8	60.9 ± 0.1	-	-
		Post-HE	-	-	-	36.2*	-	-	-
		RS	5.5	20	60.1	64.0	62.5 ± 0.2	-	-
	20 L min^{-1}	Input	6.5	15	60.0	62.4	61.2 ± 0.1	-	-
		Pre-HE	8.5	23	60.0	61.7	60.8 ± 0.1	-	-
		Return line	11	36	59.4	60.5	59.8 ± 0.2	-	-

			Time to reach:		After 60 °C was reached:			Percent oyster mortality	
			58 °C (min)	60 °C (min)	Minimum (°C)	Maximum (°C)	Mean (°C ± 95%)	0 h (± SD)	24 h (± SD)
Recirculation flow rate	20 L min^{-1}	Post-HE	-	-	-	34.7 *	-	-	-
		RS	3.5	7.5	60.0	62.9	61.6 ± 0.2	-	-
	Validation 20 L min^{-1}	Input	8.5	19.5	60.0	64.6	62.8 ± 0.2	100 ± 0	100 ± 0
		Pre-HE	12	26.5	60.1	64.1	62.4 ± 0.2	100 ± 0	100 ± 0
		Return line	18	40	60.0	62.6	61.5 ± 0.2	-	-
		Post-HE	-	-	-	38.7*	-	7 ± 5	37 ± 30
		RS	5	14.5	60	64.9	63.1 ± 0.2	-	-
		Control	-		-	-	18.6 ± 0.1	0 ± 0	0 ± 0
Cooling rate post-recirculation	Preheat to 62.5 °C, then 15 min cooling	Input	17	23.5	53.1	62.1	56.8 ± 0.7	100 ± 0	100 ± 0
		Pre-HE	12.5	27	56.8	62.9	59.1 ± 0.5	100 ± 0	100 ± 0
		Return line	14	32	37.0	61.8	52.2 ± 1.2	-	-
		Post-HE	-	-	-	36.8*	-	0 ± 0	0 ± 0
		RS	5.5	14	58.6	64.0	59.7 ± 0.5	-	-
		Control	-	-	-	-	19.1 ± 0.01	0 ± 0	0 ± 0

HE: heat exchanger, RS: raw-water strainer, *: maximum temperature observed when 60 °C was not met.

2.3.2 Ancillary seawater supply MPS

When the treatment parameters optimised for the engine-cooling MPS were applied for confirmation with the ancillary seawater supply MPS, temperatures inside pipework reached the target treatment zone at the input sampling port within 2.5 min (Figure 20). Temperatures at the post-RS sampling port attained 58 °C after 68 min (Table 6), a relatively slow rate of heating compared to those achieved for the engine-cooling MPS. Further into the MPS, the manifold and terminal sampling ports experienced little or no change in temperature. Mortality of sentinel oysters was 100% at the input and post-RS sampling ports but all sentinel oysters survived further into the MPS. Given these suboptimal heating dynamics, flow-through followed by recirculation was also trialled for this MPS.

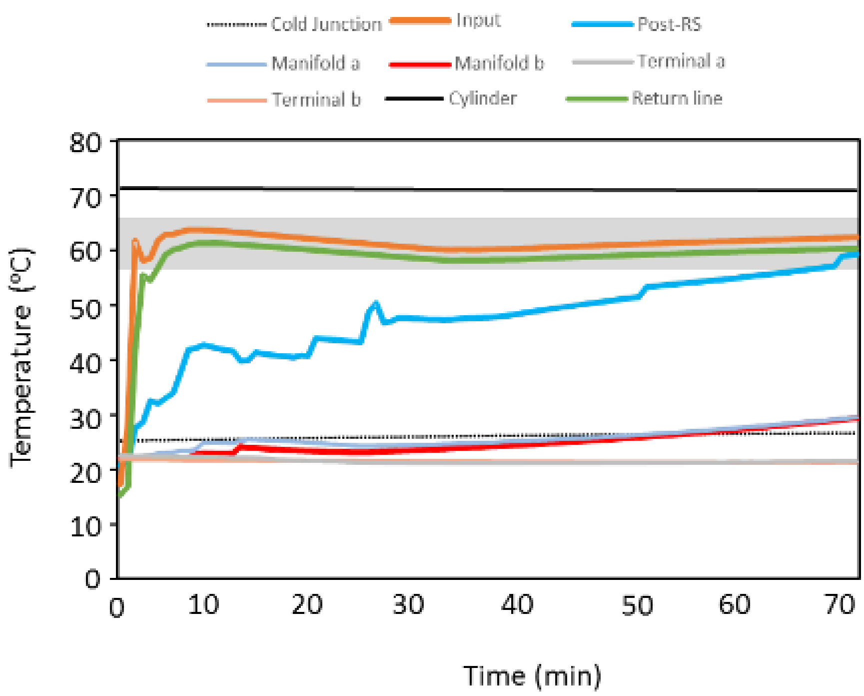

Figure 20. Temperatures recorded at sampling ports throughout an ancillary seawater supply system during a validation experiment using recirculation only. Values are means (*n* = 3).

Using flow-through, followed by recirculation, enhanced the capacity of the CRYPT to heat further into the ancillary seawater supply MPS. Temperatures reached 60 °C at the input, post-RS, and manifold sampling ports inside 5 min (Table 6). Subsequent temperature variation at the sampling ports was greater than seen for the engine-cooling MPS but temperatures were largely maintained within the target treatment zone (Figure 21). The terminal sampling ports neared the target treatment zone during the flow-through procedure, with maximum recorded values of 57.2 °C and 56.5 °C (Table 6). Once flow-through was completed, temperature stabilised at approximately 50 °C at the terminal sampling ports. These exposure conditions were sufficient to ensure 100% mortality of sentinel oysters at all sampling ports, including the terminal sampling ports.

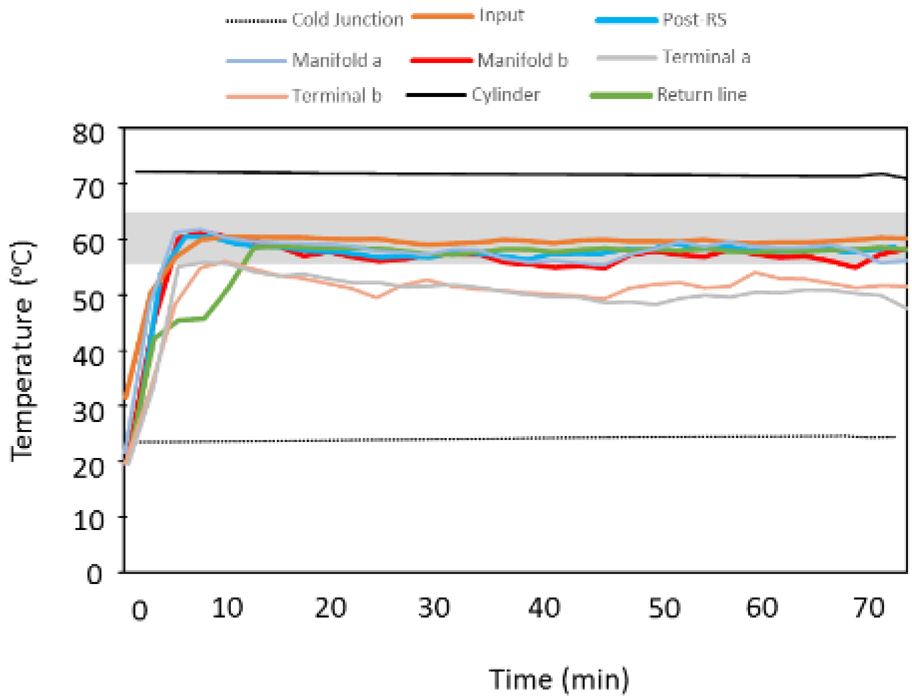

Figure 21. Temperatures recorded at sampling ports throughout an ancillary seawater supply system during a validation experiment using flow-through followed by recirculation. Values are means (*n* = 3).

2.3.3 Below-water discharge MPS

Operational parameters optimised for the engine-cooling MPS transferred easily to the below-water discharge MPS. Temperatures reached 58 °C at all sampling ports within approximately 20 min and were stable within the target treatment zone for the allotted treatment time of 60 min (Table 6, Figure 22). Correspondingly, mortality of sentinel oysters was 100% at the top, middle, and input sampling ports. The temperature of the return line was slightly lower than that of the top sampling port, which was consistently the coolest sampling port within the below-water discharge MPS.

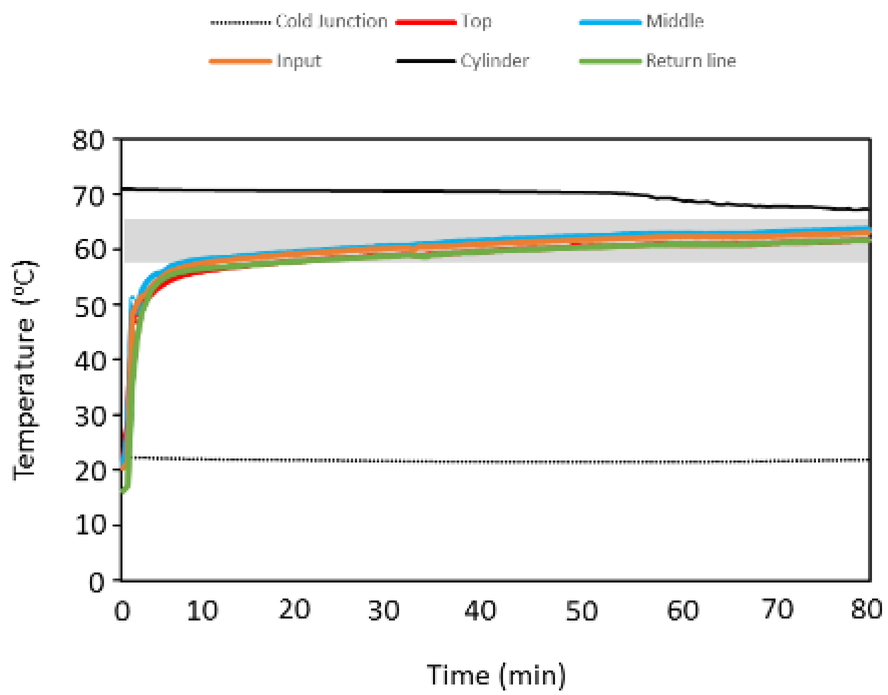

Figure 22. Temperatures recorded at sampling ports throughout a below-water discharge system during a validation experiment using an optimal combination of treatment parameters. Values are means (*n* = 3).

Table 6. Heating dynamics and associated oyster mortality within mock ancillary seawater supply and below-water discharge systems for optimised parameter variations of the CRYPT heat treatment system.

			Time to reach:		After 60 °C was reached:			Percent oyster mortality	
			58 °C (min)	**60 °C (min)**	**Minimum (°C)**	**Maximum (°C)**	**Mean (°C ± 95%)**	**0 h (± SD)**	**24 h (± SD)**
Ancillary Seawater Supply	Recirc. only	Input	2	2.5	58.3	63.6	61.4 ± 0.2	100 ± 0	100 ± 0
		Post-RS	68	-	-	-	-	100 ± 0	100 ± 0
		Manifold a	-	-	-	-	-	0 ± 0	0 ± 0
		Manifold b	-	-	-	-	-	0 ± 0	0 ± 0
		Terminal a	-	-	-	-	-	0 ± 0	0 ± 0
		Terminal b	-	-	-	-	-	0 ± 0	0 ± 0
		Return line	3	4	48.1	61.2	59.7 ± 0.2	-	-
		Control	-	-	-	-	20.4 ± 0.1	0 ± 0	0 ± 0
	Flow-through followed by recirc.	Input	5	6	59.9	62.2	59.9 ± 0.1	100 ± 0	100 ± 0
		Post-RS	3.5	4.5	56.1	61.1	58.1 ± 0.2	100 ± 0	100 ± 0
		Manifold a	3.5	4.5	53.8	62.4	58.1 ± 0.3	100 ± 0	100 ± 0
		Manifold b	4	4.5	51.6	61.4	57.3 ± 0.3	100 ± 0	100 ± 0
		Terminal a	-	-	-	57.2*	50.5 ± 0.8	100 ± 0	100 ± 0
		Terminal b	-	-	-	56.5*	51.6 ± 0.6	100 ± 0	100 ± 0
		Return line	10.5	16.1	56.6	60.2	58.0 ± 0.7	-	-
		Control	-	-	-	-	18.4 ± 0.1	0 ± 0	0 ± 0
Below-water discharge	Recirc. only	Input	10.5	25.5	60.0	63.1	61.8 ± 0.2	100 ± 0	100 ± 0
		Middle	7.5	22.5	60.0	63.7	62.2 ± 0.2	100 ± 0	100 ± 0
		Top	19.5	41.5	60.0	61.7	60.9 ± 0.1	100 ± 0	100 ± 0
		Return line	20.5	43.5	60.0	61.7	60.1 ± 0.1	-	-
		Control	-	-	-	-	19.6 ± 0.1	0 ± 0	0 ± 0

RS: raw-water strainer *: maximum temperature observed when 60 °C was not met.

2.4 DISCUSSION

The CRYPT heated 'high risk' portions of the engine-cooling MPS to the prescribed treatment temperature of 60 ± 2 °C, and maintained temperatures within this zone for 60 min without exceeding the maximum allowable temperature of 65 °C. Optimising insertion depth, cylinder temperature setting, recirculation flow rate, and cooling rate using the engine-cooling MPS decreased the initial time required to heat pipework to the target zone by almost threefold (from 87 to 27 min), and reduced subsequent temperature fluctuation during the treatment phase. The operational parameters optimised for the engine-cooling MPS transferred easily to the below-water discharge MPS, however the restrictive heating dynamics of the ancillary seawater supply MPS required a variation to procedures.

The optimisation and validation procedures are discussed separately in the following sections:

- engine-cooling MPS;
- ancillary seawater supply; and
- below-water discharge MPS.

2.4.1 Engine-cooling MPS

2.4.1.1 Insertion depth

All three of the insertion depths evaluated effectively heated 'high risk' portions of the engine-cooling MPS but the moderate insertion depth of 1 m was the most practicable and effective option. This insertion depth placed the terminus of the delivery tube at the first bend in the system, which is easily achievable when inserting the expandable test plug into the inlet of a pipework system. Inserting past the first bend is possible but requires more effort, and likely a multi-step procedure whereby the input valve is closed, the raw-water strainer opened, and the delivery tube inserted bidirectionally from the raw-water strainer. At best, inserting past the first bend results in only marginal improvements in heating efficiency and this additional effort is not warranted. In fact, the longer pathway for the water to return to the counter-current fitting means that the input sampling port takes longer to heat to effective treatment temperatures when the delivery tube is inserted past the first bend. Capacity to heat the initial portions of pipework systems is of high relative importance because biofouling is characteristically concentrated near pipework inlets or outlets (typically within the first 50 cm; Cahill et al., 2018).

There were discrepancies in heating rates between the initial experiment using the 1 m insertion depth and the subsequent validation using sentinel oysters. Time to reach target treatment temperatures at 'high risk' sampling ports almost doubled from approximately 30 to 60 min. Experimental conditions were consistent between these experiments, except that sampling ports contained sentinel oysters for the validation experiment. The addition of oysters to the sampling ports appears to act as a barrier to thermal exchange, with additional time required to heat sampling ports containing oysters. Regardless, oyster mortality was 100% at all 'high risk' sampling ports.

2.4.1.2 Cylinder temperature setting

Changing the cylinder temperature setting had impacts on both the rates of heating of pipework and the final temperatures achieved. Setting the cylinder at 67.5 °C resulted in gradual heating to temperatures well below the target treatment range, whilst a setting of 75 °C resulted in rapid overheating of pipework. The cylinder temperature setting of 72.5 °C struck a balance between efficient heating throughout the MPS with only minimal risk of overheating. This higher cylinder temperature setting, compared to the 70 °C used in the insertion depth experiments, also enhanced capacity to heat beyond restrictive pipework features. Temperatures beyond the mock engine heat exchanger reached maximum recorded values of 39.6 °C versus 32.3 °C in the insertion depth validation experiment. The enhanced capacity to heat beyond restrictive pipework features is a worthwhile advantage of the higher temperature setting. Biofouling is unlikely to be found downstream of engine heat exchangers but these results do highlight the difficulties inherent to heating beyond restrictive pipework features.

Maximum observed temperatures at sampling ports never exceeded 65 °C for the 72.5 °C temperature setting but they were consistently higher than for the 70 °C setting. Accordingly, there appears to be a slightly elevated risk of overheating pipework. This risk may be exacerbated for smaller volume pipework systems due to reduced thermal masses. Given the high diversity of pipework systems and environmental conditions that will be encountered operationally, real-time monitoring of temperatures is necessary to ensure safe operation of the CRYPT regardless of which cylinder temperature setting is used.

There was variation in the actual temperature recorded within the cylinder of up to ± 2 °C. This variation is within the range of acceptable temperatures for effective pipework treatment but it does increase the risk of overheating if cylinder temperature increases to the upper limit of the set point, or under heating if the temperature decreases to the lower limit of the set point. Accordingly, identifying temperature settings well within the optimal range is of high importance for a robust treatment protocol. As discussed, a setting of 72.5 °C strikes an appropriate balance of efficient heating with only minimal risk for damaging vessel pipework that can be controlled through real-time quality control monitoring.

2.4.1.3 Recirculation flow rate

Altering the recirculation flow rate resulted in further improvements to the overall efficiency of the CRYPT system. Rates of heating increased and there was less variation among sampling ports as flow rate increased, with the highest flow rate of 20 L min^{-1} having the best observed heating dynamics. Enhanced heat distribution associated with this higher flow rate is presumably due to greater mixing within the MPS, which improves the overall heating dynamics and reduces the risks of treatment ‘dead spots’ or overheating of pipework.

Unlike the preceding insertion depth and cylinder temperature experiments, heating rates did not deteriorate when sentinel oysters were included in sampling ports for the flow rate validation experiment. Intuitively, the higher flow rates should improve circulation of heated water in and around oysters at sampling ports. The higher flow rate also further improved capacity to heat beyond the restrictive mock engine heat exchanger, with a mean maximum temperature of 38.7 °C, which resulted in partial oyster mortality. The conditions at this ‘low risk’ sampling port appeared to be approaching the thermal tolerance of *M. gigas*, and all ‘high risk’ sampling ports recorded 100% mortality of sentinel oysters, demonstrating the suitability of the prescribed biosecurity treatment approach.

2.4.1.4 Cooling rate post-recirculation

The rate of cooling of pipework was measured to assess the possibility of a cooling step, whereby the CRYPT is deactivated once temperatures inside pipework reach a threshold value. Such an approach would effectively free the CRYPT to reheat the cylinder or be applied to another pipework system. Preheating to 60, 62.5, or 65 °C was trialled, with associated times to fall to the minimum treatment threshold (58 °C) of approximately 10, 20, or 27.5 min, respectively. Although the latter of these values appear to be useful timeframes at first glance, achieving 65 °C in pipework takes more than 60 min. Treatment parameters could conceivably be altered to rapidly achieve 65 °C in pipework but would run high risk of overheating. The highest deactivation threshold that can be realistically achieved in pipework within relevant treatment times is 62.5 °C.

When the 62.5 °C threshold for initiating a cooling step was validated with sentinel oysters, temperatures decreased steadily to reach values ranging from 53 to 59 °C at the 'high risk' sampling ports. Although sentinel organism mortality was still 100% at these sampling ports, the recorded temperature range extended below the prescribed minimum threshold of 58 °C. An inadvertent effect of the cooling step was to reduce the maximum temperature achieved at the post-HE sampling port from 39.3 to 36.8 °C (Table 5). It stands to reason that temperature increase post restrictive pipework features are curtailed once the CRYPT is deactivated.

It was deemed that the time savings of up to 20 min were insufficient to justify the inclusion of a cooling step, particularly due to temperatures at some 'high risk' sampling points falling below the prescribed minimum treatment threshold of 58 °C. It is also noted that the rate of cooling will be dependent on a range of external factors, including ambient temperature, pipework material, pipework surface area, and overall thermal mass. The experiments performed here were at a controlled ambient temperature of 20 ± 1 °C and used plastic pipework with relatively small surface area to volume ratio and correspondingly high thermal mass. The conditions likely to be encountered in the field (for example, small-diameter steel pipework or colder ambient water) are expected to increase the rate of cooling of pipework. Such circumstances pose unacceptable risk for a cooling step to jeopardise the efficacy of a treatment when temperatures fall well below effective treatment thresholds.

2.4.2 Ancillary seawater supply MPS

Using the operational parameters optimised for the engine-cooling MPS, the CRYPT struggled to heat past the raw-water strainer of the ancillary seawater supply MPS. This limitation is presumably due to restricted water movement associated with the small diameter pipework (25 mm ID), restrictive pipework features, and multiple bends in the system. Although the 'high risk' input sampling port was effectively treated, the capacity of the CRYPT to heat the ancillary seawater supply MPS using recirculation was generally underwhelming. However, the inability of actively recirculated water to penetrate further into this MPS suggests that biofouling organisms are unlikely to establish and survive in these areas as both are dependent on seawater exchange.

A modification was undertaken whereby seawater was initially flowed through each individual supply line to overcome thermal barriers within the ancillary seawater supply systems. This modification was tailored to suit the smaller diameter pipework used in this system. Sampling ports incorporated to a manifold beyond the raw-water strainer were effectively heated to the target treatment zone, but temperature fluctuations associated with small diameter pipework resulted in temperatures occasionally falling below the minimum threshold value of 58 °C. However, temperatures recorded at the manifold sampling ports were within the target treatment zone at over 80% of time points for the treatment period of 60 min, and sentinel oyster mortality was 100%. This variability increased the likelihood of conditions oscillating in and out of the target treatment zone. Temperatures recorded at the manifold sampling points were within the prescribed treatment zone for more than 80% of time points but they did occasionally fall below the minimum temperature threshold of 58 °C (Figure 21). This temperature variability likely results from a combination of restricted water movement and increased surface area to volume ratio associated with smaller diameter pipework. The former ought to add a degree of 'randomness' to how heat distributes within the pipework, while the latter will increase the rate at which heat escapes from the system.

Temperatures at the terminal sampling ports stabilised at around 50 °C. Even though the terminal sampling points are considered 'low risk' and did not meet the prescribed treatment threshold, sentinel oyster mortality was 100%. Cylinder temperature settings could conceivably be increased to ensure pipework temperatures never fall below 58 °C but this approach has a corresponding risk of periodically overheating pipework (> 65 °C). Because overheating pipework could have safety repercussions, it is preferable for temperatures to occasionally fall below 58 °C rather than exceed 65 °C. Heating capacity does diminish further into small diameter pipework systems but it can be generally concluded that a combination of flow-through and recirculation effectively treated 'high risk' portions of the ancillary sea water supply MPS.

2.4.3 Below-water discharge MPS

The operational parameters optimised for the engine-cooling MPS transferred easily to the below-water discharge MPS. Rates of heating were comparable to those recorded for the engine-cooling MPS, with approximately 20 min required to bring all sampling ports in the below-water discharge MPS to the target treatment temperature. Temperatures were subsequently well constrained within the allowable treatment zone of 58 to 65 °C and sentinel oyster mortality was 100% in all instances. The below-water discharge MPS was the simplest of the three MPS assessed, comprising a straight section of relatively large diameter pipe terminating at a vented loop. The ease of treating the below-water discharge system supports the argument that restricted recirculation dynamics are one of the primary hurdles for effective heat treatment.

2.4.4 General considerations

For any treatment approach, a reliable, real-time quality control measure is required to ensure that prescribed treatment temperatures are achieved and maintained. The laboratory experiments performed here suggest that monitoring the CRYPT return line temperature could be an operationally feasible quality control measure. Once the heating phase was completed, return line temperatures were consistently within 2 °C of the temperatures recorded at 'high risk' sampling ports for the engine-cooling, ancillary seawater supply, and below-water discharge MPS. Maintaining the return line temperature between 60 °C and 63 °C should ensure that pipework is maintained within the prescribed treatment zone, and this approach should be tested in the field. Stationing temperature probes throughout target pipework systems is an alternative option. It is considered that stationing multiple temperature probes is a generally cumbersome approach that may not be feasible in all circumstances (i.e., not all pipework systems adequately accessible).

The sentinel organism employed here, *M. gigas*, is the most heat resilient New Zealand macrofouling organism examined to date (Piola and Hopkins, 2012). It is possible that some tropical organisms are more resistant to heat than *M. gigas*. Tropical intertidal organisms that are exposed to extreme temperatures as part of their normal life history are an example of a group of organisms that may be particularly resilient to heat treatment. It is uncertain whether such intertidal organisms could ever establish within pipework systems but, to account for a range of possibilities, the current project has prescribed treatment exposure conditions of 60 ± 2 °C for 60 min in line with MPI recommendations (Growcott et al., 2017). This exposure exceeds the tolerance of *M. gigas* and, unsurprisingly, mortality was 100% when these conditions were met. Mortality was also observed at considerably lower temperatures achieved at some 'low risk' sampling ports. Exposure to approximately 40 °C for 60 min resulted in partial mortality, and treatments that reached ≥ 50 °C for considerably less than 60 min still achieved 100% mortality of *M. gigas*.

The laboratory experiments provided some evidence that biofouling itself can constrain heat distribution. The number and size of oysters housed in sampling ports corresponded to 'high' biofouling loads (i.e., sampling ports were filled almost to capacity with oysters) but natural biofouling communities with a range of biofouling organisms could affect heat distribution differently. Although 'high' biofouling loads are uncommon in pipework, field validation will directly assess the effects of natural fouling communities on heat distribution.

2.4.5 Conclusions

The CRYPT performed to expectation in the laboratory and is an adaptable system that can be adjusted to meet a range of potential scenarios. All 'high risk' areas of conservatively designed (i.e., complexity exceeding most systems likely to be encountered) engine-cooling, ancillary seawater supply, and below-water discharge MPS were effectively treated with the CRYPT apparatus.

Optimising operational parameters of the CRYPT using the engine-cooling MPS in the first instance resulted in noteworthy improvements to the efficiency of the system. The initial heating phase required to bring 'high risk' sampling ports to 60 ± 2 °C was reduced more than threefold (from 87 min to 27 min) between the first and final validation experiments performed with the engine-cooling MPS. The optimisation also improved temperature distribution within the MPS, resulting in more even heating of pipework features and associated biofouling organisms. The parameters optimised for the engine-cooling MPS transferred easily to the below-water discharge MPS; however, preliminary attempts to treat the ancillary seawater supply MPS were only partially effective. An additional flow-through step was developed, which resulted in efficient heating of 'high risk' features in this system.

A draft treatment protocol, health and safety guidance, and practical trouble-shooting information have been formulated based on the results of the laboratory optimisation (Appendix 2).

3 Field validation of a draft operational protocol

3.1 SCOPE AND OBJECTIVES

This chapter describes field validation of the draft treatment protocol developed from the results of the laboratory experiments (Appendix 2). Field validation allows for protocols to be fine-tuned and highlight any additional areas of research that may be required to yield a fully operational prototype.

Vessel pipework systems are highly confined areas that are generally poorly accessible. Operationally, it is not practical or possible to gain sufficient access to quantify biofouling viability without dismantling pipework systems. Although visual assessment of biofouling viability is achievable for accessible locations (e.g., hull surface) in clear water with sufficient light, such conditions are unlikely to be encountered within the pipework systems of vessels. With these limitations in mind, in-depth laboratory testing was prioritised to ensure accurate assessment of treatment efficacy that may be difficult to achieve in the field. The objective of the field experiments was to validate the draft operational treatment protocol developed from the laboratory experiments on a representative subset of actual recreational vessel pipework systems.

Field validation of the CRYPT was performed on six domiciled recreational vessels berthed in Nelson Marina, Nelson, New Zealand that ranged in length from 7 to 23 m. From the six vessels selected, two of each of the three main classes of pipework systems were treated using the CRYPT: engine-cooling; below-water discharge; and ancillary seawater supply. Careful selection of a range of both vessel sizes and pipework systems was undertaken to, as far as was feasible, be representative of recreational vessels commonly encountered at the New Zealand border (see Inglis et al., 2012). Where possible, qualitative assessments of biofouling extent and viability within pipework systems were made pre- and post-treatment.

3.2 METHODS

3.2.1 Vessel and pipework selection and temperature monitoring

For each of the six vessels, one pipework system was selected that could be adequately accessed from inside the vessel for positioning temperature probes at three representative locations. Many of the pipework systems encountered were excluded from the study because they were hidden behind immoveable cabinetry or fuel or water tanks.

The pipework systems selected for treatment spanned diameters from 12 to 125 mm, total lengths from 0.75 to 3 m (Table 7), and had the following primary functions:

- vessel A – engine-cooling system with secondary fire-fighting systems (Figure 23);
- vessel B – engine-cooling system (Figure 24);
- vessel C – combined below-water discharge system for the toilet and sink (Figure 25);
- vessel D – below-water discharge system for the toilet (Figure 26);
- vessel E – ancillary seawater supply system for the galley sink (Figure 27); and
- vessel F – ancillary seawater supply system for galley sink and toilet (Figure 28).

Table 7. Details of vessels selected for field validation of the CRYPT system operational protocols.

Vessel		Pipework system		
Id	**Length (m)**	**Primary function**	**Diameter (mm)**	**Total length (m)**
A	23	Engine cooling	50 – 125	3
B	7	Engine cooling	12	1.5
C	10	Below-water discharge	25 – 32	2.5
D	15	Below-water discharge	50	2
E	9	Ancillary seawater supply	25	0.75
F	9	Ancillary seawater supply	15	3

Temperature sensors were fitted externally for indirect temperature measurement because it was not possible to monitor temperatures directly from inside pipework without modifying the pipework or compromising the seal at the inlet or outlet. Thermocouple temperature sensors connected to a TC-08 multi-channel data logger (Pico Technology, St Neots, United Kingdom) were affixed to the outside of each pipework system using electrical insulation tape with a layer of 5-mm closed cell foam to isolate the sensors from the ambient environment. Where available, metal pipework or fittings were selected as monitoring points because, given high thermal conductivity, they return values representative of temperatures within pipework to ± 2 °C with only a minor time lag (Appendix 3). Where plastic pipework or fittings were used for temperature monitoring, a temperature correction of + 10 °C was applied based on laboratory validation (Appendix 3).

Of the six vessels included in this study, vessel A had the largest and most complex pipework system. Temperature sensors were affixed at four locations (Figure 23):

1. immediately adjacent to the inlet (Inlet);
2. just past the first inspection port (IP A);
3. at the terminal end of the manifold (Manifold); and
4. at the second inspection port (IP B).

Temperature sensors were affixed at three locations for the remaining vessels (Figure 24; Figure 25; Figure 26; Figure 27; Figure 28):

1. immediately adjacent to the inlet or outlet (Inlet);
2. the furthest location into the system judged to have exchange of seawater with the environment (Terminal; Cahill et al., 2018); and
3. approximately halfway between Inlet and Terminal monitoring points (Mid).

Where possible, all monitoring points were photographed during the treatment. Photography was not always possible due to the highly confined nature of some vessel interiors. Temperature monitoring points positioned on plastic pipework or fittings were: Mid and Terminal for vessel D; Mid and Terminal for vessel E; and Mid for vessel F.

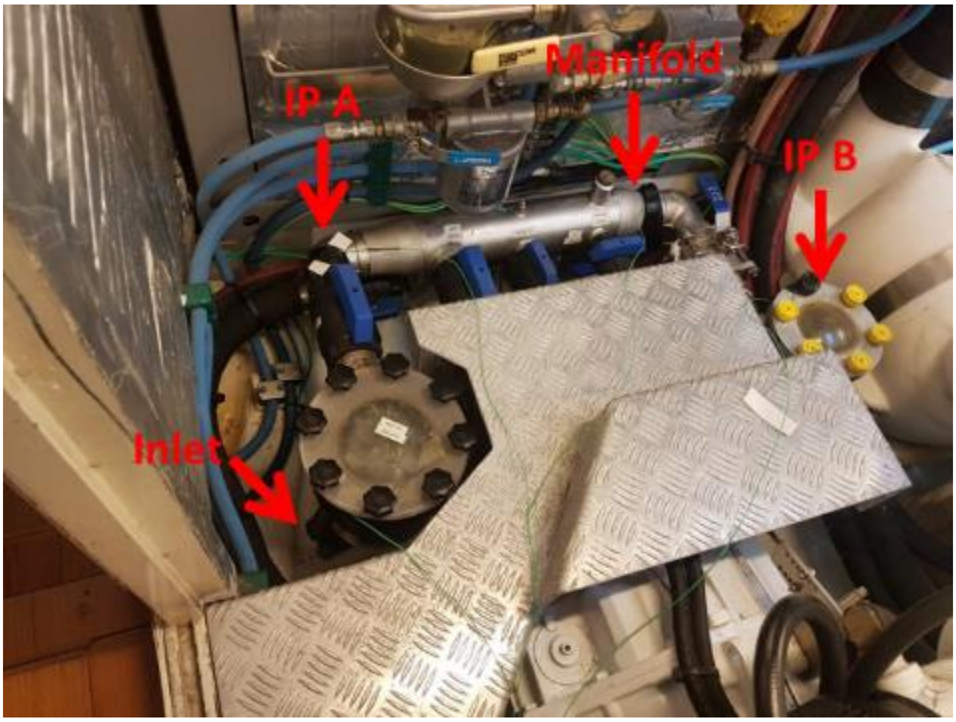

Figure 23. Temperature monitoring points on an engine-cooling system on board vessel A ('Inlet', 'Inspection Port (IP) A', 'Manifold', and 'IP B'). Image: P. Cahill.

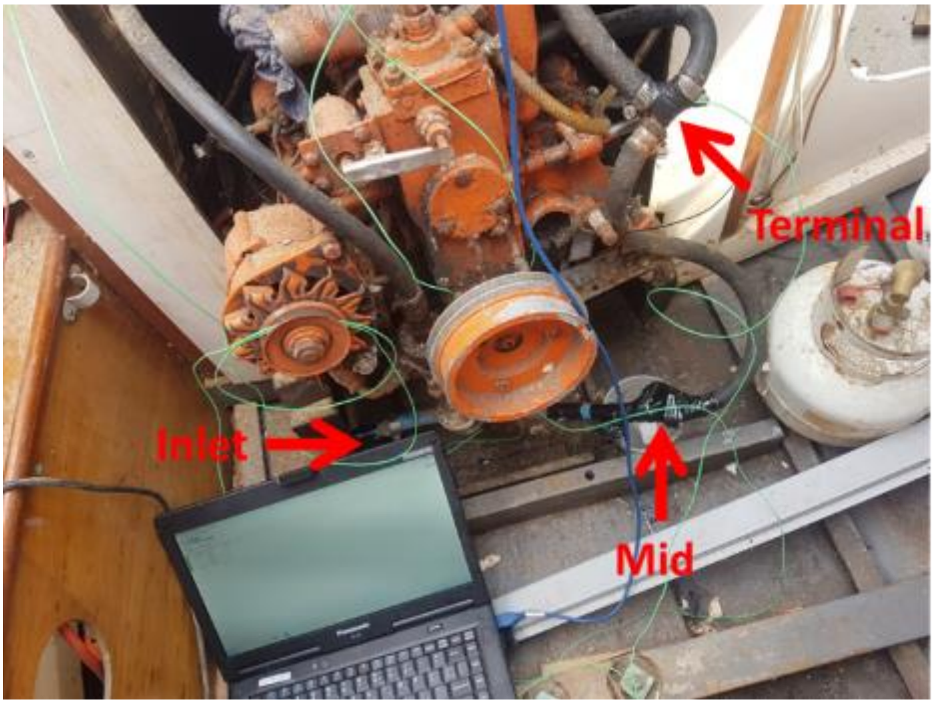

Figure 24. Temperature monitoring points on an engine-cooling system on board vessel B ('Inlet', 'Mid', and 'Terminal'). Image: P. Cahill.

Figure 25. Temperature monitoring points on a combined sink and toilet below-water discharge system on board vessel C ('Inlet' and 'Mid'). Note that the unlabelled temperature probes in this image were repositioned prior to the experiment. Image: P. Cahill.

Figure 26. Temperature monitoring points on a toilet below-water discharge system on board vessel D ('Inlet' and 'Mid'). Image: P. Cahill.

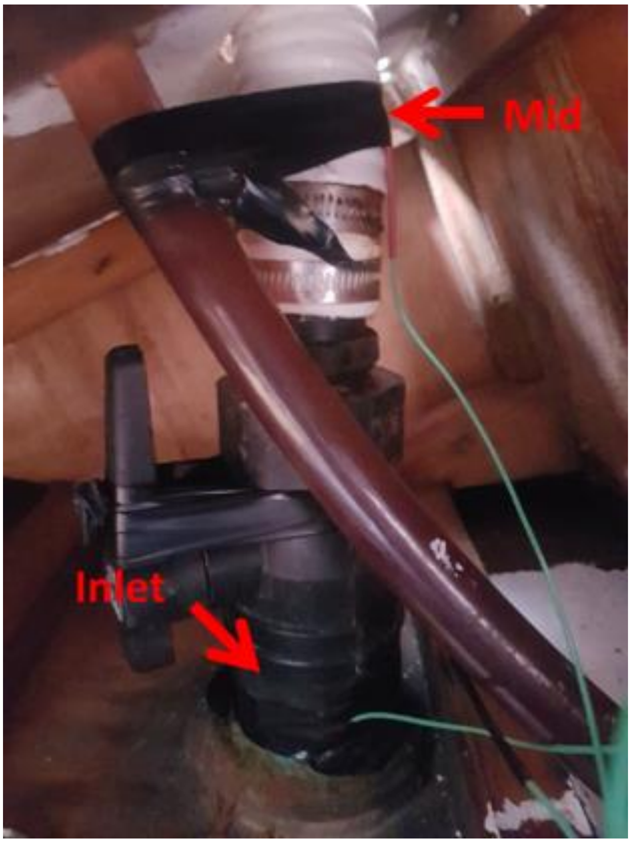

Figure 27. Temperature monitoring points on an ancillary seawater supply system on board vessel E ('Inlet' and 'Mid'). Image: P. Cahill.

Figure 28. Temperature monitoring points on an ancillary seawater supply system on board vessel F ('Inlet', 'Mid', and 'Terminal'). Image: P. Cahill.

3.2.2 Treatment application

The CRYPT was applied to vessels using a custom designed counter-current manifold as described in Section 2.2.3. Several attachments for the manifold were trialled for the various diameters and configurations of inlets and outlets encountered. Except for vessel B, a length of 12-mm Buteline™ tube corresponding to the distance from the inlet to the first bend within the pipework system was fitted to the delivery line. The laboratory trials demonstrated that this technique maximises circulation of heated water (Section 2.3.1.1). The required length of delivery tube was determined by measuring the distance from the inlet or outlet to the first bend in the pipework system from inside the vessel. This procedure took ~ 5 mins to complete. The use of the delivery tube was not possible for vessel B because an inlet grate was present.

The preferred method for attaching the counter-current manifold was the use of expandable test plugs (Haron Plumbing, Haron International Pty. Ltd.). The pipework system on vessel A was sealed with a 48 to 62 mm expandable test plug, which was inserted into the outlet and hand tightened by SCUBA divers (Figure 29).

Expandable test plugs are only commercially available for openings exceeding 37 mm, and can only be applied where no inlet grate is present. Pipework inlets or outlets of less than 37 mm in diameter, or those with an inlet grate, (vessels B to F) were sealed using a suction cup constructed from a length of 15 mm brass nippling tube and the head of a household plunger (Figure 30). Attachment of the suction cup was achieved via the following method:

1. a length of 12-mm Buteline™ tube was fitted to the delivery line of the counter-current manifold. The length of the tube corresponded to the distance from the inlet to the first bend within the pipework system;
2. the suction cup was held against the hull by divers, and ropes attached to either side of the suction cup were evenly tensioned to the port and starboard rails of the vessel (Figure 31); and
3. an additional bow or stern attachment rope was added to prevent dislodgement in cases where the submerged outlet was adjacent to the keel.

Figure 29. Expandable test plug forming an effective seal on an engine-cooling system (vessel A). Image: L. Tait.

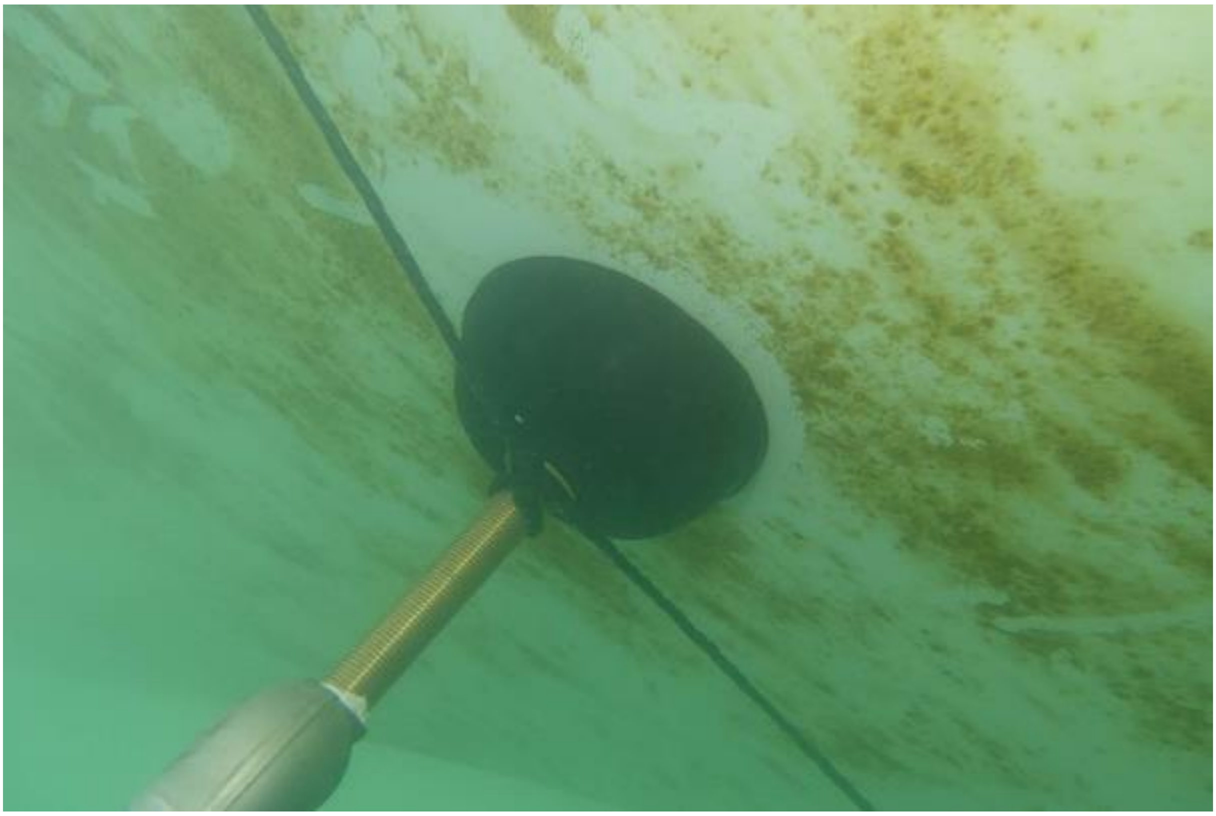

Figure 30. Effective seal for the suction cup applied to an ancillary seawater supply system (vessel E). Note the rope running across the hull to firmly position the suction cup and prevent dislodgement during treatment application. Image: L. Tait.

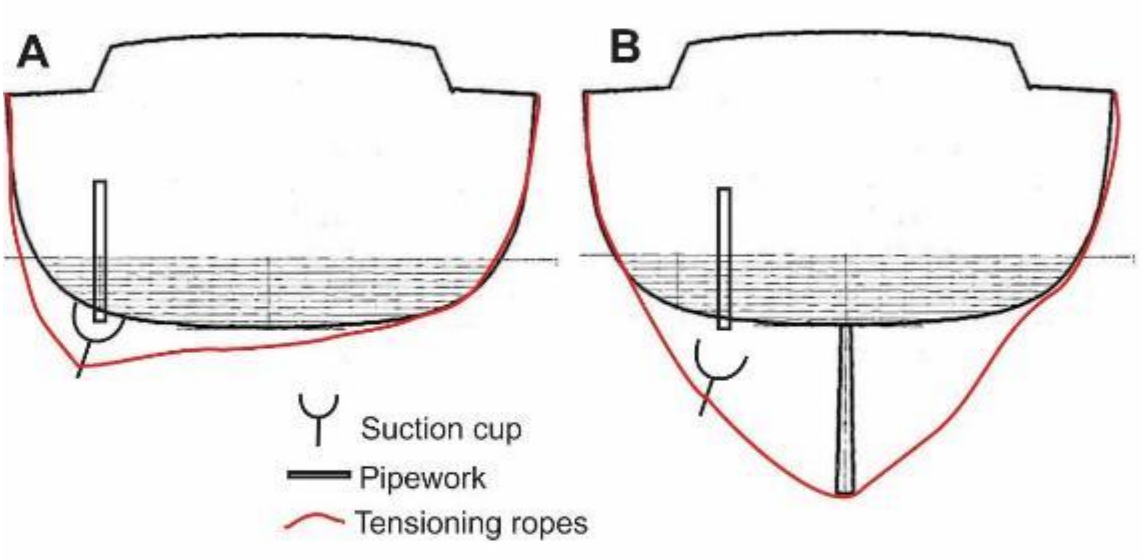

Figure 31. Suction cup applied to vessel pipework inlet or outlet without (A) and with (B) interference from the keel. Not drawn to scale.

Methods to activate, monitor, and decommission the CRYPT followed the draft treatment protocol developed from the laboratory experiments (Appendix 2). Temperatures were recorded at the monitoring points (Section 3.2.1) at 30 s intervals for the required treatment period. The temperature of the CRYPT return line was also monitored at the inlet to the main recirculation pump. Vessel A was too large to connect probes to both the pipework system and return line and, as a result, the return line temperature was not monitored for this vessel. The temperature of the CRYPT cylinder was measured immediately before and after treatment.

3.2.3 Biofouling assessment

Prior to installation of the CRYPT system, video recordings of the inside of each pipework system were made using a custom-made endoscope camera operated by SCUBA divers with a 4K video recording unit positioned topside on the adjacent pontoon (Blackmagic Video Assist 4K, Blackmagic Design). While video footage is not a reliable tool for quantifying organism viability, it was considered that the comparison of biofouling presence before and following treatment may provide useful qualitative information. Endoscopic observations were repeated approximately 24 h post-treatment to identify any visible changes to the biofouling within the pipework system. A custom-made LED lighting rod was inserted alongside the endoscope to provide illumination within pipework systems. Handheld dive torches were used in cases where pipework inlets were too narrow to accommodate the endoscope and the LED rod.

Qualitative assessments of changes in biofouling ‘load’ following treatment were made using snapshots from the video feed of identifiable features (e.g., valve seats, bends, etc.). This method was preferable to comparing unreferenced points or the video in its entirety because biases could result from, for example, prioritising dirty sections pre-treatment and clean sections post-treatment.

3.3 RESULTS

3.3.1 Engine-cooling systems

3.3.1.1 Vessel A

The engine-cooling system on vessel A had twin inlets, one of which (nearest IP B) was closed to isolate the system for treatment. The pipework was of a large diameter (50 to 125 mm) that corresponded to the comparatively large size of this vessel. A 48 to 62 mm expandable test plug with 700 mm long Buteline™ delivery tube was used to seal the pipework system. This procedure was quicker and more straightforward than for the suction cup (Figure 29), and the integrity of the seal also appeared robust (i.e., less prone than the suction cup to accidental displacement by divers). Apart from IP B, temperatures inside the pipework reached the target zone of 58 to 65 °C within 10 min and were stable for the entire treatment duration (Figure 32). The monitoring point IP B reached 41 °C by the end of the treatment. This monitoring point was adjacent to another inlet that could be treated separately to ensure all portions of pipework are heated adequately. The total time to install, heat, treat, and decommission the CRYPT (total treatment time) was approximately 1 h 49 min (Table 8).

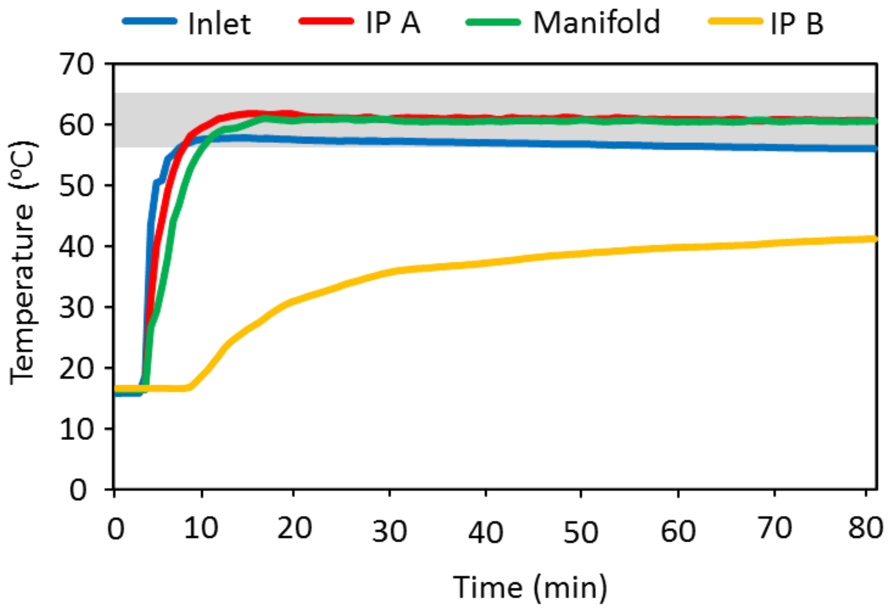

Figure 32. Temperature monitoring for treatment of an engine-cooling system on board vessel A ('Inlet', 'Inspection port (IP) A', 'Manifold', and 'IP B').

Vessel A had not been dry docked for several years but the owner of this vessel occasionally in-water cleaned the hull and regularly sailed the vessel to reduce biofouling development. Biofouling on the vessel's external hull was moderate (i.e., small patches of macrofouling), including arborescent bryozoans observed around the pipework inlet. Arborescent bryozoans and small spirorbid worms were also noticeable within the preliminary portions of the pipework system (Figure 33). Endoscopic footage confirmed that it was not possible to accurately identify and quantify the viability of biofouling organisms due to poor water quality and inability to avoid 'bumping' into organisms that may retreat into tubes or tests to bias any assessment of physiological state. The untreated pipework contained quantities of sediment and biofilm that became suspended when the endoscope was inserted, resulting in poor water clarity. This issue occurred for all six vessels. Treatment with CRYPT purged most sediment and poorly attached biofilm from the system but, nevertheless, water clarity was insufficient to accurately identify or assess mortality of organisms. Divers did observe post-treatment sloughing of biofilms and bleaching of bryozoans near the inlet (first 0.5 m visible from the inlet).

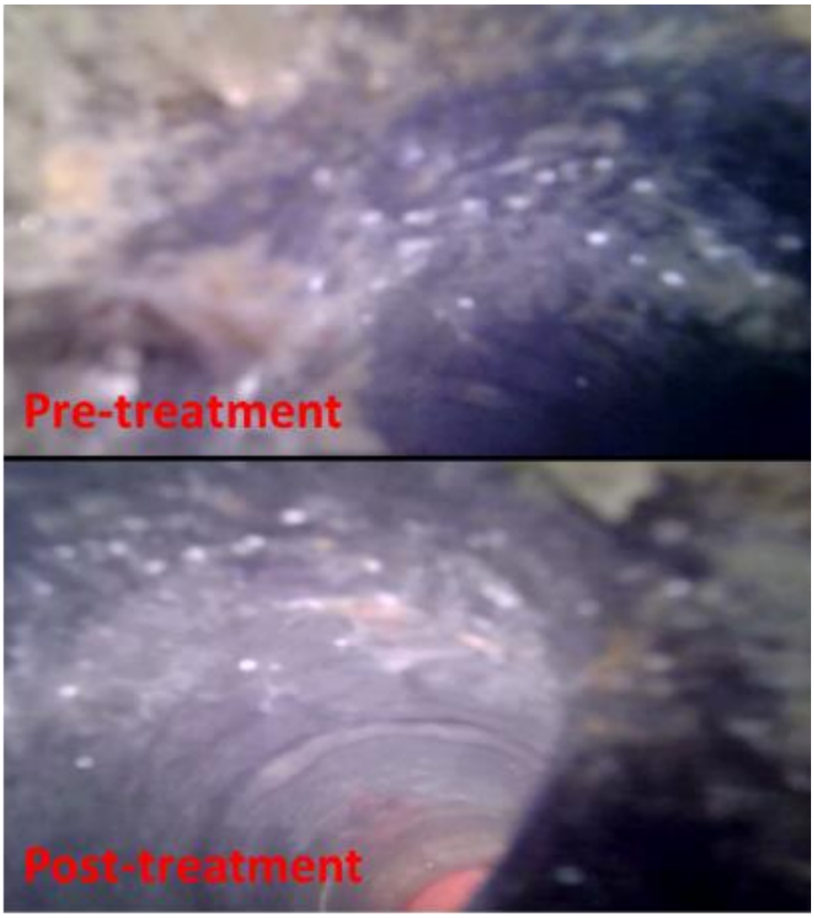

Figure 33. A section of pipe making up part of an engine-cooling system on board vessel A before and 24 h after heat treatment. Note arborescent bryozoan (out of focus) in bottom left of frame pre-treatment. Image: P. Cahill.

3.3.1.2 Vessel B

The engine-cooling system on vessel B, the smallest vessel examined, comprised small diameter tubing (12 mm) and had an inlet grate that precluded endoscopic observation of the pipework system. While attempts were made to seal the inlet grate using the suction cup, the location of the keel adjacent to the inlet posed difficulties. Dense biofouling communities surrounding the inlet were partially removed prior to positioning the suction cup but remnants of organisms (e.g., byssal plaques) and the grill itself interfered with the integrity of the seal (Figure 34). An additional bow rope was used but there was insufficient distance between the keel and intake to provide leverage to tension the suction cup hard against the hull, resulting in an imperfect seal (Figure 31B). Applying the additional bow rope added approximately 15 min to the installation time (vessels C to F). Temperature increased noticeably at the inlet monitoring point, but only achieved a maximum of 34 °C (Figure 35). After 30 min, all monitoring points had returned to ambient conditions and the cylinder temperature had dropped substantially, from 71 to 62 °C.

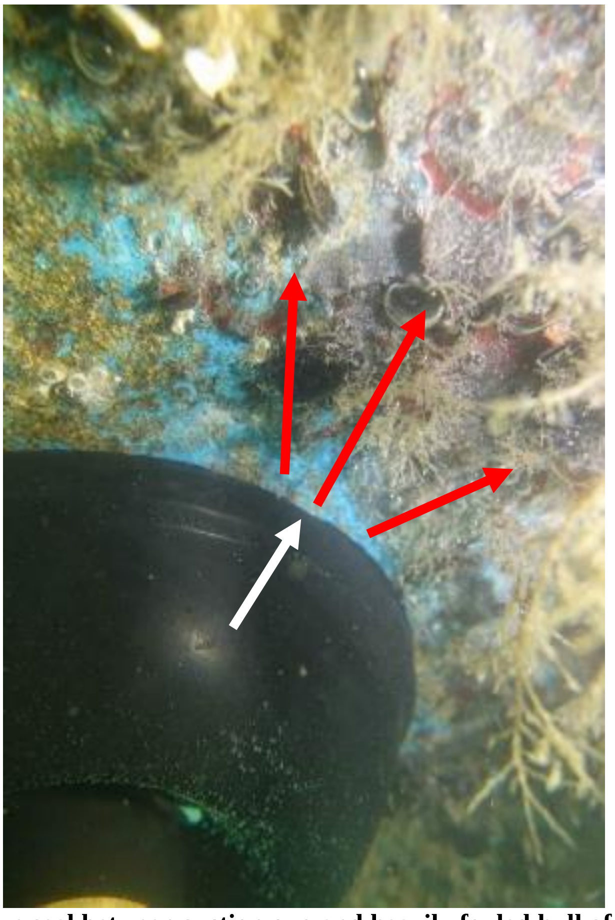

Figure 34. Partially effective seal between suction cup and heavily fouled hull of vessel B evidenced by the escape of air bubbles (red arrows) from the high point of the suction cup (white arrow). Image: L. Tait.

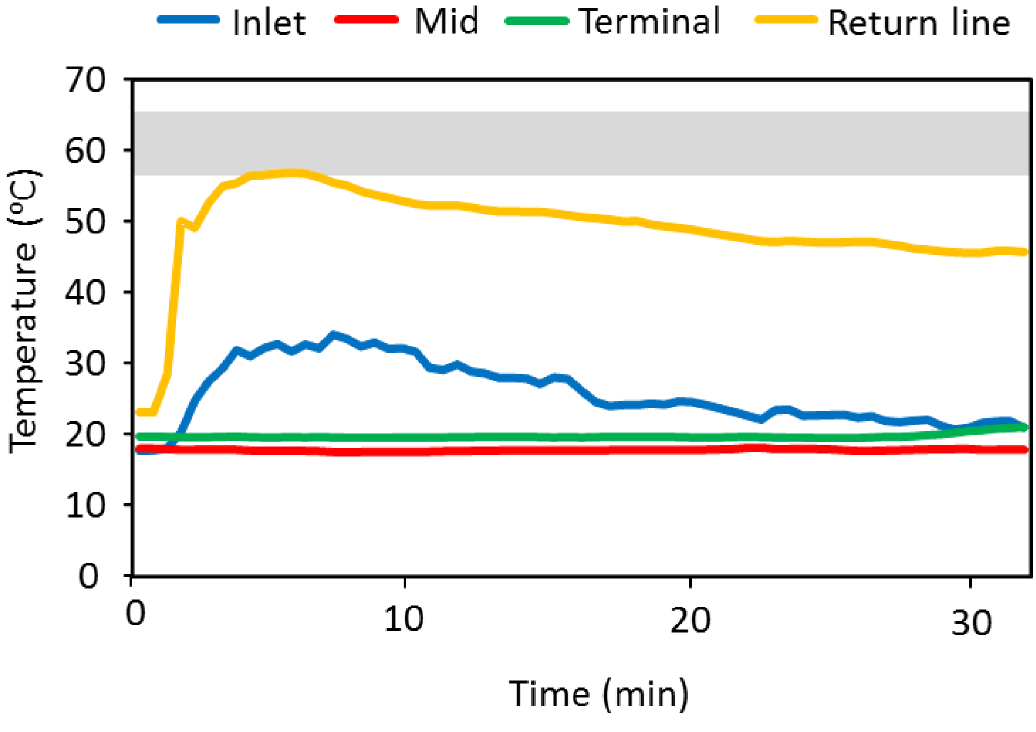

Figure 35. Temperature monitoring for treatment of an engine-cooling system on board vessel B (‘Inlet’, ‘Mid’, ‘Terminal’, and ‘Return line’).

The maintenance history of vessel B was uncertain because it had recently changed ownership. Prior to being berthed in Nelson Marina, this vessel had been on an unidentified swing mooring and was largely unmaintained for a period of several years. Vessel B had extensive hull fouling, including mature mussels, ascidians, bryozoans, and tubeworms. Fouling around the intake, which was representative of that on the vessel as a whole, was partially removed to allow positioning of the suction cup (Figure 36). Encrusting bryozoans were observed inside the intake grill before and after treatment but it was not apparent whether these organisms were alive or dead (Figure 37). Localised antifouling coating damage occurred on the intake grill during treatment (Figure 37).

Figure 36. Images of the intake grill before (left) and after (right) heat treatment for vessel B. Image: L. Tait.

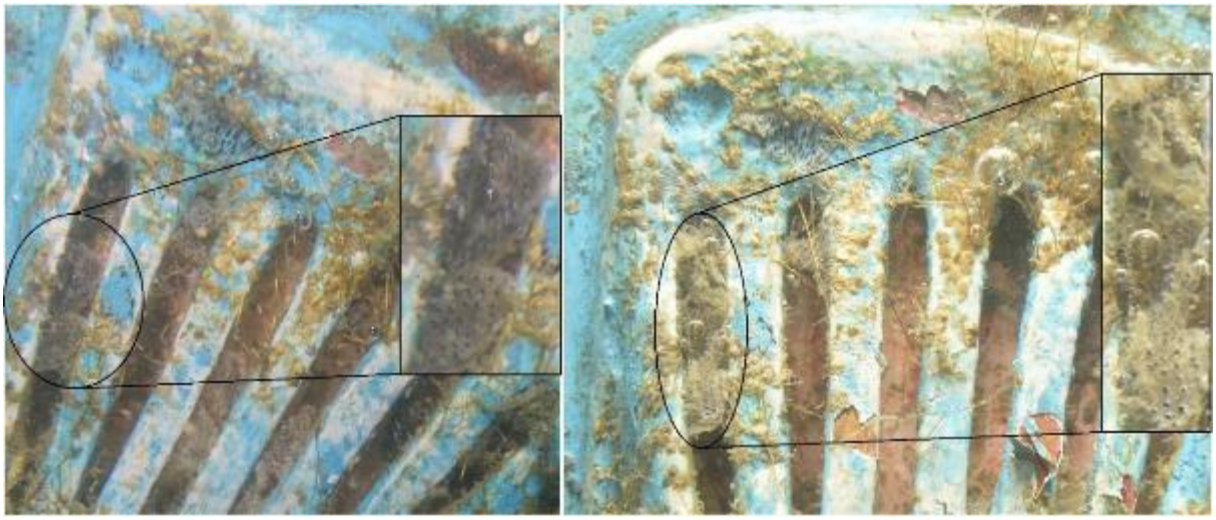

Figure 37. Encrusting bryozoan beneath the intake grill before (left) and after (right) heat treatment for vessel B. Note damage paint on grills 3rd, 5th, and 6th from the left after treatment. Image: L. Tait.

3.3.2 Below-water discharge systems

3.3.2.1 Vessel C

The below-water discharge system on vessel C was effectively treated using the CRYPT with a total treatment time of approximately 1 h 53 min (Table 8). The suction cup formed a watertight seal around the outlet, with the Buteline™ delivery tube inserted approximately 200 mm to the first bend in the system. Temperatures rose to above 58 °C at the Inlet and Mid monitoring points within 10 min of the CRYPT being activated (Figure 38). The Terminal monitoring point, which was approximately 2.5 m from the inlet, took longer to heat but once the minimum target temperature of 58 °C was achieved, temperatures were stable for the required treatment time. The temperatures recorded for the return line were slightly below those of the Inlet and Mid monitoring points, and were approximately equal to the Terminal monitoring point once the heating phase was completed. The temperature of the CRYPT cylinder decreased from 73 to 70 °C over the course of the treatment.

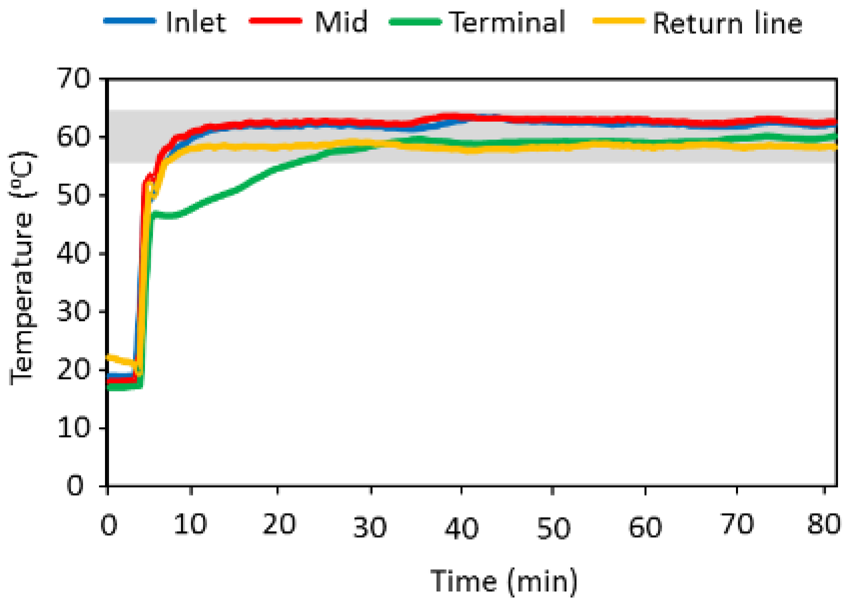

Figure 38. Temperature monitoring for treatment of a combined sink and toilet below-water discharge system on board vessel C ('Inlet', 'Mid', 'Terminal', and 'Return line').

Vessel C had recently (within 1 to 2 weeks) been in-water cleaned and biofouling on the hull was minimal (i.e., slime layer only). The pipework systems had not been included in the recent in-water clean but the pre-treatment inspection revealed no obvious macrofouling within the pipework system. The post-treatment inspection indicated dramatically improved water quality and reduction of slime layer present (Figure 39).

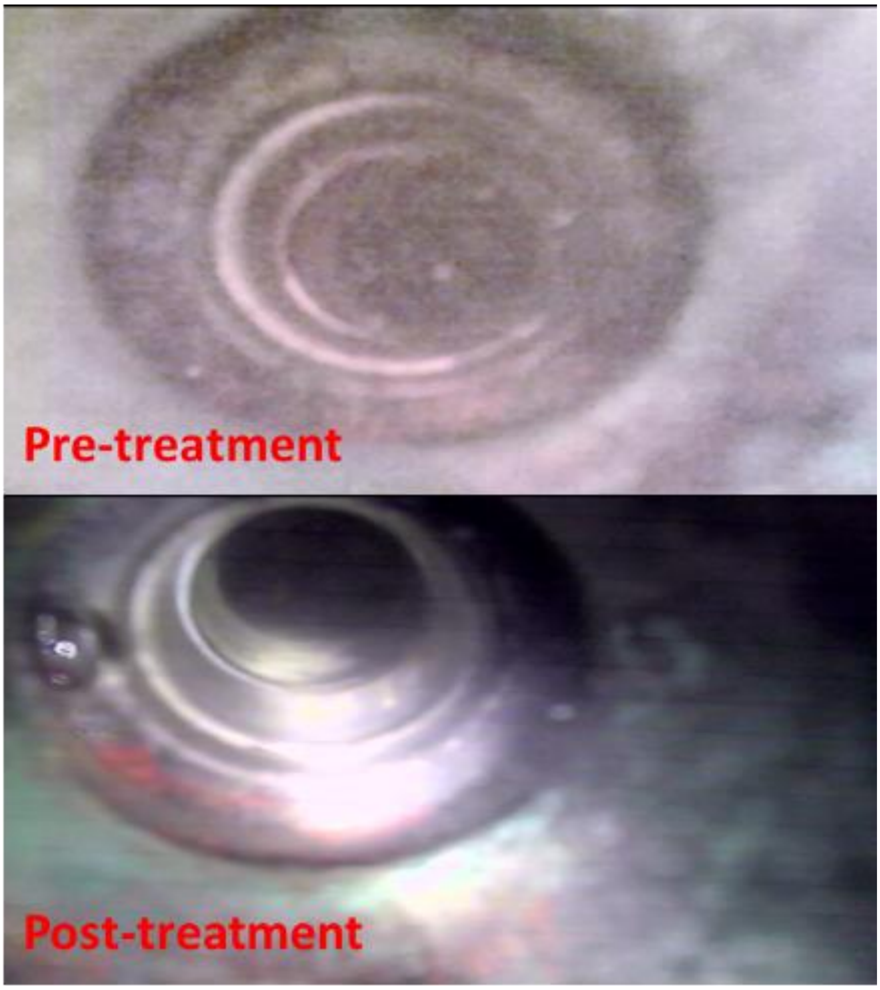

Figure 39. A valve seat inside a combined sink and toilet below-water discharge system on board vessel C before and 24 h after heat treatment. Image P. Cahill.

3.3.2.2 Vessel D

The inlet for the below-water discharge system on vessel D was too small to accommodate an expandable test plug. The suction cup with a 100 mm long delivery tube was used, which was easily positioned using port and starboard ropes to form a water-tight seal. The large overall size of this vessel and position of the outlet on the opposite side of the vessel from the marina pontoon meant that a greater length of the CRYPT delivery and return line was in the sea compared to the other vessels tested. This shortcoming provided an increased surface area available to ambient seawater resulting in heat loss. Temperatures inside the pipework increased to above 58 °C for the Mid and Terminal monitoring points within 20 min (Figure 40). The Inlet monitoring point reached 57 °C but all temperatures fell below the target zone before the required treatment time was completed (Figure 40). By the end of the treatment, temperatures were 55, 48, and 43 °C at the Inlet, Terminal, and Mid monitoring points, respectively. Including decommissioning, the total treatment time was approximately 1 h 44 min (Table 8). The CRYPT cylinder temperature was 71 °C at the beginning of the treatment and 68 °C at the end of the treatment. Although typically within ± 5 °C, temperature profiles were less stable for monitoring points positioned on plastic pipework (Mid and Terminal) compared to those positioned on metal fittings (Inlet, Figure 40).

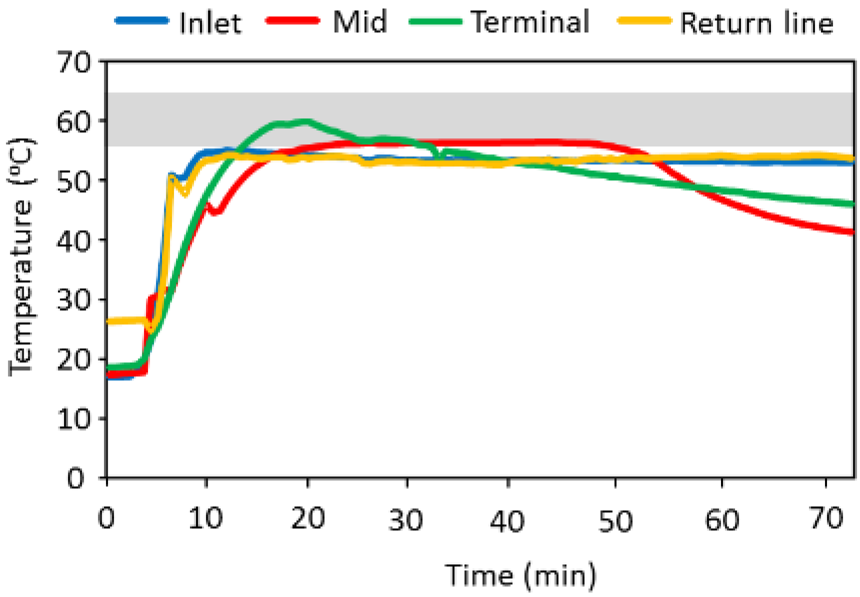

Figure 40. Temperature monitoring for treatment of a toilet below-water discharge system on board vessel D (‘Inlet’, ‘Mid’, ‘Terminal’, and ‘Return line’).

Vessel D had not been dry docked or in-water cleaned for over three years. Biofouling on the hull was moderate to heavy (i.e., many small patches of macrofouling) and arborescent bryozoans were observed around the pipework outlet. Although there was no obvious macrofouling within the pipework system, pipework appeared cleaner post-treatment due to a reduction in slime layer and sediment deposits (Figure 41).

Figure 41. A valve seat from inside a toilet below-water discharge system on board vessel D before and 24 h after heat treatment. Image P. Cahill.

3.3.3 Ancillary seawater supply systems

3.3.3.1 Vessel E

The suction cup with a 100 mm long delivery tube was used to successfully treat vessel E, with a total treatment time of 1 h 57 min (Table 8). The position of the inlet away from the keel allowed for easy attachment and a water-tight seal. Despite the short overall length of this pipework system, heating was relatively slow with 32 min required to reach the minimum treatment temperature of 58 °C at all monitoring points (Figure 42). Temperatures were subsequently stable within the target zone for the required treatment time. Temperature profiles from monitoring points positioned at metal (Inlet) and plastic (Mid and Terminal) fittings were comparable once the prescribed correction factor had been applied. The return line temperature was 1 to 5 °C lower than temperatures recorded inside the pipework system. The CRYPT cylinder temperature decreased by 3 °C over the course of the treatment from 72 to 69 °C.

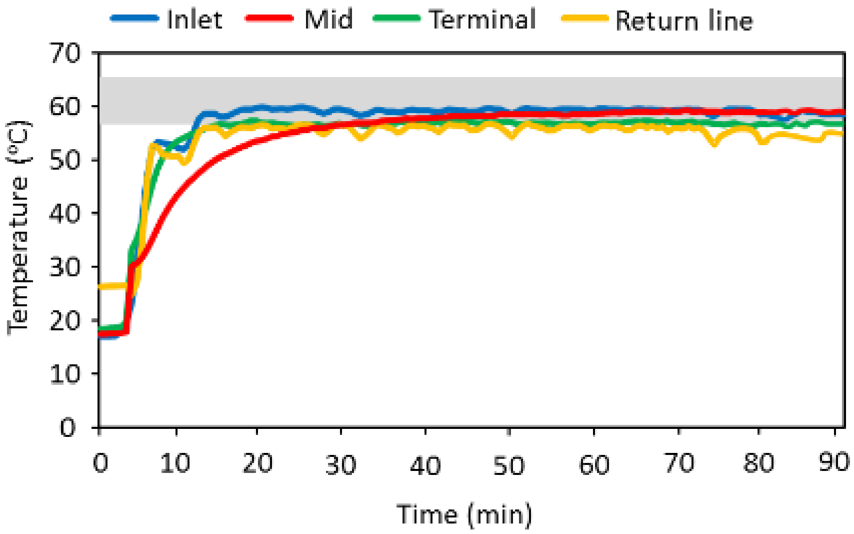

Figure 42. Temperature monitoring for treatment of an ancillary seawater supply system on board vessel E (‘Inlet’, ‘Mid’, ‘Terminal’, and ‘Return line’).

The hull of vessel E had been recently (within 1 year) refinished with a self-polishing antifouling coating and was clean, featuring only a minimal slime layer. There was no evidence of macrofouling in the pipework system. The treatment, however, purged most of the slime layer and sediment from the pipework, as indicated by increased visibility post-treatment (Figure 43).

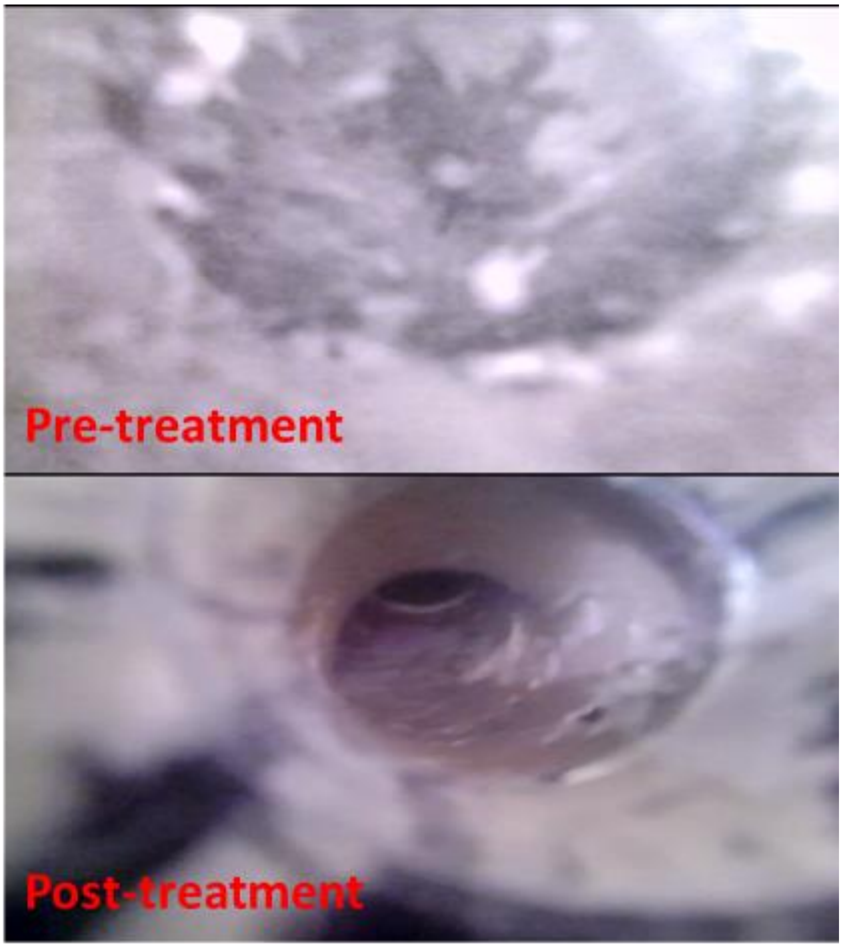

Figure 43. A section of pipe making up part of an ancillary seawater supply system on board vessel E before (clogged by biofilm and sediment layer) and 24 h after heat treatment (biofilm and sediments cleared). Image P. Cahill.

3.3.3.2 Vessel F

The suction cup with a 50 mm long Buteline™ delivery tube was used to seal the inlet of the ancillary seawater supply system on vessel F. The inlet was immediately adjacent to the keel, and an additional rope tensioned to the bow of the vessel was required to hold the suction cup in position. The first attempt to position the suction cup resulted in a partial seal— temperatures inside the pipework fluctuated widely (Figure 44) and hot water and bubbles could be seen escaping from the seal (Figure 34). The suction cup was repositioned after 35 min with additional tension applied to the securing ropes. It was decided to continue with the treatment after repositioning the suction cup to determine if the suboptimal heating was due to an ineffective seal at the pipework inlet.

The temperature profiles improved after repositioning the suction cup, but stabilised below the target zone (Figure 44). The Inlet (positioned on a metal fitting) and Mid (positioned on a plastic fitting) monitoring points had similar temperature profiles, and both stabilised at approximately 50 °C. The heating capacity of the CRYPT appears to have been overwhelmed by earlier seawater ingress to the system, with the cylinder temperature dropping from 73 °C at the start of the treatment to 66 °C at the end of the treatment. The Terminal monitoring point, which was approximately 3 m from the inlet, was not effectively heated. This pipework system was of small diameter with a bend immediately adjacent to the inlet which, combined with the initial imperfect seal of the suction cup, constrained capacity to heat further into the system.

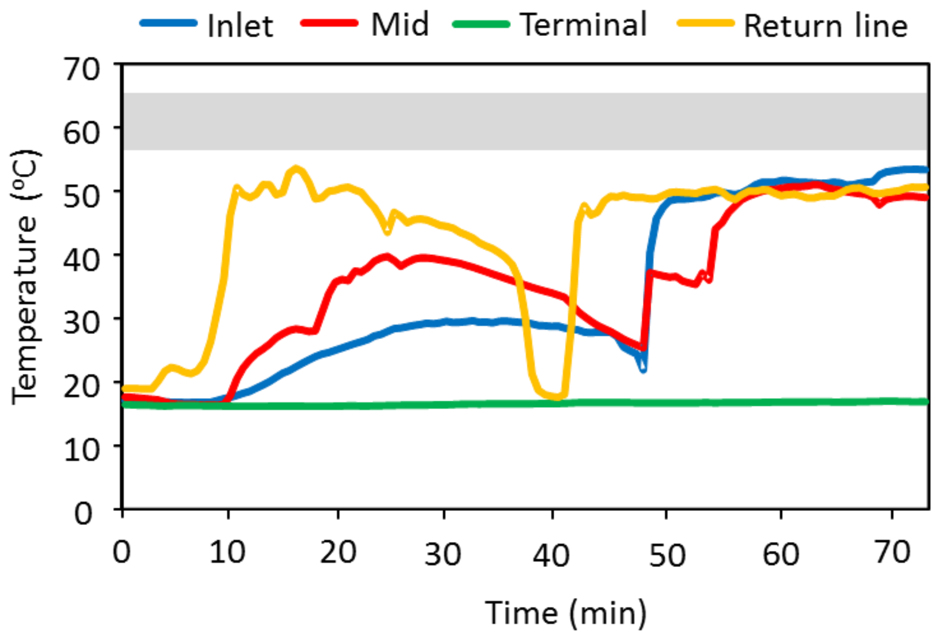

Figure 44. Temperature monitoring for treatment of an ancillary seawater supply system on board vessel F ('Inlet', 'Mid', 'Terminal', and 'Return line'). Repositioning of the suction cup occurred 35 min into the treatment.

Vessel F was well-maintained, with a relatively new ablative antifouling coating and correspondingly low biofouling loads on the hull (i.e., slime layer and small patches of macrofouling). The inlet to the ancillary seawater supply was surrounded by small arborescent bryozoans. Although there was no obvious macrofouling within the pipework, the treatment appeared to improve visibility inside the pipework post-treatment (Figure 45).

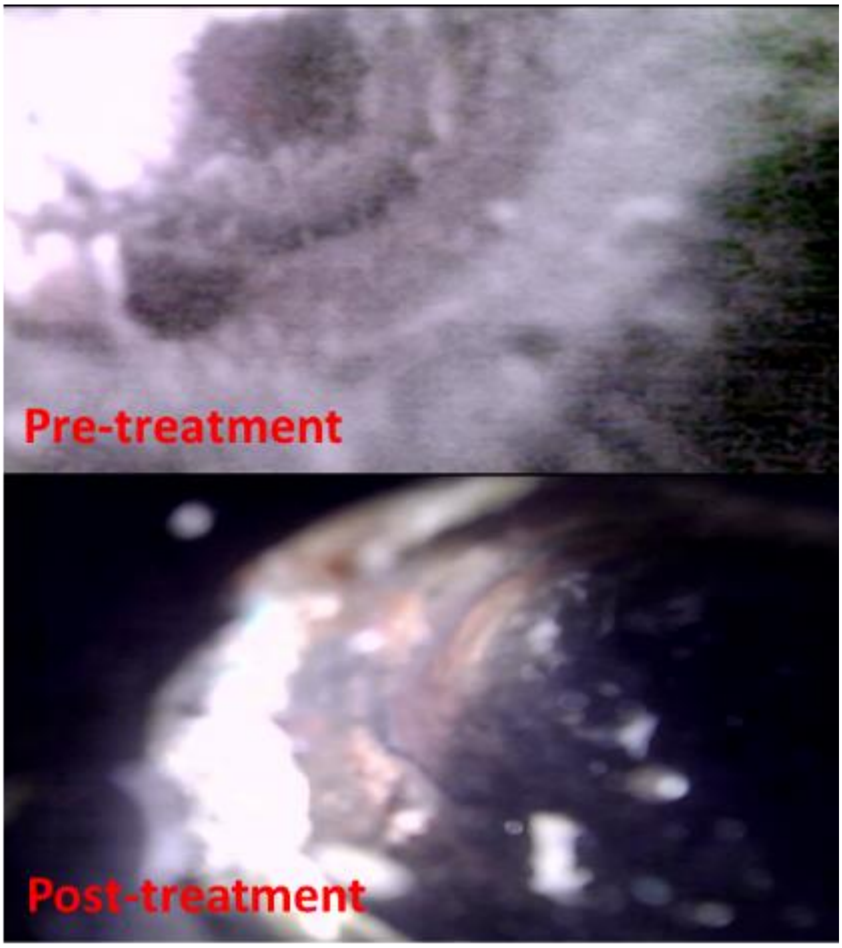

Figure 45. A section of pipe making up part of an ancillary seawater supply system on board vessel F before and 24 h after heat treatment. Image P. Cahill.

Table 8. Summary of the field trials of the CRYPT.

			Time (min)					
System targeted	**Vessel Id**	**Inlet or outlet sealing method**	**Installation[a]**	**Heating**	**Treatment**	**Decommission[a]**	**Total treatment time**	**Treatment successful?**
Engine cooling	A	Expandable test plug + 700 mm delivery tube	15	12	72	10	109	Yes
	B	Suction cup	90	-	-	10	-	No
Below-water discharge	C	Suction cup + 200 mm delivery tube	20	23	60	10	113	Yes
	D	Suction cup + 100 mm delivery tube	20	14	60	10	104	No
Ancillary seawater supply	E	Suction cup + 100 mm delivery tube	15	32	60	10	117	Yes
	F	Suction cup + 50 mm delivery tube	90[b]	-	-	-	-	No

[a] Approximate values ± 5 min; [b] two installation attempts.

3.4 DISCUSSION

The CRYPT effectively heated pipework systems of recreational vessels in the field when an effective seal was achieved at the pipework inlet or outlet and ambient heat loss was minimised. These conditions were achieved for vessels A, C, and E (Table 8). The pipework systems on vessels A, C, and E were of varied configurations with: nominal diameters of 25 to 125 mm; total lengths in the range of 0.75 to 3 m; and system functions including engine cooling, ancillary seawater supply, and below-water discharge. The temperature profiles for these effectively heated pipework systems closely matched the results observed during laboratory testing (Section 2.3).

While an effective seal was achieved for the pipework outlet of vessel D, the target temperature was not able to be maintained for the required period because ambient heat loss overwhelmed the CRYPT.

Engineering challenges prevented effective seals being achieved for vessels B and F. Even though pipework configurations were similar to systems treated effectively on other vessels, vessel layout (e.g., position of the keel and inlet grates) and condition (e.g., extent of hull fouling present around inlets) interfered with the sealing apparatus. When the seal is not effective, the CRYPT is unable to heat pipework to required conditions due to ingress of ambient seawater. Results are discussed below in terms of: (a) effectively sealed; and (b) ineffectively sealed pipework systems, respectively.

3.4.1 Effectively sealed pipework systems

3.4.1.1 Temperature profiles

An expandable test plug was used to seal the pipework system on vessel A, while a suction cup fitting was applied for vessels C and E. Temperatures stabilised at all targeted temperature monitoring points for these vessels within 5 and 30 min of activating the CRYPT system. Temperatures were subsequently held within the required zone of 58 to 65 °C for the entirety of required 60 min treatment time. The actual rate of heating would have been faster than the measured rates because there is a lag of several minutes associated with indirect temperature monitoring, particularly for plastic fittings (Appendix 3). The monitoring points that were slowest to reach the required temperature were those positioned on plastic pipework or fittings, such as Mid and Terminal for vessel E. Temperature profiles were consistent between metal and plastic fittings once the heating phase was completed, which validated the temperature correction factor applied in this instance.

The outlet of the below-water discharge system on board vessel D was effectively sealed using the suction cup but the resulting temperature profiles were unstable. Temperatures within the pipework initially met the required treatment conditions but subsequently fell during the treatment to minimum observed values of 55, 48, and 43 °C for the Inlet, Mid, and Terminal monitoring points, respectively. The Inlet monitoring point was positioned on a metal fitting, whereas Mid and Terminal monitoring points were positioned on solidly constructed plastic pipework (i.e., thicker walled than the plastic monitoring points on vessels E and F). The variability observed for these monitoring points was likely due, at least in part, to time lags associated with indirect temperature monitoring through plastic. This variability demonstrated a degree of uncertainty inherent to applying a universal temperature correction factor (Appendix 3). Although conditions achieved within the pipework system would likely kill most biofouling organisms, the temperature profiles recorded were not sufficient to fulfil the treatment requirements. Prior data have shown that 100% mortality of some resilient biofouling organisms, namely *M. gigas*, is ensured only after 60 min at ≥ 57.5 °C (Piola and Hopkins, 2012).

The temperature profiles for treatment of vessel D suggest that the heating capacity of CRYPT was overwhelmed. The relatively large size of vessel D, and position of the outlet on the side of the vessel furthest from the pier, meant that a greater length of delivery and return line was submerged in the sea compared to the other vessels assessed. Over the course of the treatment, heat loss to ambient seawater reduced treatment temperatures to levels below the required threshold. Operationally, vessels may be able to be repositioned so that the inlet or outlet is nearest the pier. Simple engineering solutions, such as insulating the delivery and return lines or increasing heating capacity, could also be investigated to minimise heat loss (Section 4.4).

The temperature profiles for the CRYPT return line of effectively sealed pipework systems are worth noting. During the laboratory trials there were indications that the temperature of the return line could be an easily monitored proxy for conditions inside treated pipework systems. In the field, return line temperatures were representative of conditions inside the pipework systems for vessels C and E but they were not able to be monitored for vessel A and they differed by more than 5 °C for vessel D (particularly as the temperatures declined). There were also time lags of up to 15 min between when temperatures stabilised inside the pipework system and at the return line. While monitoring return line temperatures might be a practical approach for treatment quality control, the inconsistencies observed in this study mean that additional validation should be undertaken.

3.4.1.2 Treatment of observed biofouling

The realities of field testing precluded assessment of biofouling viability to directly confirm the effectiveness of treatments applied but some qualitative insights were gained. Pipework systems were visibly 'cleaner' post-treatment due to purging of sediment and biofilm. It is not possible to differentiate the relative contributions of recirculation and heat using the available data. Recirculation flow rates (20 L min^{-1}) exceeded those likely to be experienced during normal operation of the systems but it could likewise be contended that flow-through operation (i.e., normal operation of these systems) should result in cleaner pipes than recirculation (i.e., CRYPT application). It is conceivable that both heat and water flow play some role in purging sediment and biofilm. Although qualitative in nature, the improved cleanliness of pipework post-treatment is consistent with effective operation of CRYPT.

Notwithstanding the limitations of poor visibility pre-treatment, the low numbers of macrofouling organisms in pipework systems would have prevented meaningful quantification of mortality rates. The literature review component of the current project suggested that significant macrofouling in pipework systems is the exception rather than the rule (Cahill et al., 2018). Although some pipework systems were only able to be partially inspected (vessel F), or not inspected (vessel B), few identifiable macrofouling organisms were observed inside pipework systems. Biofouling organisms were generally scarce inside the pipework even though some of the vessels had notably fouled hulls and had not been out of the water for more than 3 years (e.g., vessels A, B and D).

When macrofouling organisms were observed, they were in low abundance and were concentrated near pipework inlets or outlets, or inside inlet grates. For example, vessel A had sparsely dispersed spirorbid tubeworms and arborescent bryozoans near the inlet. The treatment conditions achieved were more than sufficient to kill such organisms (Piola and Hopkins, 2012). Tubeworms were too small and well adhered to assess viability via remote video but it was apparent that the larger individual bryozoans had been bleached post-treatment, suggestive of mortality. Regardless of these visual clues, the temperature profiles achieved for vessels A, C, and E were consistent with the laboratory trials (Section 2.3).

3.4.2 Ineffectively sealed pipework systems

3.4.2.1 Temperature profiles

Engineering challenges associated with sealing pipework inlets or outlets became apparent when applying CRYPT to vessels B and F. The pipework inlets of these vessels were too small to accommodate commercially available expandable test plugs, and the position of the keel adjacent to the pipework inlets on both vessels resulted in an ineffective seal by the suction cup. Additional bow ropes were used to better align the suction cup but only partial seals were achieved and the overall installation was cumbersome. These issues were confounded on vessel B by the presence of an inlet grate which further affected ability to cinch the suction cup down on the vessel surface (Figure 36).

The engine-cooling pipework system on board vessel B was not effectively heated. The only temperature increase was observed at the Inlet monitoring point, and the maximum temperature achieved was only 34 °C. The abundant biofouling around the inlet grate was removed before applying the suction cup but remnants (e.g., byssal plaques) likely contributed to an ineffective seal. The inlet grate prevented insertion of a delivery tube, and the pipework was of a small diameter. A positive displacement impeller further prevented the flow of treatment water through this engine-cooling system (see Appendix 2) – impellers are a common feature of engine-cooling systems. The combination of these factors resulted in a highly restricted circulation dynamic that potentially prevented effective treatment of the system.

Apart from methods that would pose unacceptable risk to the vessel, options to overcome such restricted circulation are not obvious. For example, removing the inlet grate in water could at worst breach the hull because the inlet seal could be compromised. There is also risk of 'burring' screws or bolts that would prevent reinstallation of the inlet grate after treatment. Some damage to the paint covering the inlet grate of vessel B occurred due to contact with the copper delivery tube during the field trial, highlighting the potential to damage vessel components in scenarios where installation is operationally difficult, or to inadvertently cause the release of biocidal coating material.

Although pipework temperatures initially increased at all three monitoring points for vessel F, they did not attain the target treatment temperature and decreased over time as seawater ingress overwhelmed the heating capacity of CRYPT. An attempt was made to better position the suction cup after approximately 40 min. A better seal was achieved but pipework temperatures stabilised below the target zone, likely because the CRYPT cylinder temperature had decreased too far when the suction cup was not sealed. The cylinder temperature was only 66 °C at the end of the treatment period for vessel F, compared to end temperatures of around 68 to 70 °C for the vessels that were effectively treated. It seems likely that if an effective seal had been achieved from the outset, the CRYPT would be able to heat this pipework system to the required temperature. Although the Terminal monitoring point saw little increase in temperature, it ought to pose low biosecurity risk because it was approximately 3 m from the inlet and the system comprised small diameter (15 mm) pipework. It is unlikely that seawater exchange would be sufficient to support biofouling organisms under normal operation of the vessel (Cahill et al., 2018).

3.4.2.2 Treatment of observed biofouling

Vessel B was inadequately treated for biosecurity purposes. While the inlet grate prevented any assessment of biofouling inside the pipework system, this was a very heavily fouled vessel and organisms were observed inside the inlet grate. Temperatures achieved at the inlet grate are likely to have approximated those at the inlet monitoring point but these temperatures were insufficient to kill most biofouling organisms. An encrusting bryozoan conspicuous inside the inlet grate was ostensibly unaltered before and after treatment. While international yacht arrivals of this small dimension (7 m) are likely rare, they are common among New Zealand's domestic boating fleet (Inglis et al., 2012) and there may be situations where biosecurity treatment is required for this class of vessel.

Treatment conditions achieved for vessel F would have been lethal to many biofouling organisms, with 50 °C being periodically met or exceeded at the Inlet and Mid monitoring points. Nevertheless, the recorded temperature profiles fell short of the required thresholds (Section 3.4.1.1). This pipework system was difficult to inspect via endoscopic camera due to a heavy slime and silt layer, and relatively small pipework diameter. At best, it can be concluded that treatment with the CRYPT removed much of the obstructing material present in the pipework. Endoscope footage was clearer post-treatment but no identifiable macrofouling organisms were seen on any of the video recordings for this vessel. The Terminal monitoring point was not effectively treated but given the system configuration this location is considered to pose low biosecurity risk (Section 4.2.1).

3.4.3 Conclusion

Robust in-water treatment of internal pipework of recreational vessels is challenging. However, with a small team of operators (field validation was performed by three divers and one Field Supervisor), the CRYPT effectively heated portions of pipework systems that posed a biosecurity risk when pipework inlets or outlets were sealed effectively and ambient heat loss was minimised. For these effectively treated pipework systems, temperatures were maintained at levels known to be effective against resilient biofouling organisms for the required treatment time. However, further development is required to yield a system and treatment protocol that accounts for the full diversity of pipework systems and environmental conditions likely to be encountered operationally. Unless all water is removed from pipework systems prior to treatment, it is likely that a recirculation system would be required to ensure uniform treatment to all high risk areas.

4 General discussion

Laboratory and field experiments were employed in the stepwise development of a functional prototype and associated protocol for the lethal thermal treatment of biofouling in engine-cooling, ancillary seawater supply, and below-water discharge pipework systems of recreational vessels. The CRYPT system performed to specification in the laboratory and for select vessels in the field. Although pipework systems were encountered that were not amenable for treatment by the CRYPT in its current form, development and validation of additional infrastructure and procedures could yield a universal CRYPT protocol.

4.1 PROTOTYPE DEVELOPMENT AND LABORATORY OPTIMISATION

The CRYPT prototype functioned adequately well even in the first iteration of laboratory testing but the optimisation process improved the efficiency of the heating procedures. Time required to bring 'high risk' sampling ports in the engine-cooling MPS to the target treatment temperature was reduced more than threefold between the first and final optimisation experiments. Temperature distribution within the MPS was also enhanced to more evenly heat pipework features. These improvements reflect an 'optimal' combination of:

- insertion to the first bend in the system;
- cylinder thermostat set to 72.5 °C; and
- main pump flow rate set to 20 L min^{-1}.

These stepwise improvements in treatment parameters highlights the value of thorough evaluation and optimisation in a laboratory setting. Applying the CRYPT to the engine-cooling MPS in the first instance allowed a range of parameters to be rapidly evaluated and replicated in a controlled setting that would be difficult or impossible to achieve in the field.

The parameters optimised for the engine-cooling MPS transferred easily to the below-water discharge MPS but attempts to treat the ancillary seawater supply MPS were only successful following the development of an additional flow-through step. The complex and small diameter pipework configuration of the ancillary seawater supply MPS restricted heat distribution, and heated water had to be driven through the system to overcome thermal barriers. Pipework with inherently restricted circulation characteristics is, however, unlikely to house biofouling because transport of water, nutrients, propagules etc. necessary for biofouling to establish and survive will be constrained (Cahill et al., 2018).

The three MPS were representative of pipework systems commonly found on recreational vessels from 10 to 25 m in length, and were designed to represent worst-case scenarios in terms of size and complexity. Likewise, exposing sentinel biofouling to 60 ± 2 °C for 60 min was designed and validated as a conservative treatment to greatly exceed the thermal tolerance of commonly encountered macrofouling organisms. Except for extremophiles dependent on hydrothermal vents (Kumar and Nussinov, 2001), we were unable to find reference to marine organisms capable of surviving under the treatment conditions defined here. Following exposure, the soft tissues of sentinel oysters were visibly 'cooked' (P. Cahill pers. obs.). At a molecular level, biologically ubiquitous proteins, membranes, and enzymes denature around 40 to 45 °C (He, 2011). Piola and Hopkins (2012), however, noted that 100% mortality of some resilient biofouling organisms, namely *M. gigas*, is ensured only after 60 min at ≥ 57.5 °C.

Although the prescribed treatment conditions do appear somewhat 'overkill', this conservative treatment approach was adopted to account, in part, for the large proportion of recreational vessels that arrive to New Zealand having transited through tropical regions (Floerl et al., 2008). The normal temperature tolerance of biofouling organisms from tropical regions should be generally higher than for organisms found in temperate New Zealand. The approach also affords a buffer to account for the likelihood that some portions of pipework systems may inadvertently fall short of the treatment thresholds (i.e., refuge areas).

4.2 FIELD VALIDATION OF A DRAFT OPERATIONAL PROTOCOL

The field validation highlighted the array of scenarios in which a reactive treatment tool, such as CRYPT, could potentially be applied. For robust biosecurity management it is necessary to define conditions under which a tool will, and will not, be effective. The outcome of the field validation provides evidence that CRYPT can be applied effectively to some engine-cooling, ancillary seawater supply, and below-water discharge systems of recreational vessels berthed in a temperate coastal location. Current limitations of the CRYPT apparatus and protocol pertain to engineering requirements associated with:

- difficult-to-seal inlets or outlets, particularly those with inlet grates;
- excess ambient heat loss; and
- small nominal pipework diameters (≤ 12 mm).

When pipework inlets or outlets were sealed effectively and ambient heat loss was minimised, the CRYPT effectively heated 'high risk' portions of engine-cooling, ancillary seawater supply, and below-water discharge pipework systems. The total time required to install, heat, treat, and decommission CRYPT for these successfully treated pipework systems was less than 2 h per system. This was an experimental treatment validation and reported times, while indicative, are likely to be streamlined through routine implementation by end users. Regardless, it could be considered that most vessels should be treated inside a standard 9 h working day assuming no more than three independent pipework systems per vessel. The initial treatment time stipulation by MPI within the Request for Proposals was ≤ 48 h, providing additional buffer for the operational implementation of the CRYPT system.

While expandable test plugs are an easy and safe method for connecting the CRYPT to vessel internal pipework, many inlets and outlets are too small to accommodate a commercially available expandable test plug and the presence of inlet grates render the plugs ineffective. The suction cup approach is more universally applicable but the associated multi-step installation procedure is influenced by vessel layout and condition. A robust seal is unlikely when: (a) the keel interferes with the suction cup tensioning ropes; (b) an inlet grate is present; or (c) fouling is present around the inlet or outlet.

Imperfect sealing can pose biosecurity risks because dislodged biofouling organisms or propagules could be discharged to the environment without having been exposed to the required treatment conditions. Although vessels with hull fouling would be required to undergo other remedial action that overrides in-water pipework treatment, any attempts to remove biofouling from around the inlet or outlet prior to applying the suction cup also poses biosecurity risks unless effectively contained. For these reasons, the suction cup in its current format is suitable only for experimental validation purpose, and additional development would be required for inclusion as an operational treatment apparatus. It may be possible to manufacture a suction cup with a double skirt that can form a seal on the hull without interference from the CRYPT recirculation (Figure 46). Multiple sizes of suction cup could cater to the range of vessels and systems likely to be encountered in the field, and further testing would be required to confirm the integrity of seals under a range of scenarios.

Engineering improvements could also counteract heat loss to the ambient environment, particularly relevant if the CRYPT were to be used in cooler environments (i.e., less than the 18 to 20 ºC where these field trials took place). A straightforward improvement would be to insulate the delivery or return lines with closed-cell foam, or similar products. A larger cylinder or more powerful heating elements could be considered, or the system could be converted to a continuous-flow gas califont. A gas califont would be a more compact and lightweight system, without the time intensive requirement to preheat the cylinder. Complete flow-through treatment of a pipework system may also be possible with a gas califont due to the greater heating capacity. A gas califont, however, would pose additional risks (i.e., flammability) to those associated with the use of electricity for pump operation.

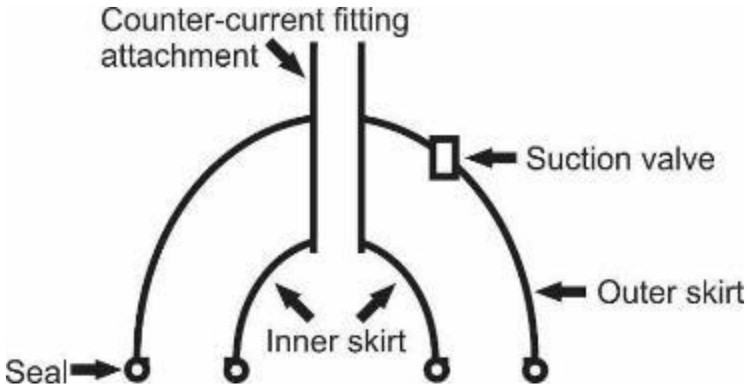

Figure 46. Concept drawing of a double-skirted suction cup. Not drawn to scale.

One aspect of the draft treatment protocol that was not validated in the field was the inclusion of a flow-through step for highly restricted pipework systems (Appendix 2, Step 1.8). The only instance where a flow-through step would have been likely to improve heating dynamics was the engine-cooling system on board vessel F. However, engine-cooling systems typically contain barriers to flow in the form of positive displacement impellers. There may be value in including this method in the operational protocol but scenarios for its application are limited and should be validated in the field. Systems that could benefit from a flow-through step are likely to be at low risk of containing fouling because seawater exchange is insufficient to sustain survival of macrofouling organisms (Cahill et al., 2018). Recirculation alone is therefore generally sufficient to mitigate biosecurity risk.

4.3 FINALISATION OF THE TREATMENT PROTOCOL

The field validation of the CRYPT informed the finalisation of a treatment protocol applicable to a subset of vessel pipework systems under defined conditions (Appendix 4). The protocol also details potential modifications to increase the diversity of pipework systems that can be treated using CRYPT, and a framework to validate such modifications. Sections 4.3.1 and 4.3.2 contain excerpts from the final treatment protocol (*in italics*) for discussion purposes.

4.3.1 CRYPT treatment protocol

Notable inclusions to the protocol based on data and practical insights gained from the field validation include:

- criteria for applying CRYPT;
- quality control monitoring methods;
- consideration of the utility of the flow-through step; and
- strategies to deal with difficult-to-seal inlets or outlets.

4.3.1.1 Criteria for applying CRYPT (Section A, Appendix 4)

The following criteria should be met to apply CRYPT in a biosecurity response scenario:

1. *vessel is berthed in water;*
2. *ambient temperatures above 16 °C;*
3. *pipework inlets or outlets are accessible by divers and do not have inlet grates;*

4. *expandable test plug can be fitted to the pipework inlets or outlets;*
5. *level of fouling around the inlet or outlet does not exceed the CRMS (minimise risk of dislodging hull fouling when applying CRYPT);*
6. *temperature probes can be stationed at three or more locations along the length of the pipework system from inside the vessel; and*
7. *pipework systems do not contain heat sensitive components (e.g., desalination plants), or heat sensitive components can be isolated.*

Because a high degree of certainty of treatment efficacy is essential in biosecurity response scenarios, the above criteria directly reflect the conditions under which the field validation was effective. Only pipework systems able to be sealed with an expandable test plug have been deemed suitable for CRYPT treatment because the suction cup installation procedure is cumbersome and poses high risk for seal failure. Likewise, the quality control measure of three temperature probes stationed along the length of the pipework system has been mandated because simpler measures (e.g., return line temperature) were not conclusively validated in the field. It is likely that the criteria detailed could be relaxed if CRYPT is assessed under a wider range of scenarios but any such changes must be grounded on robust and quantitative information (Section 4.3.2). For example, CRYPT may be suitable for use on dry-docked vessels but validation is essential and protocols may need to be tailored accordingly.

4.3.1.2 Quality control monitoring method (Section B, Appendix 4)

Monitor, in real time, temperatures at three locations along the length of the pipework system.

1. *Thermocouple temperature probes affixed externally to pipework features from inside the vessel using electrical insulation tape and a layer of 5-mm closed cell foam.*
2. *Probes should be stationed: near the inlet; near the terminus of the 'wet section' of the pipework system; and approximately equidistant between these two points.*
3. *Preferable to station probes on metallic pipework or fittings; if plastic pipework or fittings are used apply a correction of +10 °C.*
4. *Monitor temperatures real time to ensure 58 to 65 °C is achieved at all three probe locations within 45 min of activating CRYPT, and that temperatures are maintained within this zone for at least 60 min.*
5. *Record and store temperature profile information for auditing purposes.*

There were indications from the laboratory experiments that return line temperature could be used as a quality control method but field results were inconclusive. Further validation and refinement of the return line proxy could be pursued to streamline the quality control measure. Alternative approaches could also be considered, such as integrating a temperature probe to the sealing device to monitor temperatures directly inside the inlet or outlet. It is uncertain if monitoring directly at the inlet or outlet is sufficient but the even heat distribution seen for the effectively treated pipework systems suggests this approach could be useful if validated.

4.3.1.3 Strategies to deal with difficult-to-seal inlets or outlets (Step 1, Section E, Appendix 4)

Issue: seal integrity is compromised at the inlet or outlet evidenced by leaking heated water or air bubbles.

Expandable test plugs are the only validated sealing apparatus. Ensure the expandable test plug: is appropriately sized for the inlet or outlet; has been adequately expanded; has not been expanded off-of-centre; and the inlet or outlet does not have multiple lips or similar that interfere with the seal. If an intact seal cannot be achieved, abandon the treatment and pursue alternative treatment approaches.

The treatment protocol reflects currently available 'best' sealing procedures but, based on the field validation, pipework inlets or outlets not able to be sealed with expandable test plugs will regularly be encountered. Designing and constructing more and better sealing devices is the most pressing requirement for expanding the range of pipework systems that can be treated by the CRYPT.

4.3.1.4 Consideration of the utility of the flow-through step

The flow-through step has been deleted from the treatment protocol because it was not validated in the field. Pipework systems suitable for flow-through treatment were not encountered during the field validation but scenarios could conceivably be encountered operationally where this procedure allows for effective treatment of pipework systems that would otherwise be untreatable. In such instances, the testing regime to validate additions or improvements to the CRYPT (Section 4.3.2) could be applied to confirm the utility of the flow-through procedure detailed in the draft treatment protocol (Section A, Step 8, Appendix 2).

4.3.2 Testing regime to validate alterations to the CRYPT apparatus or protocol

Potential modifications to enhance the utility of CRYPT have been identified (Section 4.2), and could be developed, validated, and implemented by end users:

- wider range of pipework inlet or outlet sealing devices;
- more user-friendly quality control measures;
- applicability under a wider range of ambient environmental conditions; and
- enhanced heating capacity of CRYPT.

All four of these classes of potential modifications to the CRYPT system can be validated by testing on a representative range of vessels under suitable ambient conditions (Section F, Appendix 4). All validations should be performed on vessels that pose low or no biosecurity risk, likely domiciled vessels, to account for the possibility of failure of not yet validated apparatus or procedures.

Modifications to the CRYPT apparatus or procedures could include:

- *pipework inlet or outlet sealing devices, such as a double-skirted suction cup or custom-made expandable test plugs for small pipework diameters (< 37 mm);*
- *quality control measures, such as measuring temperatures using a thermocouple probe integrated to the sealing device;*
- *criteria for application, such as a wider range of ambient environmental conditions or for pipework systems with inlet grates;*
- *heating capacity, such as insulating delivery and return lines or higher output heating elements; and*
- *treatment procedures, such as a flow-through step for complex, small diameter pipework systems.*

In all instances, modifications must be validated on at least three vessel pipework systems that pose low or no biosecurity risk (i.e., domiciled vessels) and are representative of the intended application for the modification. For example, a sealing device intended for use on pipework systems with inlet grates and nominal diameters from x to y mm should be validated on at least three independent pipework systems with inlet grates that span the full range of nominal diameters specified.

Validation criteria as follows:

1. *At least three independent pipework systems, each on a different vessel that pose low or no biosecurity risk (i.e., domiciled vessels), are used for validation of the modified apparatus or procedure. Pipework systems should be carefully selected to represent*

the range of scenarios the modification aims to address. In some instances, more than three pipework systems spanning a greater range of scenarios may need to be included. Modifications are only validated for pipework types, configurations, and features that they have been directly tested on.

2. *External conditions match those under which the modification will be implemented operationally. External conditions to consider include: ambient air and seawater temperature; vessel location and orientation; in water versus dry docked; and level of fouling around the inlet or outlet. Modifications are only validated for external conditions under which validation experiments have been performed.*
3. *Temperature is monitored via at least three externally placed thermocouple probes according to Section B. Temperature profiles at all sampling locations should reach the target treatment zone of 58 to 65 °C within 45 min, and be maintained within this zone for the subsequent 60 min. Failure to meet these temperature requirements deems the treatment ineffective.*
4. *The integrity of the seal is monitored by divers: within 10 minutes after activating CRYPT recirculation; and just prior to deactivating CRYPT recirculation. Seal integrity must be 100%, with any leakage deeming the treatment ineffective. Leakage is evidenced by: air or heated water escaping around the seal; or unexpectedly low or fluctuating temperature profiles.*
5. *All data and analyses from validation experiments, including failed testing results, should be stored and made available for auditing purposes.*

Lethal thermal parameters for vessel biofouling are well established and remaining hurdles for applying heat for biosecurity treatment purposes centre on engineering requirements. The above detailed validation procedures will allow end users to develop additional apparatus, and associated procedures, to expand the scope and applicability of CRYPT.

4.4 CONCLUSIONS AND RECOMMENDATIONS

The CRYPT is a functioning prototype for lethal thermal treatment of biofouling in internal pipework systems of recreational vessels. The tiered experimental approach employed during the laboratory phase of the project allowed for rapid optimisation of system parameters in a controlled setting. Subsequent field validation confirmed the utility of the devices and procedures, while uncovering a range of additional operational considerations. The CRYPT functioned to specification in the field when pipework inlets or outlets could be effectively sealed and ambient heat loss was minimised.

The outcomes of the laboratory and field experiments informed a final treatment protocol for applying CRYPT to a subset of vessel pipework systems under defined conditions. Included in the treatment protocol are guidelines for validating additional apparatus or modified procedures to expand the range of pipework systems that can be effectively treated using the CRYPT. The current limitations of the CRYPT largely pertain to engineering challenges, such as the ability to form an intact seal for the highly diverse range of pipework inlets or outlets encountered in the field. The treatment protocol and validation procedures presented could eventually allow for the full diversity of pipework systems on board recreational vessels to be treated, whilst maintaining the integrity and adaptability of the CRYPT system.

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Appendix 1

Biosecurity procedures for field transfer and laboratory experiments involving oysters

Pacific oysters (*Magallana gigas*) for use in laboratory experiments associated with MPI project 405135 – Reactive Pipework Treatment for Recreational Vessels (Cawthron project 16345) will be sourced from Marlborough Oysters Limited (MOL) farms in Croisilles Harbour. A bin of live oysters (50 to 60 mm shell length, approx. 1600 individuals) will be transported via road directly from a MOL sourcing boat in Croisilles Harbour to Cawthron Institute's Halifax St campus in Nelson. Croisilles Harbour falls within the *Bonamia ostreae* Controlled Movement Area and ostereid herpesvirus type-1 µvar has been previously detected at these farms. An enquiry with MPI's Spatial Allocations Team confirmed that no permits are required for this transfer because the oysters are to be transferred within the Controlled Movement Area and the destination is not a marine farm. Regardless, Cawthron will implement best-practice biosecurity procedures to minimise the risk of disease transfer.

- All experimental work will be undertaken within the Halifax St wet laboratory. The experimental trials involve acclimating oysters to laboratory conditions, and then heat treating and monitoring oyster mortality. Containing all experimental procedures to a single laboratory space will avoid any risks associated with transferring oysters to other laboratories within Cawthron.
- A self-contained recirculating seawater system in the wet laboratory will house the oysters (and only the oysters) for the duration of the acclimation and monitoring periods (up to 1 month). The recirculating system is temperature controlled, and water is UV sterilised and sand filtered.
- Access to the wet laboratory is for approved personnel only, and the experiments for this project will be restricted to a confined and reserved area. Signs will be erected to prevent unnecessary contact with the recirculating system or the oysters.
- When tending to the oysters, personnel will avoid splashing or otherwise transferring water from the recirculating system.
- Clothing (lab coats and waterproof aprons) and footwear (gumboots) dedicated to the reserved area of the wet lab will be provided for all personnel. Clothing and footwear will be washed/disinfected at the end of the experimental period, and during the experimental period if required (e.g., dirty clothing).
- Disposable laboratory gloves will be provided, and personnel will thoroughly wash their hands at dedicated hand wash stations after contact with the oysters or recirculating system.
- At the end of all experiments the recirculating system will be: completely drained and filter material removed; flushed with hot tap water; and left unfilled for 2 weeks to desiccate.
- Water from the recirculating system will be collected, and then disposed to an unused area of gravel at the Halifax St campus. The Halifax St campus is approximately 1.5 km inland from the nearest shoreline, and there are no drains (or similar) associated with the area to which water will be dumped. After dumping water, the area of gravel will be cordoned off for at least 2 weeks, or until all water has drained away and the area is dry to touch.
- All oysters used in experiments, and any surplus oysters, will be humanely dispatched in hot water, double bagged in strong-walled plastic bags, and disposed to landfill at the

end of the experimental period. Filter material from the recirculating systems will be handled and disposed in the same manner as the oysters.
- Any additional equipment that contacts the oysters or recirculating system will be rinsed in hot tap water and left to desiccate for two weeks at the end of the experimental period.

The biosecurity procedures detailed above have been formulated specifically for this project, and are standalone from the biosecurity Standard Operating Procedures in place at Cawthron Aquaculture Park (CAP). Relevant personnel at CAP will be consulted throughout the proposed experiments to ensure that the activities associated with this project do not jeopardise biosecurity at CAP.

Appendix 2

Draft treatment protocol for field validation

In Stage 3 of MPI project 405135 – Reactive Pipework Treatment for Recreational Vessels, the operational parameters determined for the CRYPT were validated on six vessels domiciled in Nelson Marina, Nelson, NZ. This draft treatment protocol, Health and Safety guidance, and practical trouble-shooting information were formulated based on the results of laboratory experiments performed on a series of 'mock pipework systems'.

A. General procedures

1. Fill the hot water cylinder with freshwater, set thermostat to 72.5 °C, switch on heating element, and monitor cylinder temperature until 72.5 °C is achieved.
2. Where present, remove strainer basket(s) from pipework system to be treated.
3. Fit appropriately sized (to match pipework inlet being treated) expandable test plug to the counter-current fitting using thread tape to ensure a water-tight seal. From inside the vessel, measure the approximate distance from the inlet of the pipework system to the first bend in the pipework system. Subtract 20 mm from this value and cut a section of 12mm Buteline™ to this length; fit Buteline™ tube to the copper delivery tube.
4. Via SCUBA diver, insert the Buteline™ tube into the pipework inlet until the expandable test plug is flush with the hull. To seal the pipework system, expand the test plug using an appropriately sized spanner. Continue expanding the plug until 'moderate' lateral force on the counter-current fitting does not dislodge the plug. Before activating the CRYPT, SCUBA divers must standoff at least 2 m from the inlet of the pipework system being treated. Once the CRYPT has been running for several minutes, divers should visually inspect the expandable test plug for leakage; deactivate the CRYPT before any adjustment to the expandable test plug. Once satisfied that the expandable test plug is forming a water-tight seal, divers may exit the water.
5. Close the delivery line ball valve to prepare for priming of the return line. To prime the return line either: deliver seawater to the return line via the priming pump; or deliver freshwater from reticulated supply. Continue priming until the delivery tube and sight glass is completely free of air[9].
6. Activate the main pump and adjust the flow rate to the maximum of 20 L min^{-1}. Flow rate can be monitored via the sight glass flow indicator; the turbine should be spinning constantly with no air bubbles present.
7. For short pipework systems (< 1 m), pipework systems lacking multiple bends, and large diameter (> 75 mm) pipework systems, recirculation is sufficient to diffuse heat to 'high risk' locations. Monitor temperatures[10] until 60 ± 2 °C is achieved at sampling ports, and monitor that this temperature is maintained for an additional h.
8. For highly restricted pipework systems with multiple bends (> 1 bend) and small diameter pipework (≤ 25 mm), an additional flow-through step is required. After activating the main pump (i.e., system is under recirculation), open the priming valve and supply additional water to the system via the priming pump or from reticulated supply. Open each terminal end of the pipework system being treated (e.g., open taps on ancillary seawater supply facilities). Water will flow from the terminal end; monitor until temperatures have met 60 °C or have stabilised at the terminal end, and then close the terminal end. In succession, repeat the flow-through process for each individual component of the pipework system being treated. Once the flow-through procedure has

[9] Visible through transparent return line.
[10] Monitoring locations to be determined via *in situ* testing.

been applied to each individual component of the pipework system being treated, close the priming valve, deactivate the priming mechanism, and leave to recirculate as per Step 7.

9. After the treatment period of 60 min[11], deactivate the main pump. As a precaution, allow the 20 min before SCUBA divers remove the expandable test plug from the pipework inlet.

B. Health and Safety

1. Appropriate SCUBA diving protocols should be adhered to, including formulating tailored dive safety plans[12]. All divers should be appropriately qualified.
2. The CRYPT incorporates a range of electrical systems. Although these electrical systems have been designed for use outdoors and incorporate residual cut-out protection, splashes, accidental submersion, or rain ingress pose electrocution risks. Take care to protect electrical components from water and consider additional protection (e.g., water proof cover or umbrella) if operating in inclement conditions.
3. The minimum temperature that can burn human skin in a finite amount of time is 44 °C.[13] Avoid contact with the 60 to 65 °C water produced by the CRYPT, and wear appropriate personal protective equipment when contact is likely. Thick rubber gloves and full-face safety visors are sufficient in most instances. Divers should take care when removing expandable test plugs post-treatment; long-sleeved wet or dry suits and neoprene gloves should be worn.
4. Operators should have adequate indemnity insurance to cover inadvertent damage to vessels or other infrastructure.

C. Practical trouble-shooting

1. *Issue: recirculation halts unexpectedly*.
   If the sight glass flow indicator registers no flow, a blockage or air lock has formed in the return line and should be removed by: momentarily deactivating the main pump; closing the delivery line; and activating the priming mechanism. Ensure all air or blockages have been removed from the return line[14], and then recommence recirculation via the main pump. Carefully monitor the sight glass flow indicator and temperatures at relevant pipework locations, which should quickly return to the targeted treatment zone.
2. *Issue: recirculation proceeds under non-linear flow or cavitation.*
   If the sight glass flow indicator is pulsing or operating sporadically, the duty cycle of the system has been exceeded. Sequentially reduce the speed of the main pump using the variable speed drive unit until the sight glass flow indicator is turning at a constant rate.
3. *Issue: temperature of 60 ± 2 °C is not met at targeted pipework locations or the return line.*
   The target temperature may not be met for several reasons. Check the following and remedy if appropriate: the expandable test plug is forming a water tight seal at the pipework inlet; the cylinder temperature is at 72.5 ± 2 °C; the delivery and return lines are intact with no leaks; the pipework being treated is isolated from the sea (e.g., check for additional inlets or outlets); there are no unexpected barriers to flow in the pipework being treated (e.g., positive displacement impellers); and water is recirculating at a constant rate.
4. *Issue: temperatures exceed 65 °C within pipework or at the return line.*

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[11] Longer treatment periods can be specified if *in situ* testing identifies pipework systems for which thermal dissipation is not adequate inside 1 hour.

[12] Outside of scope of this document.

[13] “Pathophysiology of thermal burn injury" (DOC). Civic Plus. 2007. Retrieved 11-07-17.

[14] Visible through clear tubing.

If temperatures exceed threshold values at any point, immediately deactivate the CRYPT and drain the pipework being treated by removing the expandable test plug from the pipework inlet. Consider reducing cylinder temperature setting to below 72.5 °C for a subsequent treatment attempt.

5. *Issue: flow-through not possible.*
   Some smaller diameter pipework may not be amenable to a flow-through step due to barriers to flow or heat sensitive components further into systems (e.g., desalination plants). Because systems with inherently restricted flow characteristics are considered 'low risk', use recirculation only when flow-through is not feasible for smaller diameter pipework. In some instances, certain components of an ancillary seawater supply system will be amenable to a flow-through step while others will not. Apply flow-through step to suitable components only, and then commence recirculation.
6. *Issue: one or more circuit breakers are tripped.*
   An electrical fault has occurred. Immediately disconnect power to the CRYPT and carefully check all electrical components for obvious damage or water ingress. If all components are cleared, rectify the tripped breaker(s) and repower the CRYPT. If the electrical fault recurs, seek assistance from a registered electrician.

Appendix 3

To allow indirect assessment of temperatures inside pipework systems of recreational vessels, thermocouple temperature probes were mounted externally to pipework features. A range of pipework features were encountered in the field and this appendix describes laboratory experiments to validate correction factors for both metallic and plastic pipework features.

Metallic pipework features assessed were:

- a 25-mm stainless steel ball valve; and
- a 32-mm brass tee fitting.

Plastic pipework features assessed were:

- a 25-mm polyethylene ball valve;
- a section of 32-mm wire reinforced silicone hose; and
- a section of fibre-reinforced flexible polyvinylchloride hose.

Each pipework feature was blanked off using appropriately sized rubber bungs, so that only one end was left open. A thermocouple temperature sensor (Pico Technology, St Neots, United Kingdom) was affixed to the outside of each pipework feature using electrical insulation tape and a layer of 5-mm closed cell foam to isolate from the ambient environment. A temperature sensor was also inserted into the pipework feature through the open end. Fittings were filled with seawater preheated to 60 °C and an additional rubber bung used to seal the open end. Temperatures were stabilised (i.e., external temperature stopped increasing) and the procedure was repeated three times for each fitting. The time to stabilise gave a measure of lag, while the difference between internal and external temperatures once stabilised gave a correction factor (Table A3.1).

Table A3.1. Time for temperatures to stabilise (lag) and correction factors (internal temperature – external temperature) for indirect monitoring of seawater temperatures inside a range of pipework fittings (*n* = 3).

		Lag (min ± SD)	Internal temp. (°C ± SD)	External temp. (°C ± SD)	Correction factor (°C)
Metal fittings	Stainless steel ball valve	1.0 ± 0.2	50.6 ± 1.5	49.3 ± 0.5	1.3
	Brass tee	0.7 ± 0.1	48.2 ± 1.1	48.4 ± 0.5	0.2
Plastic fittings	Polyethylene ball valve	11.4 ± 2.1	48.4 ± 0.8	38.2 ± 1.1	10.2
	Silicone hose	4.8 ± 0.2	49.6 ± 0.3	41.7 ± 2.1	7.9
	Polyvinylchloride hose	7.4 ± 0.4	48.8 ± 1.4	39.3 ± 1.4	9.5

Appendix 4

Final treatment protocol post-field validation

Protocol for applying CRYPT system to recreational vessel pipework systems in reactive biosecurity response scenarios that was developed based on the outcomes of laboratory experiments and field validation.

A. Scenarios for CRYPT treatment

The following criteria should be met to apply CRYPT in a biosecurity response scenario:

1. vessel is berthed in water;
2. ambient temperatures above 16 °C;
3. pipework inlets or outlets are accessible by divers and do not have inlet grates;
4. expandable test plug can be fitted to the pipework inlets or outlets;
5. level of fouling around the inlet or outlet does not exceed the CRMS (minimise risk of dislodging hull fouling when applying CRYPT);
6. temperature probes can be stationed at three or more locations along the length of the pipework system from inside the vessel; and
7. pipework systems do not contain heat sensitive components (e.g., desalination plants), or heat sensitive components can be isolated.

B. Quality control measure

Monitor, in real time, temperatures at three locations along the length of the pipework system.

1. Thermocouple temperature probes affixed externally to pipework features from inside the vessel using electrical insulation tape and a layer of 5-mm closed cell foam.
2. Probes should be stationed: near the inlet; near the terminus of the 'wet section' of the pipework system; and approximately equidistant between these two points.
3. Preferable to station probes on metallic pipework or fittings; if plastic pipework or fittings are used apply a correction of + 10 °C.
4. Monitor temperatures to ensure that 58 to 65 °C is achieved at all three probe locations within 45 min of activating CRYPT, and that temperatures are maintained within this zone for at least 60 min.
5. Record and store temperature profile information for auditing purposes.

C. General procedures

1. Fill the hot water cylinder with freshwater, set thermostat to 72.5 °C, switch on heating element, and monitor cylinder temperature until 72.5 °C is achieved.
2. Where present, remove strainer basket(s) from pipework system to be treated.
3. Before sealing the inlet or outlet, prime the delivery and return lines. With the counter-current fitting submerged in the sea, close the delivery line ball valve and open the priming valve. Prime the return line by delivering: seawater from the priming pump; or freshwater from reticulated supply. Continue priming until the return line is completely free of air. Repeat process for the delivery line by closing return valve and opening the delivery valve.
4. Use expandable test plugs to seal inlets/outlets – this is the only validated sealing method.
    i. Fit appropriately sized (to match pipework inlet being treated) expandable test plug to the counter-current fitting using thread tape to ensure a water-tight seal.
    ii. From inside the vessel, measure the approximate distance from the inlet of the pipework system to the first bend in the pipework system. Subtract 20 mm from

this value and cut a section of 12-mm Buteline™ to this length; fit Buteline™ tube to the copper delivery tube.

iii. Via SCUBA diver, insert the Buteline™ tube into the pipework inlet until the expandable test plug is flush with the hull. To seal the pipework system, expand the test plug using an appropriately sized spanner. Continue expanding the plug until 'moderate' lateral force on the counter-current fitting does not dislodge the plug.

5. Once the sealing device has been applied, SCUBA divers must standoff at least 2 m from the inlet/outlet of the pipework system being treated.
6. Activate the main pump and adjust the flow rate to the maximum of 20 L min^{-1}. Flow rate can be monitored via the sight glass flow indicator; the turbine should be spinning constantly with no air bubbles present.
7. Once the CRYPT has been recirculating for several mins, divers visually inspect the sealing device for leakage as evidenced by escaping air bubbles or heated water. Deactivate the CRYPT before any adjustment to the sealing device. Once satisfied that the sealing device is forming a watertight seal, divers may exit the water.
8. Monitor temperatures until 58 to 65 °C is achieved at the temperature monitoring points, and ensure that this temperature range is maintained for an additional 60 min (Section B).
9. After the treatment period of 60 min, deactivate the main pump. Divers remove the sealing device from the pipework inlet or outlet.

D. Health and Safety

1. Appropriate SCUBA diving protocols should be adhered to, including formulating tailored dive safety plans[15]. All divers should be appropriately qualified.
2. The CRYPT incorporates a range of electrical systems. Although these electrical systems have been designed for use outdoors and incorporate residual cutoff protection, splashes, accidental submersion, or rain ingress pose electrocution risks. Take care to protect electrical components from water and consider additional protection (e.g., waterproof cover or umbrella) if operating in inclement conditions.
3. The minimum temperature that can burn human skin in a finite amount of time is 44 °C[16]. Avoid contact with the 60 to 65 °C water produced by the CRYPT, and wear appropriate personal protective equipment when contact is likely. Thick rubber gloves and full-face safety visors are sufficient in most instances. Divers should take care when removing expandable test plugs post-treatment; long-sleeved wet or dry suits and neoprene gloves should be worn.
4. Operators should have adequate indemnity insurance to cover inadvertent damage to vessels or other infrastructure.

E. Practical trouble-shooting

1. *Issue: seal integrity is compromised at the inlet/outlet evidenced by leaking heated water or air bubbles.*
   Expandable test plugs are the only validated sealing apparatus. Ensure the expandable test plug: is appropriately sized for the inlet or outlet; has been adequately expanded; has not been expanded off-of-centre; and the inlet or outlet does not have multiple lips or similar that interfere with the seal. If an intact seal cannot be achieved, abandon the treatment and pursue alternative treatment approaches.
2. *Issue: recirculation halts unexpectedly*.

[15] Outside of scope of this document.
[16] "Pathophysiology of thermal burn injury" (DOC). Civic Plus. 2007. Retrieved 11-07-17.

If the sight glass flow indicator registers no flow, a blockage or air lock has formed in the return line and should be removed by: momentarily deactivating the main pump; closing the delivery line; and activating the priming mechanism. Ensure all air or blockages have been removed from the return line[17], and then recommence recirculation via the main pump. Carefully monitor the sight glass flow indicator and temperatures at relevant pipework locations, which should quickly return to the targeted treatment zone.

3. *Issue: recirculation proceeds under non-linear flow and/or cavitation.*
   If the sight glass flow indicator is pulsing or operating sporadically, the duty cycle of the system has been exceeded. Sequentially reduce the speed of the main pump using the variable speed drive unit until the sight glass flow indicator is turning at a constant rate.
4. *Issue: temperature of 60 ± 2 °C is not met at targeted pipework locations and/or the return line.*
   The target temperature may not be met for several reasons. Check the following and remedy if appropriate: the expandable test plug is forming a water tight seal at the pipework inlet or outlet; the cylinder temperature is at 72.5 ± 2 °C; the delivery and return lines are intact with no leaks; the pipework being treated is isolated from the sea (e.g., check for additional inlets or outlets); there are no unexpected barriers to flow in the pipework being treated (e.g., positive displacement impellers); and water is recirculating at a constant rate.
5. *Issue: temperatures exceed 65 °C within pipework or at the return line.*
   If temperatures exceed threshold values at any point, immediately deactivate the CRYPT and drain the pipework being treated in a biosecure manor. Consider reducing cylinder temperature setting to below 72.5 °C for a subsequent treatment attempt.
6. *Issue: one or more circuit breakers are tripped.*
   An electrical fault has occurred. Immediately disconnect power to the CRYPT and carefully check all electrical components for obvious damage or water ingress. If all components are cleared, rectify the tripped breaker(s) and repower the CRYPT. If the electrical fault recurs, seek assistance from a registered electrician.

F. Testing regime to validate additions or modifications

Modifications to the CRYPT apparatus or procedures could include:

- pipework inlet or outlet sealing devices, such as a double-skirted suction cup or custom-made expandable test plugs for small pipework diameters (< 37 mm);
- quality control measures, such as measuring temperatures using a thermocouple probe integrated to the sealing device;
- criteria for application, such as a wider range of ambient environmental conditions or for pipework systems with inlet grates;
- heating capacity, such as insulating delivery and return lines or higher output heating elements; and
- treatment procedures, such as a flow-through step for complex, small diameter pipework systems.

In all instances, modifications must be validated on at least three vessel pipework systems that pose low or no biosecurity risk (i.e., domiciled vessels) and are representative of the intended application for the modification. For example, a sealing device intended for use on pipework systems with inlet grates and nominal diameters from x to y mm should be validated on at least three independent pipework systems with inlet grates that span the full range of nominal diameters specified.

[17] Visible through clear tubing.

Validation criteria as follows:

1. At least three independent pipework systems, each on different vessel that pose low or no biosecurity risk (i.e., domiciled vessels), are used for validation of the modified apparatus or procedure. Pipework systems should be carefully selected to represent the range of scenarios the modification aims to address. In some instances, more than three pipework systems spanning a greater range of scenarios should be included. Modifications are only validated for pipework types, configurations, and features that they have been directly tested on.
2. External conditions match those under which the modification will be implemented operationally. External conditions to consider include: ambient air and seawater temperature; vessel location and orientation; in water versus dry docked; and level of fouling around the inlet/outlet. Modifications are only validated for external conditions under which validation experiments have been performed.
3. Temperature is monitored via at least three externally placed thermocouple probes according to Section B. Temperature profiles at all sampling locations should reach the target treatment zone of 58 − 65 °C within 45 min, and be maintained within this zone for the subsequent 60 min. Failure to meet these temperature requirements deems the treatment ineffective.
4. The integrity of the seal is monitored by divers: within 10 minutes after activating CRYPT recirculation; and just prior to deactivating CRYPT recirculation. Seal integrity must be 100%, with any visible leakage deeming the treatment ineffective. Leakage is evidenced by: air or heated water escaping around the seal; or unexpectedly low or fluctuating temperature profiles.
5. All data and analyses from validation experiments, including failed testing results, should be available for auditing purposes.