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In-water Treatment of Biofouling in Internal Systems: Field Validation of Quaternary Ammonium Compound (QAC) Chemical Treatment Protocols

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ABSTRACT

Mussel growth in the internal sea water systems of Navy vessels can result in significant performance issues and biosecurity concerns for affected vessels. The primary in-water treatment method for mussel fouling of the internals of Navy vessels is to flush with a 1% detergent solution containing quaternary ammonium compounds (QAC). Parameters for the application of this treatment are based on previous research; however, much of the research has been conducted at small-scales under laboratory conditions. This study examined the efficacy of two commercial QAC solutions for treating mussel biofouling under realistic field conditions using experimental sea water piping systems. The efficacy of the QAC solutions was found to be highly dependent on the size of the mussels present. All treatment solutions were effective at killing large sized mussels in the pipework and sea chest of the system following a 24 h dosing period. In contrast, small mussels appeared resilient to the majority of treatment regimes tested. Changes in water temperature and increased exposure time to treatment chemicals did not enhance efficacy of treatment.

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Executive Summary

Ship internal sea water systems have long been recognised as high risk mechanisms for non-indigenous species (NIS) transfer due to their propensity to readily accumulate and shelter sessile and mobile marine species. Fouling in sea chests and sea water pipework is also an operational issue for marine engineers, as it restricts and changes water flow regimes to essential vessel systems and may enhance biocorrosion. In the past five years, there have been ten instances of unwanted mussel biofouling on RAN vessels, five of which involved sea chests and/or internal sea water system fouling.

While dry docking vessels is the surest method of effectively minimising biosecurity risks, the process is expensive, time consuming and has the potential to impact operational availability of vessels. In contrast, in-water treatment of vessel biofouling is significantly more cost effective. Non-oxidising disinfectant/sanitiser solutions containing quaternary ammonium compounds (QACs) are a recognised method for treating biofouling in sea water pipework systems.

The overall aim of the present study was to field-validate previous DSTO research assessing the usage parameters of QAC solutions for the control of mussels occurring in vessel sea water systems, using a replica experimental piping system. The study examined the effectiveness of two commercially available QAC disinfectants formulations, '*Conquest TGA*' and '*Quatsan*', in killing the southern Australian blue mussel *Mytilus galloprovincialis planulatus*. *Conquest* is currently recommended to the RAN for emergency biosecurity response treatment and the use of *Quatsan* as a biosecurity response agent has been previously studied by DSTO, NT Fisheries and Neil and Stafford [1, 2]. These agents were therefore chosen for further examination during this study.

This study showed that the efficacy of two commercially available QAC formulations (*Conquest* and *Quatsan*) in treating mussel biofouling of sea water systems was highly dependent on the size of the mussels present. Treatment solutions of both *Quatsan* and *Conquest* appeared very effective at killing large sized (50 – 90 mm) mussels in the pipework and sea chest environments following a 24 h dosing period. 100% mortality of large mussels was achieved in all treatment groups, with the exception of the 1% *Conquest* treatment group. In contrast, small (0 – 30 mm) sized mussels appeared quite resilient to the majority of treatment regimes tested, with 100 % mortality throughout the entire test

system only recorded for one of the treatment regimes (5% *Quatsan*). Changes in water temperature and increased exposure time to treatment chemicals did not enhance efficacy of treatment.

Despite this study showing that the efficacy of QAC treatment varies with respect to mussel size, its use as an effective biosecurity emergency response tool should not be discouraged. Rather, QAC treatment should be used in conjunction with other management strategies to ensure its effectiveness against unwanted mussel species. Despite the additional intervention steps required of this approach, the cost of treatment and disruption to operational availability would still be significantly less than if the vessel were to be placed in dry dock and treated.

Based on the findings of the current study assessing the efficacy and usage parameters of QAC solutions for the control and eradication of mussels in sea water systems under field conditions, we recommend the following:

1. Revise the recommended RAN QAC emergency response dosing protocols for controlling mussel biofouling to 5% v/v disinfectant solution for 24 h, rather than the currently recommended protocol of 1% v/v for 14h.

2. Mandatory follow-up inspections and monitoring of vessels found to contain unwanted mussel species to ensure resistant organisms have not survived and grown after initial QAC treatment.

3. Procurement and storage of sufficient quantities of a selected commercial QAC disinfectant across all RAN bases where vessels are berthed.

4. QAC treatment of mussel fouling should be viewed as an emergency response option, not an on-going management strategy.

Authors

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Richard Piola joined the Environmental Research and Biotechnology Group in 2010. After completed his PhD in Marine Ecology and Ecotoxicology from the University of New South Wales in 2007, Richard worked for several years as a research scientist and consultant with the Cawthron Institute in New Zealand, specialising in the fields of marine biosecurity, vessel biofouling assessment and management, and the development of tools for the control and eradication of unwanted marine pests. His primary research interests at DSTO continue to be biofouling and marine pest management, biofouling control, and the improvement of biosecurity inspection and incursion response protocols.

Clare Grandison
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Clare Grandison joined DSTO in 2009 after completing a BEnvSc(Hons) at RMIT University. She also holds a MSocSc (International Urban and Environmental Management). Since joining the Environmental Research and Biotechnology Group, she has been involved in research into marine pollution control and management (including ship board waste auditing), fouling prevention strategies for internal seawater systems, marine biosecurity management as well as performing environmental and technological risk assessments for new acquisitions and fleet-in-being.

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1. Introduction

The International Maritime Organisation (IMO) considers the introduction of marine non-indigenous species (NIS) as one of the major threats to the world's marine environments, together with marine pollution, the exploitation of marine resources and the physical alteration/destruction of marine habitats [3]. Concern primarily centres around on the potential for NIS to reach pest densities in new environments, making them a major threat to the diversity, health and economic potential of coastal regions worldwide [4-8]. Of the numerous human-mediated transport vectors of NIS within the marine environment, shipping is almost certainly the most prevalent and important [4, 9], having the capacity to transport suites of viable organisms across international and domestic borders via mechanisms such as ballast water and internal and external vessel biofouling [10].

In response to marine biosecurity risks associated with shipping, the Australian federal and state/territory governments, along with marine industries and marine scientists, have implemented the Australian National System for the Prevention and Management of Marine Pest Incursions (hereafter referred to as the National System) [11]. The National System aims to prevent new marine pests arriving, coordinate and support a response when a NIS does arrive in a region, and minimise the spread and impact of those marine pests already established in Australia [11]. In the case of shipping, this is largely achieved through the development of guidelines and frameworks for the effective management of ballast water and biofouling, port monitoring and surveillance, and the coordination of incursion response events when unwanted species are detected.

The Royal Australian Navy (RAN) has long acknowledged the importance of sound environmental management and in recent years has faced an increasingly complex range of environmental issues and regulations that impact on the way it operates. Among these, is the problem of NIS introductions to Australian waters, primarily as a result of biofouling on Navy vessels returning from overseas operations. Over recent years the Navy has taken a proactive approach to addressing risks associated with marine biosecurity and has developed a comprehensive marine biosecurity management framework [12].

Even when a vessel is painted with a biocidal coating and maintained to specifications, biofouling can still establish in vessel niche areas, such as gratings, bow thrusters and propeller shafts [13-17]. Primary niche areas on larger vessels are the sea chests and internal sea water pipework systems. Internal sea water systems have been identified as high risk mechanisms for non-indigenous species (NIS) transfers due to their propensity to readily accumulate and shelter both sessile and mobile marine species [14]. Fouling in sea chests and sea water pipework is also an operational issue for marine engineers, as it restricts water flow to essential vessel systems and may enhance biocorrosion [18, 19].

In the past five years, there have been ten instances of unwanted mussel biofouling on RAN vessels [20]. On five of these occasions the unwanted mussels were found within the sea chests and/or internal sea water systems of the infected vessels. In all cases the priority unwanted species in question were mussels, of which particular concern was given to the Asian green mussel, *Perna viridis* (AGM). The AGM appears on the Australian Government

trigger list for unwanted marine species[1], due its potential for economic, ecological and human health impacts [21].

In the event that an unwanted NIS is discovered, RAN incursion response actions are initiated (as described in [22]), with available biofouling treatment options ranging from in-water inspection and treatment of the vessel to removal of the vessel to land. While dry docking vessels is the surest method of effectively eliminating both external (i.e. hull) and internal (i.e. sea water systems) biofouling biosecurity risks, the process is expensive, time consuming and has the potential to impact operational capability and scheduling of affected vessels. In contrast, in-water treatment of vessel biofouling is significantly more cost effective; however, given the current ban on in-water hull cleaning in Australian waters [23], in-water treatment options are typically limited to the small scale treatment of internal niche areas (e.g. sea water systems).

Non-oxidising disinfectant solutions containing quaternary ammonium compounds (QACs) are a recognised method for treating biofouling in sea water pipework systems. QACs have long been known to contain anti-bacterial properties and are commonly used as surface sanitisers and disinfectants [24]; however, some formulations in particular have also been shown to be very effective molluscicides. These include QAC formulations containing active substances such as benzalkonium chloride (BAC; also known as alkyl dimethyl benzyl ammonium chloride (ADBAC)) and didecyl dimethyl ammonium chloride (DDAC). Some advantages of using QACs for mussel biofouling control include an inability of molluscs to readily detect the toxicant in the water [25] and the minimal damage these chemicals cause to exposed materials and infrastructure [26]. QACs have a history of being used for control of biofouling in the cooling water systems of industrial plants [27], but have similarly been used to eradicate mussel species from vessel sea water systems [1, 2].

Current RAN protocols for the in-water treatment of mussel fouling in internal vessel sea water systems prescribe the chemical treatment of affected systems with a 1% solution of QAC for an exposure period of 14 h. These recommendations are based on previous research [2, 28], including a study conducted by DSTO [1]. However, to date, much of the research into the molluscicide properties of QACs are based on small-scale trials conducted under controlled laboratory conditions. As such, there remains uncertainty as to the efficacy of QAC treatment with respect to numerous real-world factors, including:

- different mussel size classes,
- varying sea water temperature regimes,
- difficulties associated with dosing complex, high volume environments (i.e. actual sea water pipework systems),
- the effects of variable water quality parameters,
- seasonal variability, and
- biophysical complexity inherent in natural systems.

[1] http://www.marinepests.gov.au/national_system/how-it-works/emergency_management/trigger_list

2. Scope of study

The overall aim of the present study was to field-validate previous DSTO research assessing the efficacy and usage parameters of QAC solutions for the control and eradication of mussels occurring in vessel sea water systems, using a replica experimental piping system. In particular, there was a desire to simulate the actual treatment protocols that would be likely to occur under realistic treatment-response conditions (i.e. 'bucket chemistry'). The influence of mussel size and water temperature on the efficacy of QAC as a molluscicide was also examined, as these factors may have an influence on bivalve survival [1, 2, 26, 29], but have yet to be thoroughly investigated.

3. Materials and methods

3.1 Test organisms

Australian blue mussels, *Mytilus galloprovincialis planulatus*, were selected as the test organism for all experimental treatment trials. Mytilid mussels are a ubiquitous component of inter- and subtidal marine communities worldwide and are considered as key species and important habitat engineers in benthic communities [30]. They possess high productivity, high fecundity and wide ecological tolerances that allow them to adapt to various environments [31], which may in part explain their proliferation as a ship fouling species [14, 15, 32, 33].

Fouling by mussels of ship internal sea water systems is a significant problem on many Royal Australian Navy (RAN) vessels, with *Mytilus galloprovincialis planulatus* often the primary culprit. This is particularly true for vessels based at *HMAS Stirling* (Fleet Base West), largely due to commercial farming of Australian blue mussels within the adjacent Cockburn Sound. However, RAN vessels are also prone to fouling by non-native mussel species.

Given both *Mytilus galloprovincialis planulatus* and *Perna viridis* belong to the family Mytilidae, and possess similar habits and physiologies, the native blue mussel provides a suitable test organism for better understanding and refining parameters of use of QAC solutions for the eradication of problematic non-native mussel fouling species.

3.2 Field experimental setup

Chemical dosing experiments were conducted in replica, purpose-built once-through seawater systems, situated pier-side at the DSTO Marine Coatings and Corrosion Test Facility, BAE Williamstown Shipbuilding Facility. During all trials, one system was dosed with the chemical treatment solutions while the second acted as a seawater-only control system. Each system operated completely independently and comprised:

- A 35 L grated sea chest (0.35 x 0.35 x 0.3 m^3) submerged at a constant depth of approximately 1m (Figure 1a),
- A Grundfos JP5 self-priming centrifugal pump (GRUNDFOS Holdings A/S, Bjerringbro, Denmark (Figure 1b),
- 6 m of 50 mm diameter flexible reinforced hosing (connecting the sea chest to the inlet port of the pump)

- 8 m of 50 mm diameter PVC piping (connected to the outlet port of the pump; Figure 1c), and
- Three 90 mm diameter in-line screw-top access ports inserted equidistant along the length of the PVC pipework (Figure 1d).

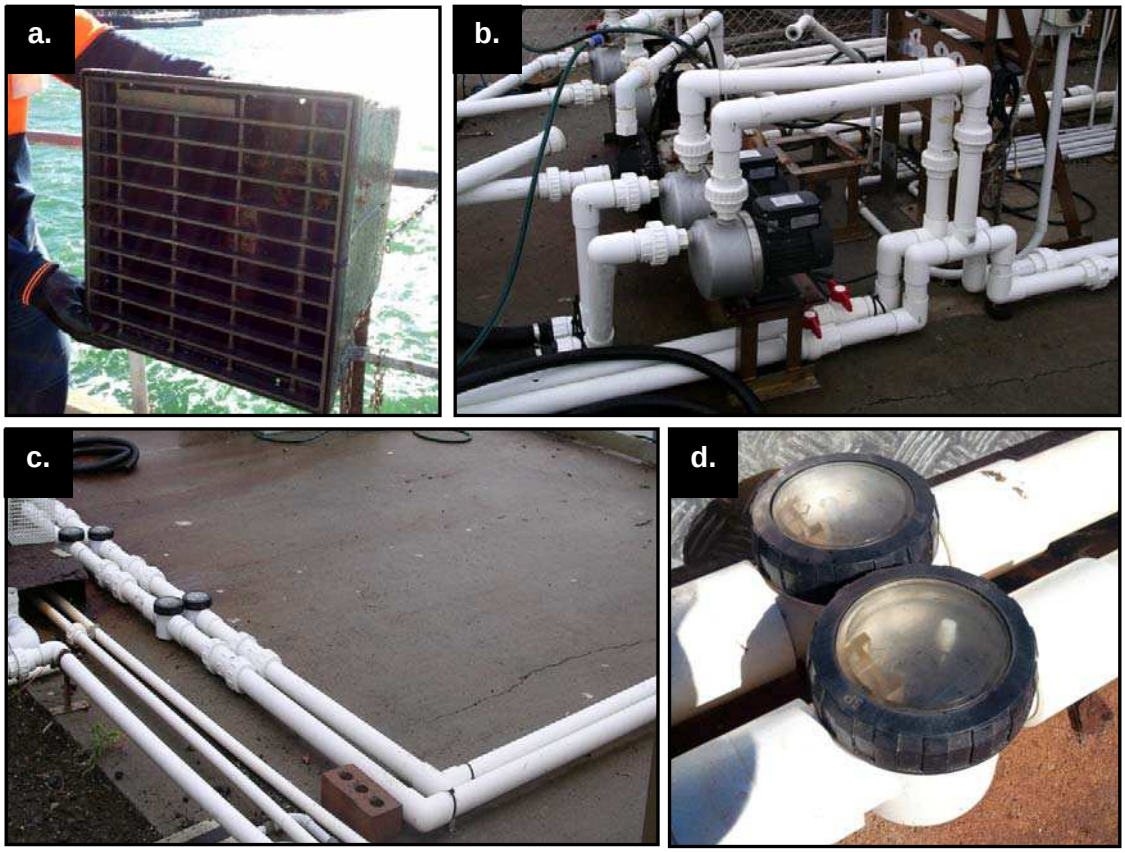

Figure 1. Elements of the purpose-built experimental sea water system, including (a) the grated sea chest, (b) pumps providing sea water, (c) 50 mm PVC piping comprising the pipework of the systems, and (d) two of the in-line screw-top access ports into which test mussels were placed for dosing

Treatment and control sea chests were fitted with a bleeder tap (attached via 4 m of 15 mm diameter hose) to facilitate removal of air from the system during operation. In addition, the sea chest of the chemical treatment system was fitted with a 32 mm hose inlet to facilitate introduction of the chemical treatment solution during dosing (see *Dosing Procedure* section 3.3). The seawater systems were designed in such a way that the pipework remained flooded with sea water even when the pumps were not operating (e.g. during a chemical dosing cycle). During all experimental trials, water temperatures within treatment and control sea water systems were recorded using UTBI-001 Tidbit v2 temperature loggers (Onset Computer Corporation, Bourne, MA), with one logger placed into the sea chests of chemical treatment and control systems, two loggers placed in the pipework of the treatment system, and one logger placed in the pipework of the control system.

3.3 Dosing procedure

When chemically dosing the sea water system of large vessels, a typical approach is to seal-off the sea chest from the water column using a blanking plate fitted with a hose leading to the surface. Once the blank is secured, a pre-mixed treatment solution is pumped via the hose into the sea chest (and associated internal sea water piping) until the solution is seen flowing from overboard discharges.

A similar method was used when chemically dosing the experimental sea water system in the present study (Figure 2). Prior to dosing, the seawater pump feeding the treatment system was switched off and a 15 mm plywood blank was used to seal the opening of the sea chest (Figure 2a). On the pier, a 100 L dosing drum was filled with the appropriate volumes of sea water and treatment chemical to achieve the desired dosing concentration (factoring in the volume of water already present in the system pipework; Figure 2b). Next, the treatment sea water system was converted to a closed system by placing the free end of the 32 mm sea chest dosing hose in the dosing drum, along with system outflow pipe (Figure 2c). Finally, the sea water pump was switched on for ~30 min to allow even mixing of the chemical solution throughout the entire system, after which the pump was switched off and the system allowed to stand idle for the prescribed dosing period. Following dosing, the sea chest blank was removed and the system flushed with fresh seawater for approximately 1 h prior to the removal and survival assessment of test mussels.

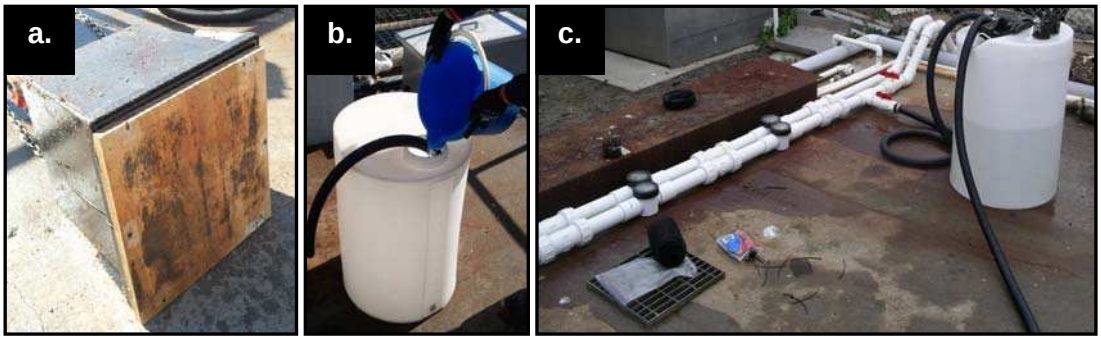

Figure 2. Elements of the chemical dosing procedure, including (a) application of a plywood blank to the open face of the sea chest, (b) mixing of the treatment chemical in the 100 L dosing drum, and (c) recirculation of the chemical solution throughout the sea water system

3.4 Experimental design

This study examined the efficacy of various concentrations of two commercially available QAC solutions, *Conquest*[2] and *Quatsan*[3], in killing small and large sized mussels over an exposure period of 24 h. *Conquest* is currently recommended to the RAN for emergency biosecurity response treatment and the use of *Quatsan* as a biosecurity response agent has been previously studied by DSTO, NT Fisheries and Neil and Stafford [1, 2]. These agents were therefore chosen for further examination during this study. The primary active molluscicide in both these formulations is benzalkonium chloride (BAC). Concentrations of

[2] *Conquest* sanitiser: http://www.shamrockchemicals.com.au/products/94-*Conquest*-sanitiser
[3] *Quatsan* disinfectant: http://www.northernchemicals.com.au/disinfectants

treatments solutions used were based on the findings of previous studies [1], and comprised 1, 2 and 5% v/v solutions prepared with field sea water.

Small (10 – 30 mm) and large (50 – 92 mm) mussels used during the experiment were collected from the lower intertidal zone on wooden structural supports of Booth Pier in Hobsons Bay (northern Port Phillip Bay), Victoria (Department of Primary Industries Fisheries Victoria General Research Permit RP963). Mussel experimental units comprised mesh cages containing either ten small or six large individuals (Figure 3a). Given detached mussels are more susceptible to the effects of toxic compounds [34], transplanted caged mussel were allowed to recover in a flow-through sea water tank (Figure 3b) for approximately 7 d prior to dosing (sufficient time for reattachment to occur; [35]). Prior to each dosing event, one mussel cage was placed in the sea chests of treatment and control sea water systems (Figure 3c), and three cages were placed at regular intervals along the pipework of each system (Figure 3d). Each treatment solution was tested across three replicate dosing events. The overall experimental design is shown in Table 1.

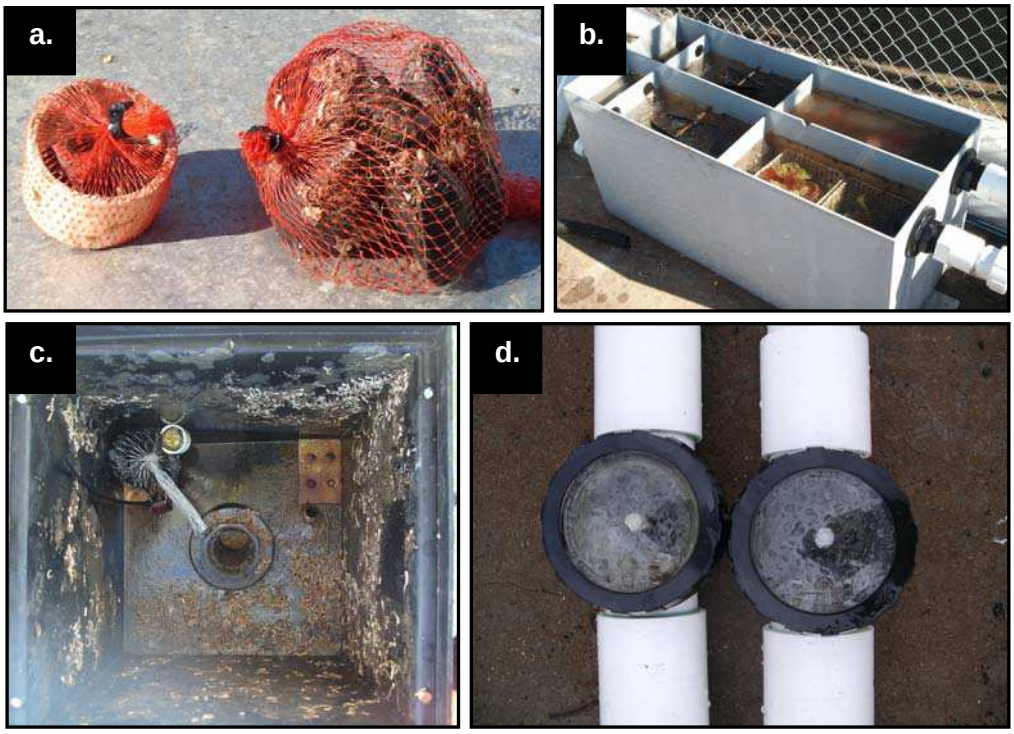

Figure 3. Photos showing (a) the small (left) and large(right) mussel experimental units used during the dosing trials, (b) the flow-through sea water holding tank used to hold mussels pre- and post-treatment, (c) mussels placed in the experimental sea chest prior to dosing, and (d) mussels placed in the pipework system prior to dosing.

Table 1. Summary of the experimental design for the main experiment, consisting of two chemical formulations at three concentrations, used to treat small and large mussels over a 24 h period, with post-treatment survival assessment made immediately after 24 h exposure and 5 – 7 d post-treatment.

Chemical	Concentration (v/v)	Mussel size	Exposure duration	Survival assessment (post treatment)
Conquest TGA® *Quatsan*®	1%, 2%, 5%	Small (10-30 mm) Large (50-90 mm)	24 h	24 h 5-7 d

Following completion of the aforementioned experiment it was decided to further examine the effects of dosing small mussels with 2% *Conquest* solution over a 48 h period. This was done to determine if a longer exposure period resulted in increased efficacy of treatment for lower concentration solutions. This additional experiment was conducted using the same methods and experimental design outline above.

3.5 Survival assessment

Following each replicate 24 h dosing event, mussels were examined for mortality. Determination of mortality comprised an assessment immediately following the 24 h chemical dosing period (the *24 h assessment*), followed by a repeat assessment 5-7 d later (the '*Post-treatment assessment'*) to determine any delayed mortality. The criterion assigned to assess mussel mortality was shell valve gape with no sign of closing in response to external stimuli. After 5-7 d, dead mussels were easily identified by either empty open shells, or partially open shells containing decaying tissue.

3.6 Water quality and chemical analysis

Water quality parameters of field site waters were recorded prior to the commencement of each dosing event. Parameters were measured using a Hydrolab DS5X sonde (Hydrolab Corp., Austin, TX) and included temperature, salinity, pH, turbidity and dissolved oxygen (DO). In addition, the pH of the prepared chemical treatment solution was recorded at the time of dosing. Water quality measurements of the dosing medium were not recorded at the completion of the 24 h dosing period because the water quality probe was too large to access the pipework system.

During the course of the experiment, a total of five discrete batches of *Conquest* and two discrete batches of *Quatsan* were used for dosing. Given that QAC concentrations present in these chemicals can be variable, with the manufacturers' specification for QAC content stated as <10% w/v for *Conquest* and between 10 and 60% for *Quatsan* and BAC as <10% for Quatsan, replicate (n = 2) samples (60 mL) of undiluted (neat) chemical were collected from individual batches to determine the initial levels of active benzalkonium chloride (BAC) present in each and identify possible variability among batches. Initial chemical analyses of neat solutions were done by DSTO staff using NMR spectroscopy [36]. Samples (60 mL) of diluted dosing QAC solutions were also collected at the time of dosing to determine the levels of active benzalkonium chloride present in actual experimental treatment solutions. Dilute

treatment solutions were unable to be analysed using the NMR spectroscopy method described above and were instead sent to Leeder Consulting, a National Association of Testing Authorities (NATA) accredited commercial laboratory for analysis. At least two samples of all neat and diluted chemical concentrations were sent for analysis using the following method (details supplied by Yan Wang, Senior Chemist, Environmental Services, Leeder Consulting).

Seawater samples which contain benzalkonium chloride at various levels were diluted appropriately with pure water and diluted samples were then analysed using Reverse-phase High Performance Liquid Chromatography with UV detection (HPLC-DAD). Benzalkonium chloride reference standards were acquired from Sigma-Aldrich. 5 level benzalkonium chloride calibration standards, ranging from 1.0 mg/L to 100 mg/L, were used to quantify the benzalkonium chloride results in seawater samples. The laboratory quality control samples, including reagent blanks, sample duplicates and method spikes were co-analysed with samples and all these QC/QA samples are within our laboratory acceptance criteria.

3.7 Data analysis and interpretation

A one-way Analysis of Variance (ANOVA) was used to determine if there was a significant difference in the size (total shell length) of mussels from different chemical concentration treatment groups within the small and large size classes. An independent samples t-test was then used to examine if there was any difference in the shell length between treatment group (pooled data) and control group mussels belonging to the small and large size classes. The significance level was set as $\alpha = 0.05$.

The efficacy of the two chemical treatment agents, *Conquest* and *Quatsan*, on the post 24 h mortality of mussels under different experimental conditions (chemical concentration, size of mussels, and mussel location within the test system) was examined using a three-way ANOVA. Where applicable, post hoc analysis was performed using Tukey's Least Significant Difference (LSD) test. Similar analysis on the 5-7 d post treatment mortality of mussels was not conducted, due to insufficient variance (i.e. 100% mortality of mussels) across many of the treatment groups. A two-way Repeated Measures Analysis of Variance (RM-ANOVA) was used to examine the effect of 24 h versus 48 h chemical exposure periods (Exposure time) and location of the mussels in the experimental sea water system (Location) on mussel survival.

Separate three-way ANOVAs were used to compare the maximum temperature and temperature range experienced by small and large mussels from different chemical treatment groups at different locations in the experimental sea water system over a 24 h dosing period. Regression analysis was then used to determine if maximum temperatures and temperature ranges experienced in different treatment groups were significantly related to recorded mussel survival. Lines were fitted that best represented the data; in some cases, this was a curve.

Prior to analysis, all data were tested for normality using residual frequency histograms and probability plots (P-P plots) and for homogeneity using side-by-side boxplots and plots of residuals against predicted values (as per [37]). Analyses were performed using the statistical analysis package SPSS v19.0.

4. Results

4.1 QAC treatment solutions

Results presented here for chemical analysis of neat and dilute QAC solutions are based on chemical analysis conducted by Leeder Consulting. Chemical analysis of neat *Conquest* and *Quatsan* solutions indicated differential concentrations of BAC between the two formulations. Undiluted *Conquest* recorded an average BAC concentration of 45.0 g L^{-1} (± 2.9 SD; n = 8), while BAC levels in *Quatsan* were approximately 10% higher at an average of 49.8 g L^{-1} (± 7.3; n = 4). However, it should be noted that there was some intra- and inter-batch variability in BAC levels recorded for both *Conquest* and *Quatsan* (Table 2). For *Conquest*, intra-batch BAC concentrations differed by between 2.1 and 12.2%, while mean batch concentrations varied by up to 12% (between Batch A and D; Table 2). For *Quatsan*, intra-batch differences were only observed in one batch (Batch A, 7.1%); however, a large (c. 22.4%) inter-batch difference in BAC was observed (Table 2).

Amounts of active BAC in treatment solutions ranged from 223 – 1767 mg L^{-1} for 1 and 5% *Conquest* and 858 – 1650 mg L^{-1} for 2 and 5% *Quatsan* respectively (Table 3).

Table 2. Results of chemical analysis to determine the amounts of active benzalkonium chloride (BAC) present in different formulations and supply batches of QAC treatment chemicals. Data presented is from chemical analysis conducted by Leeder Consulting.

Treatment chemical	Batch	Replicate	BAC conc. (g L^{-1})	Intra-batch variability (%)
Conquest TGA	A	1	48	2.1
		2	49	
	B	1	46	12.2
		2	41	
	C	1	47	6.8
		2	44	
	D	1	42	2.4
		2	43	
Quatsan	A	1	45	7.1
		2	42	
	B	1	56	0
		2	56	

Table 3. Nominal and measured treatment concentrations of benzalkonium chloride (BAC) in dosing treatment solutions used in field experiments.

Nominal treatment Concentration (%)	BAC (mg/L)	
	Conquest TGA	*Quatsan*
1	223	
2	632	858
5	1767	1650

4.2 Mussel sizes

The average shell length of mussels in the five treatment groups was 19.8 mm (range = 10 – 30 mm) for small mussels and 59.7 mm (range = 49.0 – 92.0 mm) for large mussels. Small size class mussels from treatment groups were comparable to those from controls (*t*-test, df = 1194, t = -1.23, p = 0.22; Fig. 5b), as were large mussels from treatment and control groups (*t*-test, df = 718, t = -0.53, p = 0.60; Fig. 5b).

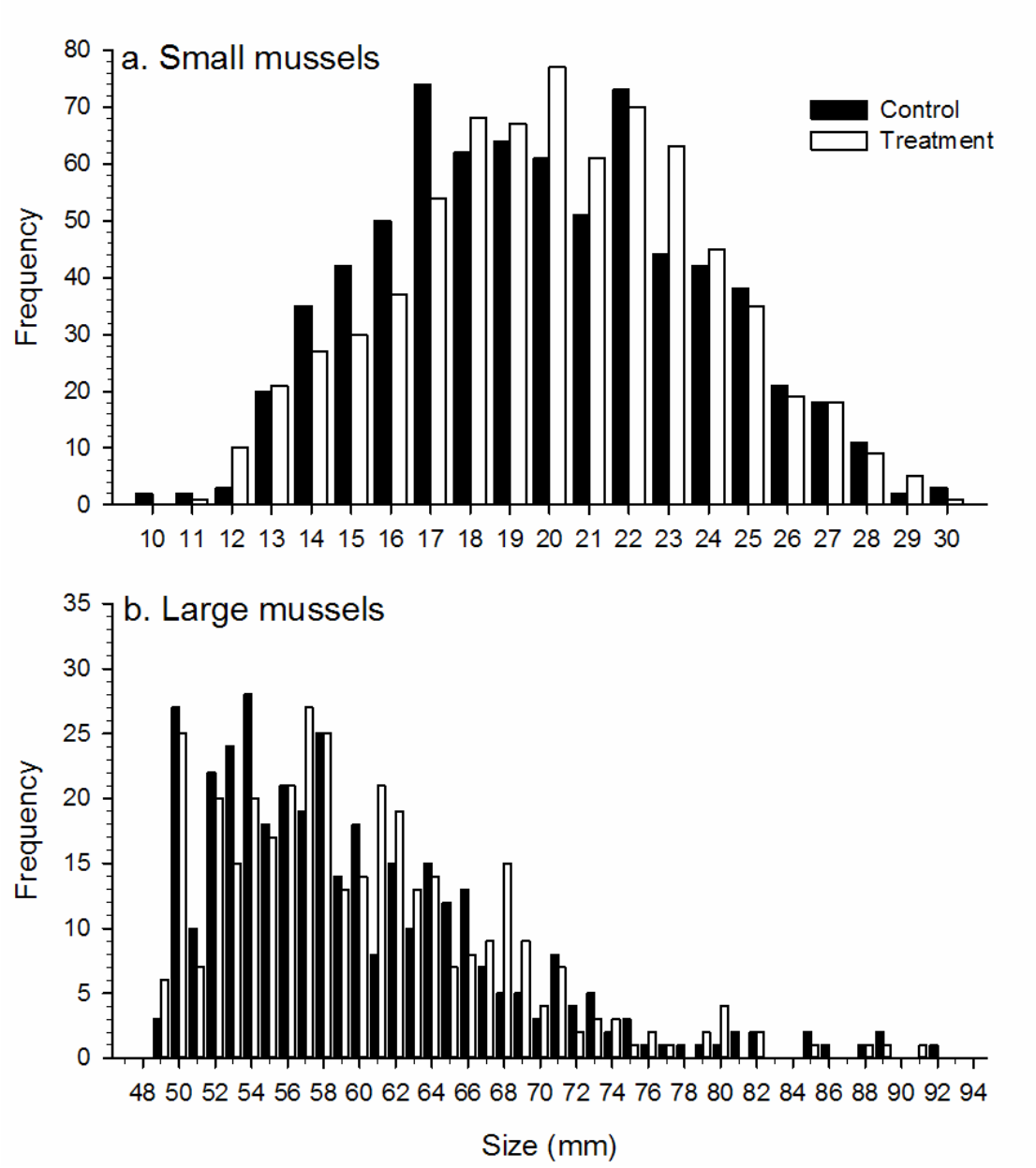

Figure 5. Comparison of the size (shell length) distribution of (a) small and (b) large test mussels from experimental treatment and control groups

Shell length of small mussels differed statistically between treatment groups (ANOVA, $F_{4,598}$ = 4.28, p = 0.002; Fig. 6a). However, in reality the total size range between the treatment group with the smallest mean mussel size (*Quatsan* 5%, mean = 18.8 ± 0.34 mm) and largest mean

mussel size (*Quatsan* 2%, mean = 20.5 ± 0.34 mm) was only 1.7 mm. The shell length of large mussels also differed significantly between treatment groups (ANOVA, $F_{4, 359} = 7.83$, $p < 0.001$; Fig. 6b). The total size range between the treatment group with the smallest mean mussel size (*Conquest* 1%, mean = 56.1 ± 0.67 mm) and largest mean mussel size (*Conquest* 1%, mean = 63.0 ± 0.92 mm) was small (6.9 mm) relative to the size range of mussels used.

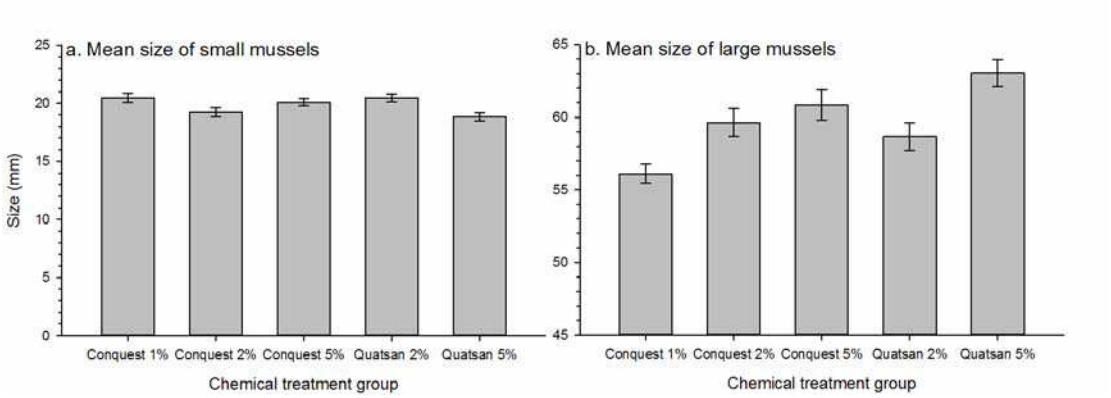

Figure 6. Comparison of the mean size of (a) small and (b) large test mussels from the five chemical treatment groups tested. Values represent mean ± 1SE.

4.3 Mussel survival

Mortality of mussels within experimental control treatments was low, with less than 2% of control mussels (23 of a total of 1081) dying during the experiment.

None of the chemical treatments (i.e. chemical and concentration) recorded 100% mussel mortality immediately following the 24 h dosing period (Figure 7a; Table 4). However, ANOVA results did indicate mussel survival varied significantly with respect to the type of chemical treatment ($F_{4,119} = 17.44$, $p < 0.001$; Table 5). Post-hoc analysis showed that mussel survival in *Conquest* 1% and 2% treatments did not differ significantly to each other (Tukey's LSD, $p = 0.676$), but was significantly greater than that recorded for *Conquest* 5% (Tukey's LSD, $p \leq 0.02$) and *Quatsan* 2% and 5% (Tukey's LSD, $p < 0.001$). Similarly, survival in *Quatsan* 2% and 5% treatments were similar (Tukey's LSD, $p = 0.241$), and significantly reduced compared to all *Conquest* treatments (Tukey's LSD, $p \leq 0.01$). ANOVA also revealed that mussel size also had a significant effect on treatment efficacy ($F_{1,119} = 4.13$, $p = 0.045$), with small mussels showing greater survival across most treatment (Figure 7a; Table 5).

When assessing delayed mortality of mussels 5 – 7 d post-treatment, large mussels recorded 100% mortality in four of the five chemical treatment groups tested (Figure 7b; Table 4). The only chemical treatment in which large mussels survived was *Conquest* 1%, and this only occurred in the sea chest compartment of the sea water system (Table 4).

Table 4. Percentage survival (mean ± 1SE) of small and large mussels located in the pipework (Pipework 1, 2 and 3) and sea chest of the experimental test rig following exposure to the five chemical treatments tested. Assessments of survival were conducted immediately following 24 h treatment, and five to seven days post-treatment.

Treatment	Concentration	Mussel size	24 hours post-treatment (% survival)				5–7 days post-treatment (% survival)			
			System location				System location			
			Pipework 1	Pipework 2	Pipework 3	Sea chest	Pipework 1	Pipework 2	Pipework 3	Sea chest
Conquest	1%	Small	73.3 ± 8.8	76.7 ± 13.3	56.7 ± 6.7	56.7 ± 12.0	33.3 ± 14.5	13.3 ± 8.8	23.3 ± 12.0	33.3 ± 12.0
		Large	33.3 ± 9.6	50.0 ± 9.6	33.3 ± 16.7	50.0 ± 9.6	**0**	**0**	**0**	27.8 ± 11.1
	2%	Small	60.0 ± 15.3	48.3 ± 15.2	56.7 ± 13.3	86.7 ± 3.3	23.3 ± 3.3	3.0 ± 3.0	13.3 ± 8.8	30.0 ± 15.3
		Large	50.0 ± 9.6	50.0 ± 19.2	50.0 ± 9.6	50.0 ± 9.6	**0**	**0**	**0**	**0**
	5%	Small	30.0 ± 17.3	36.7 ± 12.0	42.1 ± 20.4	50.0 ± 23.1	6.7 ± 6.7	**0**	12.7 ± 6.4	6.7 ± 6.7
		Large	27.8 ± 20.0	50.0 ± 25.5	44.4 ± 22.2	22.2 ± 14.7	**0**	**0**	**0**	**0**
Quatsan	2%	Small	31.9 ± 13.2	6.7 ± 3.3	16.7 ± 12.0	43.3 ± 8.8	11.1 ± 11.1	**0**	**0**	16.7 ± 8.8
		Large	5.6 ± 5.6	16.7 ± 9.6	22.2 ± 5.6	27.8 ± 11.1	**0**	**0**	**0**	**0**
	5%	Small	3.3 ± 3.3	10.0 ± 10.0	6.7 ± 6.7	23.3 ± 12.0	**0**	**0**	**0**	**0**
		Large	5.6 ± 5.6	33.3 ± 9.6	5.6 ± 5.6	22.2 ± 5.6	**0**	**0**	**0**	**0**

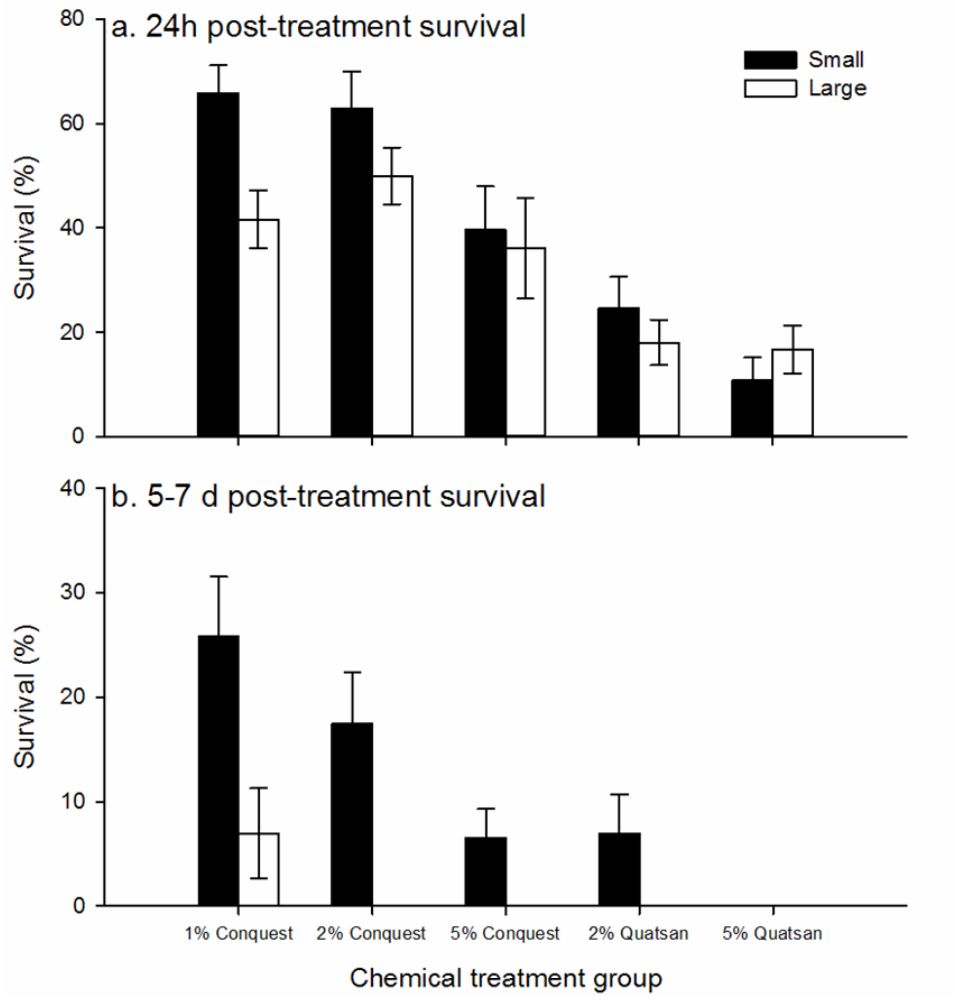

Figure 7. Percentage survival (mean ± 1SE) of small (black bars) and large (white bars) mussels from the five chemical treatment groups tests, when assessed (a) immediately following 24 h treatment, and (b) five to seven days post-treatment.

Table 5. Summary of analysis of variance (ANOVA) examining the effects of chemical treatment type (Chemical & Conc.), mussels size (Size) and location of the mussels in the experimental sea water system (Location) on mussel survival, as assessed upon immediate completion of 24 h dosing. Values in bold represent significant differences (at a = 0.05).

Source	df	MS	*F*	p
Chemical & Conc.	4	8667.963	17.444	**0.000**
Size	1	2053.441	4.132	**0.045**
Location	3	758.125	1.526	0.214
Chemical & Conc. x Size	4	747.055	1.503	0.209
Chemical & Conc. x Location	12	367.017	0.739	0.710
Size x Location	3	767.652	1.545	0.209
Chemical & Conc. x Size x Location	12	258.585	0.520	0.896
Error	80	496.905		

In contrast, small mussels recorded survival in all but one of the chemical treatment groups, with the exception being the *Quatsan* 5% treatment (Figure 7b; Table 4). Interestingly, survival in 2% *Quatsan* treatments (6.9 ± 4.3%) was similar to that recorded in 5% *Conquest* treatments (6.5± 2.8%), while 2 and 1% *Conquest* treatment survival was progressively greater (at 17.4 ± 5.0 and 25.8± 5.7%, respectively; Figure 7b).

Given the greater survival observed for small mussels relative to large, closer investigation was made into the size distribution of live and dead mussels within this small size class, to ascertain if surviving individuals were clustered within a particular size range. When all treatment groups were pooled, the sizes of surviving small mussels were distributed relatively evenly across the entire size range (Figure 8). When examined with respect to individual treatment groups, there was again no partitioning of survivors to any particular size range (Figure 8). Evidently, the size-dependent threshold for consistent 100% mortality of test mussels exposed to QAC solutions lies between 30 and 50 mm shell length (i.e. between the upper and lower bounds of the small and large size class, respectively; Table 4).

Prolonged 48 h exposure to low concentrations of chemical agent did not result in any long-term improvement to treatment efficacy. RM-ANOVA results did show a significant overall decrease in survival of small mussels exposed to 2% *Conquest* solution for 48 h rather that 24 h ($F_{1,16}$ = 5.305, p = 0.035; Figure 9; Table 6) with lower survival (c. 32%) recorded for mussels in 48 h exposure treatments relative to individuals in 24 h exposure treatments (c. 62% survival; Figure 9). However, survival of mussels 5 – 7 days post-treatment was very similar in both 24 and 48 h exposure treatments (at 17.4 and 19.9%, respectively; Figure 9). This disparate pattern of survival for 24 and 48 h exposure treatments mussels over time was the primary driver of the significant Time x Exposure period interaction observed ($F_{2,32}$ = 9.194, p = 0.001; Figure 9; Table 6). Location of the mussels in the experimental system had no significant effect on the efficacy of treatment (Table 6).

4.4 Effect of temperature on mussel mortality

The average 24 h dosing temperature profile of sea chest and pipework locations of the experimental sea water system sea chest and pipework environments for each experimental treatment group is presented in Figure 10. Temperature profile patterns were similar across all treatments. Sea chest water temperatures for any given treatment remained constant throughout the 24 h dosing period, ranging between approximately 13°C and 19°C depending on the seasonal variations (Figure 10). Pipework temperatures fluctuated markedly over the same 24 h period, reflecting the influence of ambient air temperatures on exposed system components. Typically, water temperatures in the system pipework rose above sea chest temperatures during daylight periods, and dipped below sea chest temperatures at night (Figure 10). Again, average maximum (c. 21 – 31°C) and minimum (c. 8.5 – 16°C) pipework temperatures varied with respect to seasonal changes, but also as a result of the daily climatic conditions.

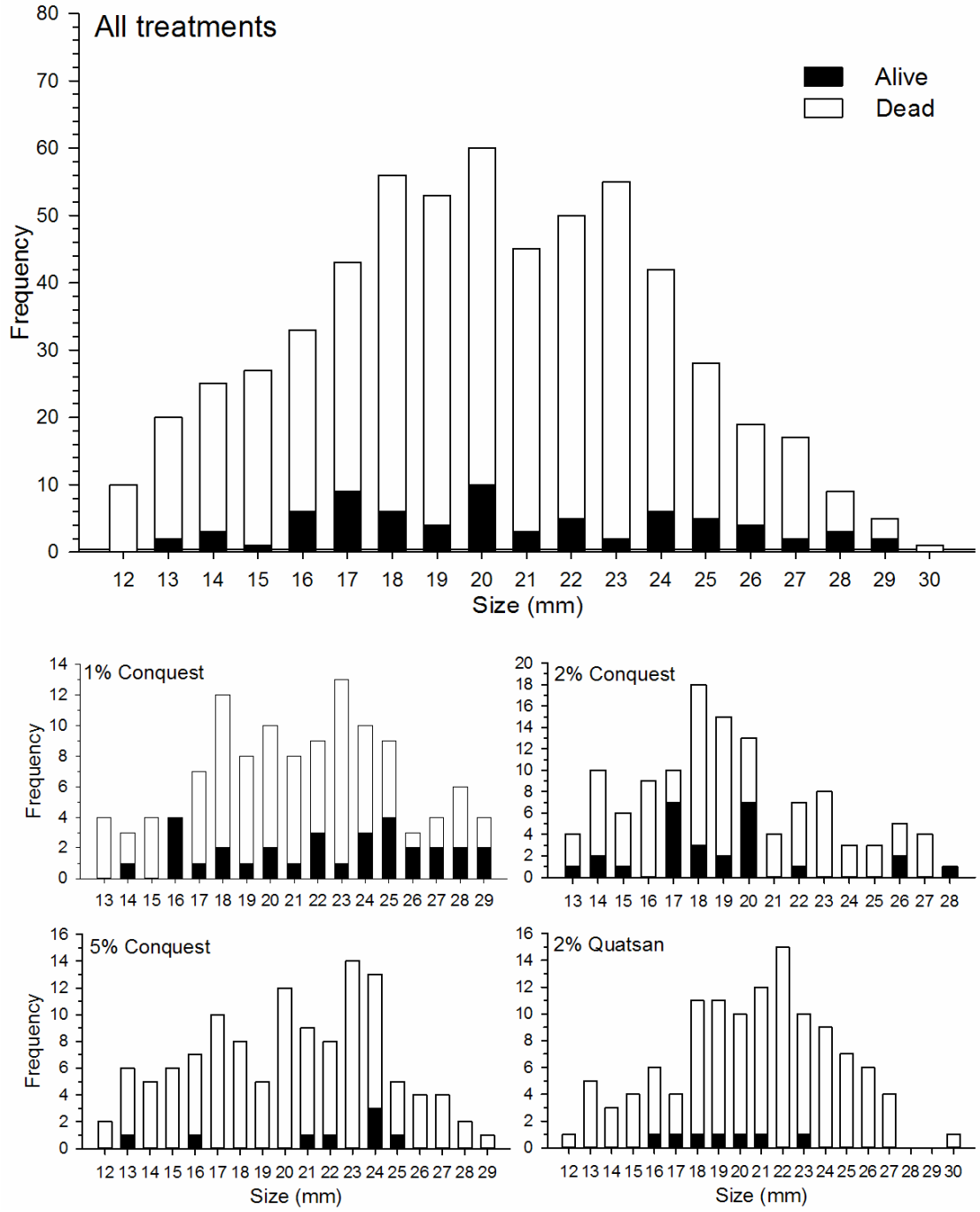

Figure 8. Size distribution of alive (black) and dead (white) small test mussels from experimental treatment groups, as recorded 5 – 7 d post-treatment. Data are presented as a consolidation of all treatments (large plot) and individual treatment groups (small plots).

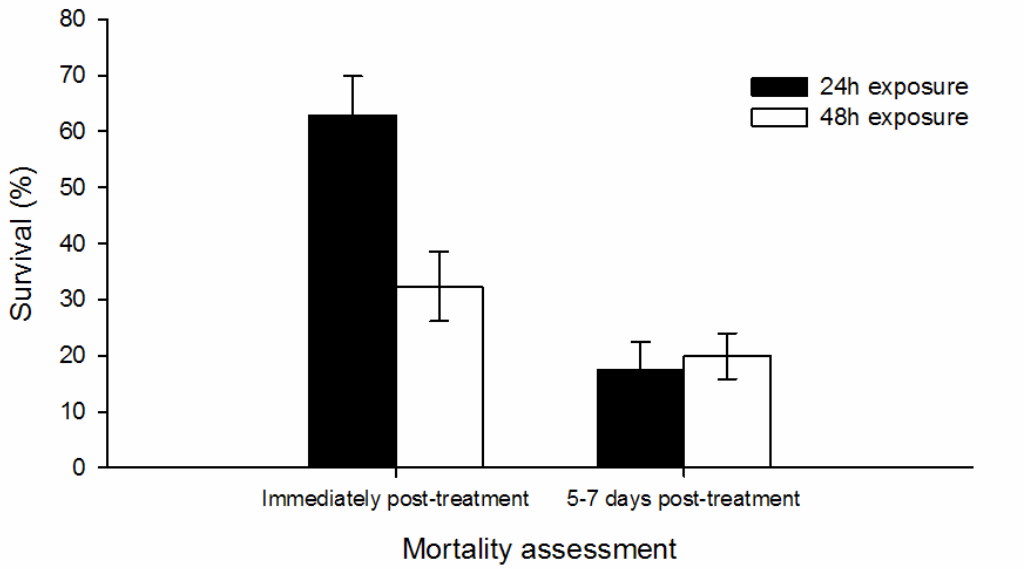

Figure 9. Percentage survival (mean ± 1SE) of small mussels exposed to 2% Conquest treatment solution for a dosing period of 24h (black symbols) and 48h (white symbols). Survival assessment occurred immediately following the dosing period and five to seven days post-treatment.

Table 6. Summary of repeated measures analysis (RM-ANOVA) examining the effect of 24 h versus 48 h chemical exposure periods (Exposure time) and location of the mussels in the experimental sea water system (Location) on mussel survival. Values in bold represent significant differences (at a = 0.05).

Factors	Source	df	MS	*F*	p
Between	Exposure time	1	1568.000	5.305	**0.035**
	Location	3	469.852	1.590	0.231
	Exposure time x Location	3	604.445	2.045	0.148
	Error	16	295.586		
Within	Time	2	40787.817	185.144	**0.000**
	Time x Exposure time	2	2025.500	9.194	**0.001**
	Time x Location	6	146.624	0.666	0.678
	Time x Exposure time x Location	6	176.434	0.801	0.577
	Error	32	220.303		

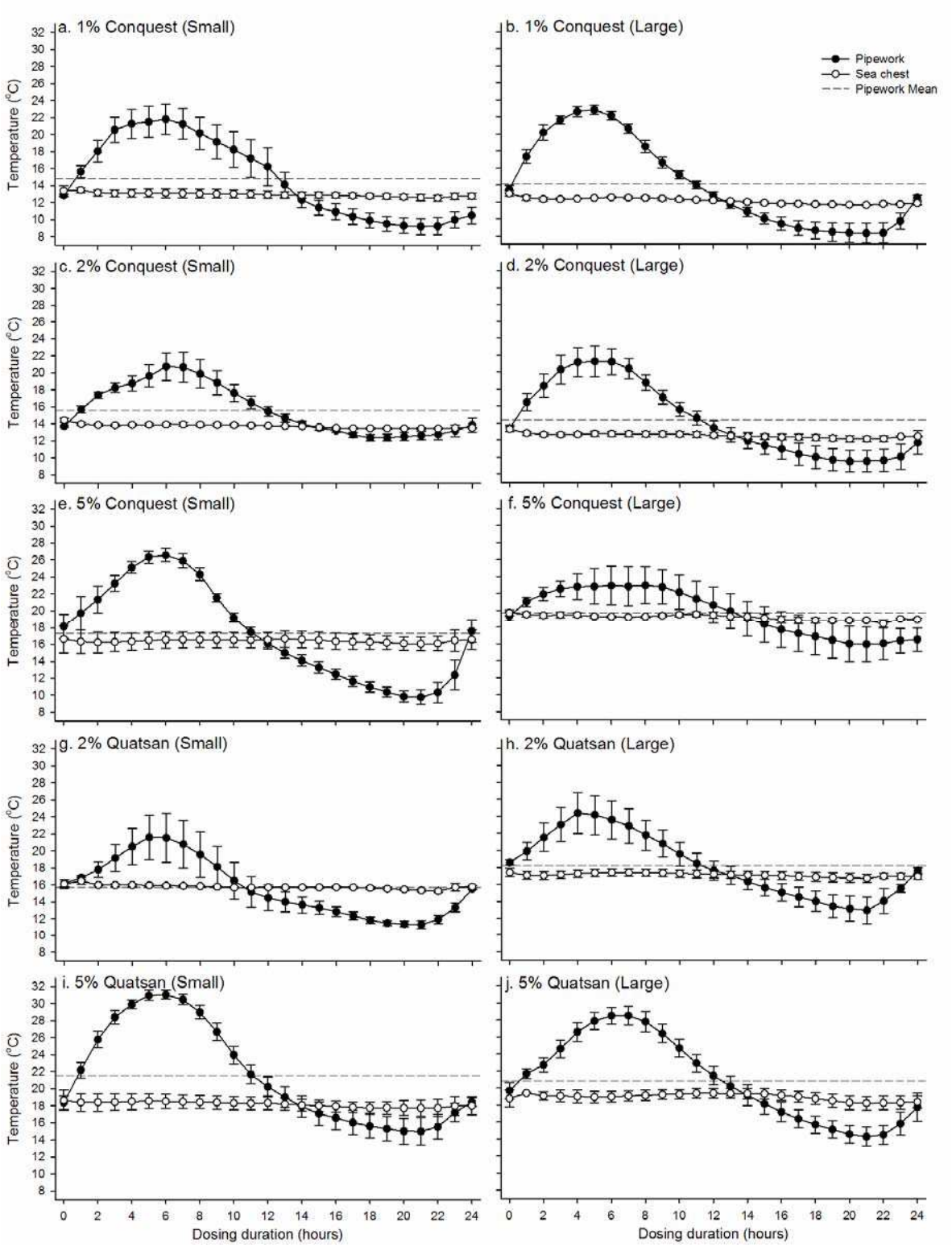

Figure 10. Water temperatures (mean ± 1SE) recorded hourly in the pipework (black symbols; n = 6) and sea chest (white symbols; n = 3) of the experimental sea water system over a 24 h dosing period during chemical treatment trials on small and large mussels. Dotted line represents 24 h average temperature in the system pipework.

To determine if there was an effect of temperature on treatment efficacy of QAC solutions, mussel survival was compared between sea chest and pipework locations. This method was considered justified based on temperature parameters recorded for the system during the trials. The average maximum temperature experienced by mussels in the sea chest of the experimental sea water system over a 24 h dosing period was significantly lower than that experienced by mussels in the system pipework ($F_{2,60}$ = 85.624, p < 0.001; Table 7a; Figure 11a). Similarly, the average temperature range endured by sea chest mussels (1.5 – 1.8°C) across the different treatments groups over a 24 h dosing period was markedly less than that experienced by pipework mussels (11.3 – 16.3°C) over the same period ($F_{2,60}$ = 213.653, p < 0.001; Table 7b; Figure 11b). Both maximum temperature and temperature range differed significantly among chemical treatment groups (Chemical & Conc.; p < 0.001; Table 7a and b) as a result of seasonal changes in ambient air and water temperatures during the course of the experimental period (Figure 11). Analysis of temperature range also revealed a significant interaction between chemical treatment group (Chemical & Conc.) and size ($F_{4,60}$ = 6.410, p < 0.001; Table 7b), which was driven by the reduced temperature range observed in large mussel 5% *Conquest* and *Quatsan* treatments (Figure 10f and j) relative to their respective small mussel treatments (Figure 10e and i). No difference was observed in the maximum temperature or temperature range between the two pipework locations (Tukey's LSD, p = 0.572; Figure 11).

Table 7. Summary of analysis of variance (ANOVA) comparing the (a) maximum temperature and (b) temperature range experienced by small and large (Size) mussels from different chemical treatment groups (Chemical & Conc.), at different locations in the experimental sea water system (Location) over a 24 h dosing period. Values in bold represent significant differences (at a = 0.05).

a. Maximum Temperature

Source	df	MS	*F*	p
Chemical & Conc.	4	161.197	18.559	**0.000**
Size	1	1.511	0.174	0.678
Location	2	743.714	85.624	**0.000**
Chemical & Conc. x Size	4	15.649	1.802	0.140
Chemical & Conc. x Location	8	5.199	0.599	0.775
Size x Location	2	0.322	0.037	0.964
Chemical & Conc. x Size x Location	8	7.133	0.821	0.587
Error	60	8.686		

b. Temperature Range

Source	df	MS	*F*	p
Chemical & Conc.	4	28.054	4.047	**0.006**
Size	1	2.196	0.317	0.576
Location	2	1480.912	213.653	**0.000**
Chemical & Conc. x Size	4	44.428	6.410	**0.000**
Chemical & Conc. x Location	8	6.351	0.916	0.510
Size x Location	2	0.911	0.131	0.877
Chemical & Conc. x Size x Location	8	12.662	1.827	0.090
Error	60	6.931		

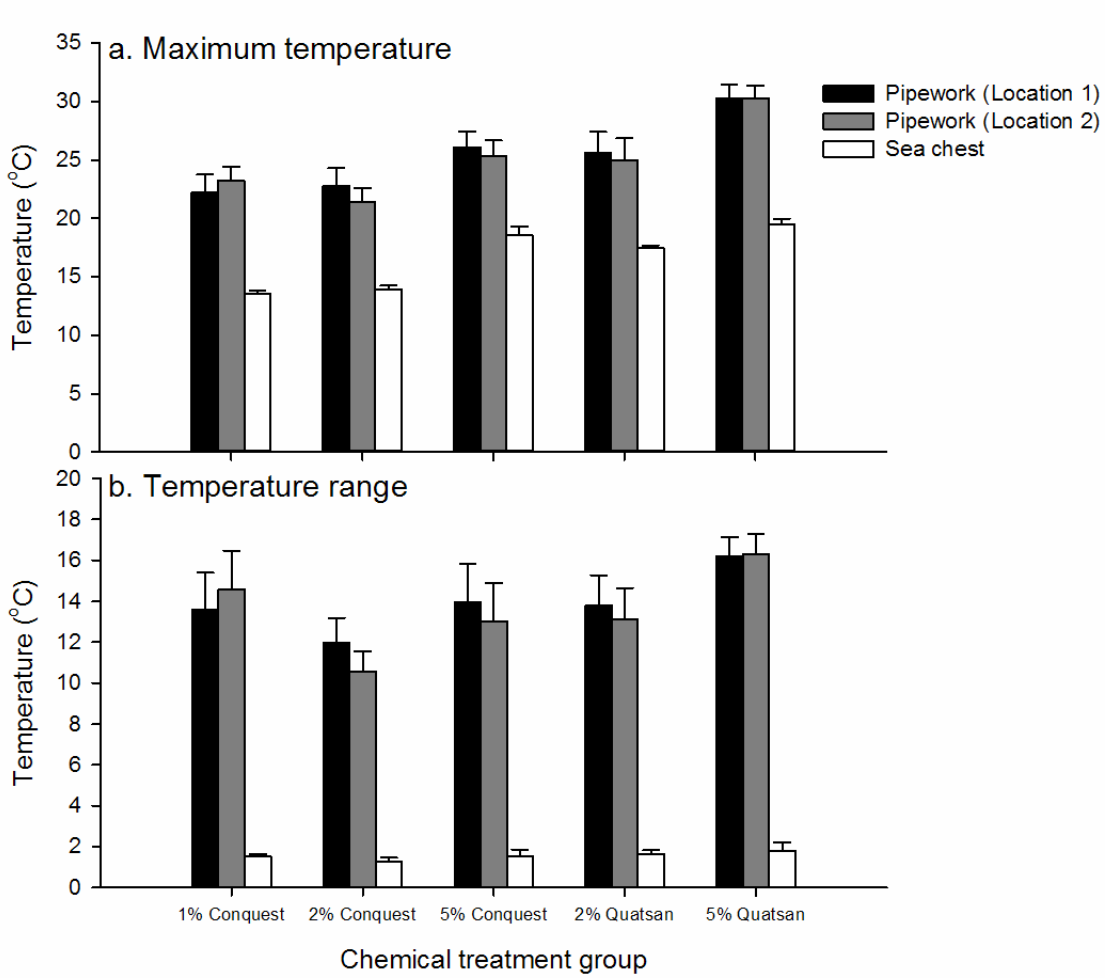

Figure 11. (a) Maximum temperature and (b) temperature range recorded in the pipework (at two locations, black and grey bars) and sea chest (white bars) of the experimental sea water system over a 24 h dosing period during chemical treatment trials.

Regression analyses showed little effect of temperature on the efficacy of chemical treatments, with only three treatment groups showing a statistically significant relationship. Percentage survival of large mussels dosed with 5% *Conquest* was negatively related to increases in both maximum temperature and temperature ranged experienced (Table 8; Figure 12a and c). Survival of small mussels exposed to 5% *Quatsan* was also negatively related to maximum temperature (Table 8; Figure b). In all these cases, however, the strength of the relationship between temperature and mussel survival was weak (Table 8).

Table 8. Regression analysis (r^2-values and p-values) for mussel percentage survival and (a) maximum temperature and (b) temperature range recorded in the sea chest and pipework of the experimental sea water system. Values in bold represent significant differences.

a. Maximum temperature

Treatment	Concentration	Mussel size	*n*	r^2	p
Conquest	1%	Small	9	0.044	0.587
		Large	9	0.011	0.789
	2%	Small	9	0.599	0.064
		Large	9	0.040	0.885
	5%	Small	9	0.165	0.278
		Large	9	0.444	**0.050**
Quatsan	2%	Small	9	0.243	0.177
		Large	9	0.241	0.179
	5%	Small	9	0.450	**0.048**
		Large	9	0.044	0.588

b. Temperature range

Treatment	Concentration	Mussel size	*n*	r^2	p
Conquest	1%	Small	9	0.364	0.257
		Large	9	0.158	0.598
	2%	Small	9	0.331	0.105
		Large	9	0.002	0.907
	5%	Small	9	0.032	0.646
		Large	9	0.509	**0.031**
Quatsan	2%	Small	9	0.228	0.193
		Large	9	0.154	0.296
	5%	Small	9	0.324	0.110
		Large	9	0.221	0.201

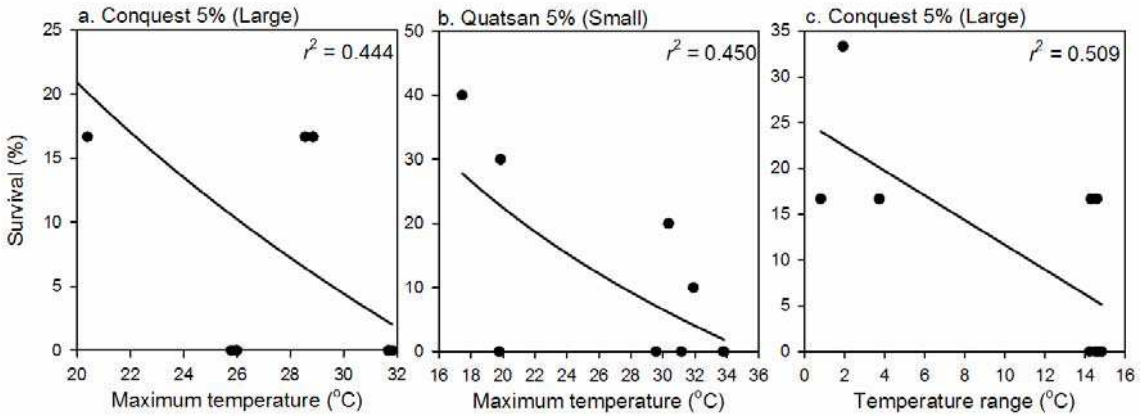

Figure 12. Regression analyses for mussel percentage survival and (a-b) maximum temperature and (c) temperature range recorded in the sea chest and pipework of the experimental sea water system. Results are presented for $p \leq 0.050$ only.

4.5 Water quality parameters

Baseline water quality data was collected for treatment solutions immediately prior to the addition of mussels and treatment chemicals to the sea water system. Given that control and treatment sea water system intakes were located side-by-side, it was assumed that starting water quality parameters for both systems were the same.

Dissolved oxygen levels at the commencement of small mussel treatments ranged between 8.3 to 6.8 mg L^{-1} (for 1% *Conquest* and 5% *Quatsan*, respectively) and 8.2 to 7.5 mg L^{-1} in large mussel treatments (for 1% *Conquest* and 5% *Quatsan*, respectively; Figure 13a). Salinity levels recorded during small and large mussel treatments ranged from 31.0 to 33.5 ppt, with differences being attributable to freshwater input following heavy rainfall events (Figure 13b). The baseline turbidity of system water was relatively uniform (c. 1 – 6 NTU) among treatment groups, with the exception of large mussel 5% *Conquest* and 2% *Quatsan* treatments groups, which recorded spikes in turbidity readings following large rainfall events that occurred during the treatment period (Figure 13c). The largest individual spikes in turbidity recorded during each of these treatment periods was 116.6 and 78.2 NTU for 5% *Conquest* and 2% *Quatsan* trials, respectively.

While the baseline pH recorded for sea water across treatment groups was uniform (c. pH 8.0; Figure 13d), pH did vary following the addition of treatment chemicals. The addition of *Conquest* increased the pH of treatment system waters by between 0.8 (1% solution) and 1.4 (5% solution) units over baseline levels (Table 9). In contrast, addition of *Quatsan* resulted in slightly decreased sea water system pH relative to baseline levels, with pH remaining constant (at 7.7) irrespective of the volume of chemical added (Table 9).

Table 9. The pH levels (mean ± 1SE) of sea water in experimental test systems during chemical treatment trials, as recorded before (Baseline) and after (Chemical added) the addition of treatment chemicals.

Treatment	Concentration	pH	
		Baseline	Chemical added
Conquest	1%	7.9 ± 0.0 (6)	8.7 ± 0.0 (6)
	2%	7.9 ± 0.1 (6)	8.8 ± 0.0 (6)
	5%	7.7 ± 0.3 (6)	9.1 ± 0.1 (4)
Quatsan	2%	8.0 ± 0.0 (6)	7.7 ± 0.1 (6)
	5%	8.1 ± 0.0 (6)	7.7 ± 0.0 (6)

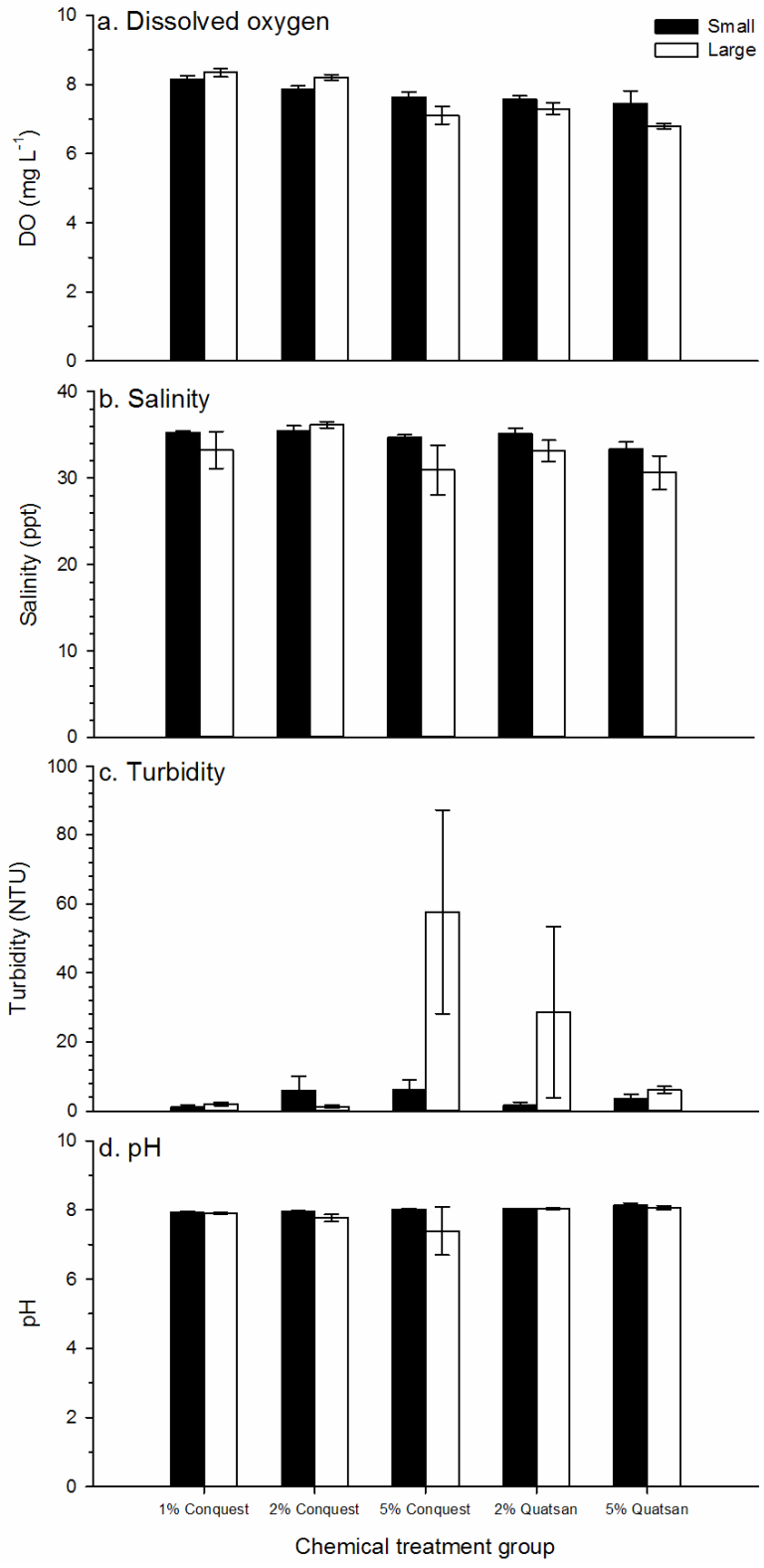

Figure 13. Baseline water quality data (mean ± 1SE) for (a) dissolved oxygen, (b) salinity, (c) turbidity, and (d) pH recorded at the commencement of chemical treatment trials, just prior to the addition of treatment chemical solutions.

5. Discussion

5.1 Efficacy of QAC chemical treatment

This study showed that the efficacy of two commercially available QAC formulations in treating mussel biofouling of sea water systems was highly dependent on the size of the mussels present. Treatment solutions of both *Quatsan* and *Conquest* appeared very effective at killing large sized (50 – 90 mm) mussels in the pipework and sea chest of the experimental test systems over a 24 h dosing period. 100% mortality of large mussels was achieved in all treatment groups, with the exception of the 1% *Conquest* treatment group, in which 17 – 50% of mussels consistently survived in the sea chest compartment of the test system during each of the replicate trial runs. In contrast, small (0 – 30 mm) sized mussels appeared quite resilient to the majority of treatment regimes, with 100 % mortality throughout the entire test system only recorded for one of the treatments regimes (5% *Quatsan*). Findings from this study indicate that changes in water temperature throughout the duration of the treatment period have little effect on treatment efficacy. Similarly, increased exposure time to treatment chemicals did not enhance efficacy of treatment.

Toxicity of *Quatsan* solutions was higher than that of *Conquest* mixtures, with greater mortality observed between comparable treatment percentage dilutions. Regardless, efficacy of both treatment solutions did improve as treatment concentration increased. According to the manufacturer's Safety Data Sheet (SDS) for *Conquest* (Appendix A), the undiluted solution contains <10% QAC (CAS No. 63449-41-2), which primarily comprises the active molluscicide agent ADBAC (with the alkyl group having a chain length of 8-18 atoms). The SDS for *Quatsan* (Appendix B) indicates the presence of two QAC components, the first being the same QAC ingredient found in *Conquest*, albeit in greater concentrations (10-60%), and the second being an ingredient itemised as Benzalkonium Chloride (CAS No. 68989-00-4), which is essentially the same except the alkyl chains contain 10-16 atoms. The additional BAC present in *Quatsan* would explain its greater toxicity to the test mussels. Another possible explanation for the decreased efficacy of *Conquest* (relative to *Quatsan*) is the additional ingredients in its formulation (Appendix A), namely the organic solvent 2-butoxy ethanol and the compound sodium metasilicate (a coagulant/flocculant agent). The possibility exists that these additional ingredients may be readily detected and considered "unpleasant" by mussels, causing them to close their valves and resulting in diminished exposure to the BAC compounds.

The findings of the present field-based study support earlier DSTO laboratory work investigating the use of commercial QAC solutions as treatments against mussel fouling. [1] tested the toxicity of both *Conquest* and *Quatsan* against specimens of *Mytilus galloprovincialis plannulatus* comparable in size (mean = 45 mm; range = 25 – 65 mm) to mussels classified as 'large' in this study. These laboratory studies found that after 14 h immersion in *Conquest* treatment solutions of 1, 5 and 10%, all mussels from all treatments groups were dead within 48 h of the cessation of dosing. Similarly, mussels exposed to 1, 5 and 10% *Quatsan* solutions for 14 h were all dead within 24 h post-treatment. These findings are largely consistent with the results recorded for large mussels in this study, with the exception of the 1% *Conquest* treatment, where [1] recorded 100% mortality post 14 h exposure, yet the present study recorded consistent survival up to 5 –7 days post-treatment. The most probable explanation

for this disparity in results lies in the respective scale of each experiment. Experimental test units used during the laboratory investigations by [1] comprised simple containment vessels (glass beakers) holding small volumes of water (3 L), making the preparation of accurate treatment solutions easy. In contrast, test units used in this field study comprised a complex sea water system holding relatively large volumes of water (~100 L), of which the operational volume was difficult to accurately define. As such, actual chemical treatment concentrations in the present study were consistently less than the target nominal concentrations (Table 3). Clearly, at low chemical concentrations (1%) this underestimation would have had a greater impact on overall treatment toxicity compared to higher concentration treatments (5 and 10%). This difference in results is a valuable reminder of the importance of field validation of laboratory results, and highlights the need to understand real-world systems in order to develop effective management tools.

Numerous other studies have demonstrated the efficacy of QAC against a range of freshwater and saltwater mollusc taxa. Bax et al. [28] found that a 1% v/v solution of *Conquest* resulted in 100% mortality of black striped mussels (*Mytilopsis sallei*) after 7 h exposure. Similarly, treatment solutions containing small concentrations (>2.5 mg L^{-1}) of BAC have been shown to be highly effective (99.5% mortality) at controlling freshwater zebra mussels [25]. Laboratory assessments by Britton and Dingman [26] showed that 10 s exposures to a 3% solution of a commercial QAC formulation (containing the active compounds BAC and diecyl dimethyl ammonium chloride (DDAC)) resulted in 100% mortality of freshwater Quagga mussel (*Dreissena rostiformis begnsis*) veligers within 60 min. Oplinger and Wagner [38] determined that ~500 mg L^{-1} of BAC (equivalent to a the 1 – 2% solutions used in this study) was effective at killing 100% of New Zealand mud snails (*Potamopyrgus antipodarum*) following 15 min exposure.

In contrast, laboratory trials by Neil and Stafford [2] found that *Quatsan* was largely ineffective at killing test specimens of the Sydney rock oyster (*Saccostrea glomerata*), with 12 h exposure to 5 and 10% treatment solutions resulting in only ~10 and ~20% mortality, respectively. These findings may be comparable to research by Waller et al [29], who found a commercial QAC molluscicide formulation (containing 13% BAC) was more toxic to zebra mussels relative to a native unionid mussel species (*Obliquaria reflexa*).

5.2 Effects of mussel size

One of the more unexpected outcomes of the present study was the greater observed tolerance of small (10 - 30 mm) mussels to QAC relative to larger (50 – 92 mm) individuals. This finding is in contrast to observations made for freshwater zebra mussels (*Dreissena polymorpha)*, where a QAC molluscicide formulation (containing 13% BAC) was more toxic to small sized mussels (5 – 8 mm) compared to larger adults [20 – 25 mm; 29].

One possible reason for the greater tolerance to QACs observed for small mussels in the present study may have to do with differences in the surface area of gill tissue, relative to overall body size, in small versus large mussels. The toxic effect of QAC on molluscs works by absorbance into and disruption of soft tissue components of the organism, primarily the gills [24]. A recent study examining the blue mussel (*Mytilus edulis*; a closely related species to *Mytilus galloprovincialis*) showed that the exposed surface area of the gills in this species

increases exponentially with respect to increasing shell length [Figure 14; 39]. If we assume that the ratio of gill surface area to shell length is similar for the mussels used in this study, then the gill surface area of the average small sized mussel tested (mean = 19.8 mm) is approximately nine times less that of the average large sized (59.7 mm) mussel, at ~5 and ~45 cm^2, respectively (Figure 14). This means that the available gill tissue surface area on which the toxicant can act and be absorbed by the organisms is disproportionately less for small mussels (relative to overall mussel size) compared to large mussels. This could potentially lead to decreased uptake of the toxicant (relative to overall size) in small mussels, resulting in greater perceived tolerance.

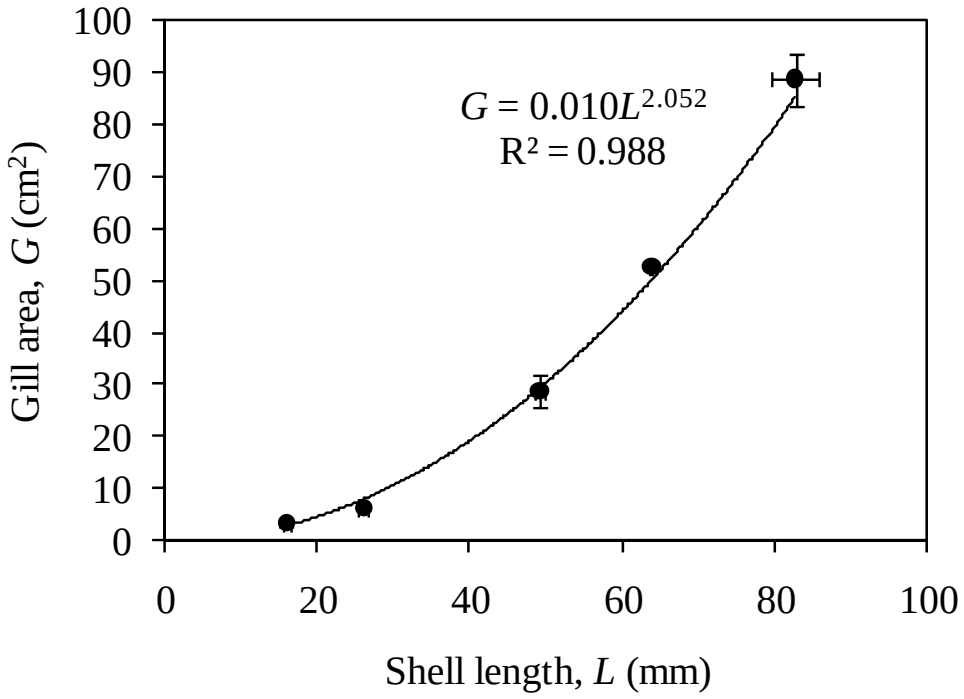

Figure 14. Plot showing the gill surface area (G) of the blue mussel, Mytilus edulis, as a function of shell length (L). Figure reproduced from Riisgård et al. (2011) with permission from the authors.

Another possible explanation for the increased observed tolerance of small sized mussels to BAC is that they are more sensitive to the presence of the toxicant in the water column, and react by closing their shells to avoid exposure. Mussels have sensitive chemoreceptors that can detect small changes in water chemistry, including the presence of toxicants and harmful chemicals, and have the ability to tightly close their bivalve shells and isolate themselves from the ambient water conditions for long periods of time (days to weeks) by switching from aerobic to anaerobic metabolism [40, 41]. However, one of the stated strengths of QAC (in particular BACs) as an effective molluscicide is the inability of mussels to detect the chemical in the water [41], thereby keeping their shells open and exposed. While this scenario appears to be the case for large mussels tested in this study, it is possible that a proportion of small

mussels were more sensitive to the presence of QACs and remain closed for the duration of the exposure period. If this were really the case, however, one would expect to see greater mussel survival with increasing QAC concentration, as the chemical would become more 'easily' detectable, but this was not the case. Further investigation into the exact mechanisms of differential tolerance to QACs in different sized mussels is required.

5.3 Effect of water temperature

It has previously been stated that the effectiveness of QACs as molluscicide is heavily dependent upon the temperature of waters used during dosing [24, 27]. This is thought to be due to an increase in the physiological activity of the target organisms under warmer conditions, resulting in a greater uptake of the toxicant [42]. However, the present study found that significant increases in the environmental temperature experienced by mussels in the pipework sections of the test system during dosing had little effect on overall mussel mortality when compared to mussels in the constant temperature environment of the sea chest location of the system. There are several possible factors contributing to this contrary finding. Firstly, the magnitude of the difference in maximum temperatures experienced by pipework mussels relative to sea chest mussels (between 7.2 and 10.9^{o}C depending on treatment group; Figure 11a) was not enough to result in increased mortality over the 24 h dosing period. Secondly, it is possible that the time period of increased water temperature experienced by pipework mussels (approx. 12 h) was of insufficient duration to have any meaningful influence on QAC toxicity (Figure 10). Finally, it must be acknowledged that, while the pipework of the test system certainly did experience higher maximum temperatures over a 24 h relative to the sea chest environment, it also experienced periods of decreased temperature over this period (Figure 10). It is possible that these two differing temperatures regimes effectively 'balanced' each other out, negating any overall effect of increased temperature on QAC toxicity. In fact, the average temperature over a 24 h period in the system pipework was ≥ 3^{o}C compared to that in the sea chest region (Figure 10). This last explanation is likely the most plausible reason for the results observed in this study, since greater QAC toxicity associated with increased mussel physiological activity during higher temperature periods would almost certainly be counteracted by decreased toxicity during periods of lower physiological activity at lower temperatures.

5.4 Effects of dosing duration time

The current study chose 24 h for the preferred chemical dosing period, in an effort to strike a balance between maximum efficacy of treatment and a realistic turnaround time for vessel disinfection. As previously mentioned, laboratory studies by Lewis and Dimas (2007) determined that 14 h exposure to 1, 5 and 10% solutions of *Conquest* and *Quatsan* killed 100% of large sized mussels. However, the present study found that, under field conditions, 1% *Conquest* did not kill 100% of large mussels in the test system, and only 5% *Quatsan* killed 100% of small mussels. Prolonged (48 h) exposure to low concentrations (2%) of *Conquest* did not result in any long-term improvement to treatment efficacy against small mussels (Figure 9). The 5 – 7 day-post treatment mortality levels were largely identical for both 24 h and 48 h dosing regimens. Therefore, greater mortality observed in the 48 h treatment group immediately following the cessation of dosing likely reflects the additional 24 h time period allocated to this treatment prior to initial mortality assessment, rather than enhanced

mortality due to extended chemical exposure. This assumption is supported by the results of Lewis and Dimas [1] who recorded 40 – 60% mortality in mussels treated with 1 – 10% *Conquest* in the first 24 h following treatment.

These results indicate the most effective treatment duration for 100% mortality of large mussels using *Conquest* (at 1 – 10% concentrations) and/or *Quatsan* (at 2 – 10% concentrations) under field conditions is between 14 and 24 h.

5.5 Application of QAC treatment

In the past five years, there have been ten known instances of RAN vessels being infected with priority unwanted mussel biofouling [20]. On five of these occasions the unwanted mussels were found within the sea chests and/or internal sea water systems of the infected vessels. In all cases, the priority unwanted species in question was AGM, which appears on the Australian Government trigger list for unwanted marine species[4] due to its potential for economic, ecological and human health impacts [21].

Traditional emergency response and remediation procedures for dealing with such biosecurity incidences can be expensive and time consuming, with the potential to impact operational capability by preventing the vessel from getting underway when required. For example, when AGM were discovered on HMAS ANZAC in 2011, both it and the nearby vessel HMAS ARUNTA were dry docked for inspection and cleaning [22]. Similarly, HMAS BATHURST underwent emergency dry docking in 2012 following the discovery of AGM during a routine pre-deployment diver inspection [20]. In the case of HMAS ANZAC and ARUNTA, costs associated solely with the biosecurity components of the dockings were ~ $900K per vessel (M. Whitehouse, pers. comm.). The minimum turn-around for one of these biosecurity dry docking events was 7 days (for HMAS BATHURST).

In comparison, the emergency in-water treatment of unwanted mussel fouling, using techniques such as chemical dosing with QAC, can be cheaper, faster and less of an impact to vessel operations. For example, when AGM were discovered in a sea chest of HMAS TOOWOOMBA in 2011, it was determined that dry docking of the vessel for inspection and cleaning was a non-preferred treatment option due to the highly adverse impact it would have on Navy fleet operating schedules [22]. Instead, all vessel sea chests and sea water pipework (up to and including the sea chest strainer boxes) were chemically treated in-water by sealing off each external sea chest grating and flooding the cavity and pipework with a 1% QAC solution (*Conquest* TGA) diluted with sea water [as per 1]. Successful treatment of the entire vessel (a total of 13 sea chests) was achieved in fewer than 48 h, at a total cost of ~ $38K (M. Whitehouse, pers. comm.).

Despite this study showing that the treatment efficacy of QAC varies with respect to mussel size (Section 5.2), its use as an effective biosecurity emergency response tool should not be discouraged. Rather, QAC treatment should be used as a complementary strategy in conjunction with other management options to ensure its effectiveness against unwanted mussel species. For example, if the sea chests and/or sea water system of a vessel were found

[4] http://www.marinepests.gov.au/national_system/how-it-works/emergency_management/trigger_list

to be infected with an unwanted mussel species (e.g. AGM), QAC dosing would likely eliminate any immediate biosecurity risks, as it would kill any large mussels that may be reproductively mature or ready to spawn. Any remaining residual biosecurity risk, resulting from the possible survival of small mussels, could be managed through follow-up monitoring and inspection of the vessel. If required, repeated QAC dosing could be carried out to treat any remaining individuals (preferably once they have reached size conducive to effective treatment). Despite the additional intervention steps required of this approach, cost of treatment would still be significantly less than if the vessel were to be dry docked and treated on land.

One concern related to the use of QACs for the in-water treatment of biofouling is its persistence in the environment following discharge and subsequent potential impacts on non-target marine organisms. QACs are not metabolized by aquatic organisms; they are accumulated in the consumable parts of fish; they are immobilized in the soil and do not pass into ground water [Schroenig et al., 1995 in 24]. Degradation of ADBAC has been measured by carbon dioxide production tests, showing 66% is degraded within 29 days under aerobic conditions based on an initial concentration of 10 mg L^{-1} [Dobbs et al., 1995 in 24]. Being surfactants, QACs are readily adsorbed on suspended matter in water or on colloids such as humic acids [24]. As such, bentonite clay is commonly added to treatment water (at a concentration of 5 – 40 mg L^{-1}) prior to discharge, resulting in immediate detoxification [24]. Alternatively, adequate dilution of discharged treatment water may be sufficient to minimise any detrimental effects on non-target organisms. Neil and Stafford [2] determined that ~8000 L of sea water would be needed to dilute 1 L of 5% *Quatsan* effluent sufficiently for safe release into the environment, and they suggest that sufficient dilution may be achieved if the treatment water is released to a vast body of water held in a bay or inlet. Provided QAC treatment events were an infrequent occurrence and the target vessel was situated in a large, well flushed body of water, this discharge option appears feasible. In all cases, however, permission to discharge any volume of treatment effluent should first be sought from the appropriate environmental regulator of the region (e.g. State Environment Protection Authority). If sufficient environmental dilution is not possible, it may be preferable to discharge the treatment water to an onshore dilution facility prior to release into the environment [2].

6. Recommendations

Based on the findings of the current study assessing the efficacy and usage parameters of QAC solutions for the control and eradication of mussels in sea water systems under field conditions, we recommend the following:

1. **Revise the recommended RAN QAC dosing protocols for controlling mussel biofouling to 5% v/v disinfectant solution for 24 h.** The current dosing guidelines recommend treatment with a 1% disinfectant solution for 14 h; however, this study indicates that, under variable field conditions, this dosing mixture is insufficient to reliable ensure 100% mortality throughout an entire sea water system.

2. **Mandatory follow-up inspections and monitoring of vessels found to contain unwanted mussel species even after QAC treatment.** The current study indicates that small sized mussels are more resilient to QAC solutions compared to large mussels, with some individuals able to survive 24 h exposure to 5% QAC solutions. While QAC treatment of a vessel mussel-infected vessel is certainly important (and recommended) for the effective elimination of large mussels that pose the greatest biosecurity risk (given their reproductive and spawning potential), small mussels may survive treatment and continue growing. As such, a follow-up inspection for potential survivors is important; with the recommendation of additional QAC treatments should it be required.

3. **Procurement and storage of sufficient quantities of a pre-determined commercial QAC disinfectant in ports and facilities harbouring Defence vessels.** The current study shows that different brands of commercial QAC formulations can have varying amounts of active ingredient(s). Therefore, the same formulation/brand of QAC should be used across Navy for all treatment events, in order to provide assurance of outcomes. Since availability of sufficient volumes of QAC may be manufacturer and/or time dependent, DSTO recommends stockpiling adequate amounts of QAC solution at each RAN base where it may be required. The amount required for stockpiling will depend on the number of vessels home-ported at each location, and the size of the various sea water systems of different craft; however sufficient quantities to treat several vessels would be preferable. Unpublished DSTO data shows undiluted QAC solutions do not lose toxicity after 12 – 18 months on the shelf.

4. **QAC treatment of mussel fouling should be viewed as an emergency response option, not an on-going management strategy.** QAC treatment of problematic and unwanted mussel biofouling should not be viewed as an acceptable fouling control strategy. Rather, it should be used as an emergency response tool for the rapid remediation of an infected vessel. Preferred strategies that should be employed for the sustained long-term prevention, control and management of problematic biofouling in vessel sea waters systems include suitable marine growth protections systems (MGPS) on board every vessel, pre- and post-deployment diver inspections, and education and awareness training for crew and base staff.

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Appendix A: Safety Data Sheet (SDS) for *Conquest* TGA

MATERIAL SAFETY DATA SHEET

1. IDENTIFICATION OF THE MATERIAL AND SUPPLIER

Product Name: CONQUEST TGA
Other names
Recommended Use: Hard surface cleaner and sanitiser.

Supplier Name Shamrock Chemicals (NT) Pty Ltd.
Address 2/94 Coonawarra Road, Winnellie, NT. Australia. 0820
Emergency Telephone (08) 8927 8948
Telephone (08) 8947 1777 **Facsimile** (08) 8947 2388

2. HAZARD IDENTIFICATION

HAZARD CLASSIFICATION: Classified as non-hazardous according to criteria of NOHSC

DANGEROUS GOODS CLASSIFICATION: Not classified as Dangerous Goods according to the ADG Code.

HAZARD CATEGORIES: Not categorised as Hazardous according to the criteria of NOHSC.

RISK PHRASES:
None specified

SAFETY PHRASES:
None specified according to criteria of NOHSC, however, good practice for chemical handling recommends the following precautions:
S2 Keep out of reach of children.
S24/25 Avoid contact with skin and eyes.
S38 In case of insufficient ventilation, wear suitable respiratory equipment.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

3. COMPOSITION/INFORMATION ON INGREDIENTS

Name	Proportion (w/v)	CAS Number
Quaternary ammonium compounds	<10%	Not classified as hazardous
Non-ionic surfactants	<5%	63449-41-2
Sequestrants	<10%	Not classified as hazardous
2-butoxy ethanol	<10%	111-76-2
Sodium Metasilicate	<10%	6834-92-0
Food dye	<1%	Not classified as hazardous
Water	balance	7732-18-5

4: FIRST AID MEASURES

For Advice, contact a Poisons Information Centre on 13 1126 (Australia wide) or a doctor.

Swallowed : If swallowed, do NOT induce vomiting.

Eye: If in eyes, wash out immediately with water.

Skin: If skin or hair contact occurs, remove contaminated clothing and flush skin and hair with running water.

Inhaled: If affected, remove from contaminated area. Apply artificial respiration if not breathing.

Advice to doctor: Treat symptomatically.

5: FIRE FIGHTING MEASURES

Non-flammable

Specific Hazards:	No specific hazards known. If strongly heated in a fire, decomposition products may include oxides of carbon and nitrogen.
Extinguishing Media:	Water spray. (Non-flammable.)
Unsuitable Extinguishing Media:	None known or expected.
Protective Equipment for Firefighters:	Wear full protective clothing and self-contained breathing apparatus.
Additional Advice:	
Hazchem Code	No Hazchem code is set for this product under ADG code.

6: ACCIDENTAL RELEASE MEASURES

Observe all relevant local, federal and international regulations.

Protective measures: Avoid contact with spilled or released material. Immediately remove all contaminated clothing. For guidance on selection of personal protective equipment see Chapter 8 of this Material Safety Data Sheet. For guidance on disposal of spilled material see Chapter 13 of this Material Safety Data Sheet. Shut off all leaks if this is possible without personal risk. Contain to prevent environmental contamination. Prevent from spreading or entering drains, ditches or rivers using sand, earth or other inert barriers.

Spills: Spills are slippery. Clean up all spills immediately.
For small liquid spills (< 1 drum), mop up all possible material and place in a sealable container for re-use or disposal. Rinse off remainder with water. Dispose in accordance with Federal, State and Local regulations.
For large spills (> 1 drum), transfer by mechanical means, such as vacuum truck, to a salvage tank for recovery or safe disposal. .Rinse off remaining residue with water. Dispose in accordance with Federal, State and Local regulations.

7: HANDLING AND STORAGE

Precautions: Avoid contact with skin and eyes.
Wear suitable protective clothing and equipment (PPE) to prevent skin and eye contact.
Ensure ventilation is adequate and that air concentrations of components are controlled below the Exposure Standard. If ventilation is inadequate, wear a respirator fitted with cartridges approved for organic vapours or air supplied breathing apparatus.

Storage: Store, tightly sealed, away from foodstuffs. Ensure containers are correctly labelled, protected from potential damage and properly sealed when not in use.

8: EXPOSURE CONTROLS/PERSONAL PROTECTION

Exposure limits: No exposure limits available for this product, however, limits for its hazardous ingredient 2 butoxy ethanol, is as follows:

Ingredient	Reference	TWA		STEL	
		ppm	mgm^{-3}	ppm	mgm^{-3}
2 butoxy ethanol (Ethyleneglycol monobutyl ether)	ACGIH	20	96.9	50	242

Recommended Minimum Personal Protective Equipment:

EYE PROTECTION:	Safety glasses with side shields or splash proof chemical goggles.
GLOVE TYPE:	PVC or Neoprene gloves
RESPIRATOR:	Not normally required, however if using in a confined space or if ventilation is poor, such that exposure limits are likely to be exceeded, wear a respirator fitted with cartridges approved for organic vapours or supplied-air breathing apparatus.
OTHER:	Suitable protective clothing to prevent skin contact, e.g. impervious overalls and PVC Apron.

9: PHYSICAL AND CHEMICAL PROPERTIES

Appearance	Clear, red mobile liquid.
Boiling Point	100°C
Vapor Pressure	not determined
Specific Gravity	1.0 g/cm^3
Flash Point	none
Solubility in water	Miscible in all proportions
pH	11.0 (neat)

10: STABILITY AND REACTIVITY

Chemical Stability:	Stable under ambient storage conditions.
Conditions to Avoid:	Avoid storing at temperatures above 40°C or below 5°C. Storing in temperatures outside this range may lead to instability. This instability is not hazardous however, and once the product is returned to a temperature within the range above, gentle mixing will return it to a uniform state.
Material to Avoid:	Do not mix with strong oxidising agents or strong, concentrated acids.
Decomposition:	If heated to decomposition, e.g. in a fire, oxides of carbon and nitrogen may be evolved.
Hazardous Reactions:	May react violently with strong, concentrated acids and strong oxidising agents.

11: TOXICOLOGICAL INFORMATION

Possible Health Effects:

Acute:

Swallowed:	Irritant. Ingestion of large quantities is likely to induce spontaneous vomiting and/or diarrhoea.
Eye:	Irritant.
Skin:	Prolonged exposure, especially to the concentrate, may lead to de-fatting of the skin, resulting in dryness, possible cracking and dermatitis. Absorption through the skin may be a significant source of exposure.
Inhaled:	Vapours may be irritant to the respiratory tract. If finely atomised and inhaled, spray mist, especially of strong concentrations of Conquest TGA, may be irritant to the respiratory tract, resulting in coughing and discomfort. Chronic exposure to high vapour concentrations may lead to central nervous system depression, liver and kidney damage and blood effects.

Toxicity Data:
No data exists for this product, however, animal toxicity data for the ingredient 2-butoxy ethanol are as follows:

NOTE: Toxicity may differ significantly depending upon the age of the animals studied Weanling rats are less sensitive than older ones.
LD50 (oral, weanling rat): 3000 mg/kg
LD50 (oral, 6 week old rat): 2400 mg/kg
LD50 (oral, 1 year old rat): 560 mg/kg
LD50 (oral, mouse): 1200 mg/kg
LD50 (oral, rabbit): 320 mg/kg
LD50 (dermal, rabbit): 0.40 g/kg (material confined to the skin for 24 hours)
LD50 (dermal, rabbit): 1.8 g/kg (material applied by gentle massage)
LD50 (dermal, rabbit): 0.11 mL/kg
LC50 (inhalation, rat): 500 ppm;/duration of exposure: 4 hours
LC50 (inhalation, mouse): 700 ppm;/ duration of exposure: 7 hours.

ACUTE TOXICITY COMMENTS: Changes in the kidney, liver, spleen, and lung were found in animals exposed by ingestion, inhalation, and skin absorption Deaths usually resulted from central nervous system depression, lung damage, and kidney injury.

EFFECTS OF BLOOD CELLS: Long-term exposure to 2-butoxyethanol can cause blood changes, including anaemia, in rats Both 2-butoxyethanol and its metabolite butoxyacetic acid can cause breakdown of red blood cells Humans appear to be less sensitive to this effect than rats.

REPRODUCTIVE EFFECTS: 2-Butoxyethanol has been found to produce toxic effects in pregnant rats at about 200 ppm, with no apparent increase in congenital defects among the offspring It did not cause testicular atrophy in males.

12: ECOLOGICAL INFORMATION

Environment: Avoid release to the environment. Refer to Chapter 6 of this Material Safety Data Sheet.

Ecotoxicity: Considered to have a low impact on the environment under normal use conditions.

Persistence/Degradability: This product contains surfactants that are readily biodegradable according to standard test methods.

13: DISPOSAL CONSIDERATIONS

Waste Disposal: Wear protective equipment. Refer to local authorities to ensure compliance with legislative requirements for correct disposal. Recover and recycle if possible.

Container Disposal: Drain container thoroughly. After draining, rinse with water until effluent is pH neutral.

Local Legislation: Disposal should be in accordance with applicable regional, nation and local laws and regulations. Local regulations may be more stringent than regional or national requirements and must be complied with.

14: TRANSPORT INFORMATION

Not classified as a Dangerous Good according to the criteria of the ADG Code.

UN Number	none allocated
Proper Shipping Name:	none allocated
DG Class	not classified as a dangerous good.
Subsidiary Risk Class	none
Packaging Group	none
Hazchem Code	No Hazchem code allocated (non-hazardous product)

Storage and Transport: Transport in original container or one that is correctly labelled. Store away from food stuffs and in accordance with government regulations. Keep container tightly closed when not in use.

15: REGULATORY INFORMATION

Poisons Schedule: Not a scheduled poison - According to the criteria of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) – National Drugs and Poisons Schedule Committee.

AICS: All ingredients listed on the Australian Inventory of Chemical Substances.

16: OTHER INFORMATION

LAST REVISION OF MSDS: February 2008

Appendix B: Safety Data Sheet (SDS) for *Quatsan*

Material Safety Data Sheet.
Date Issued 13/03/2008

Product: Quatsan

MATERIAL SAFETY DATA SHEET

NORTHERN CHEMICALS PTY LTD
A.C.N. 010 495 039
157 Hartley Street, Cairns, Qld. 4870
P.O. Box 1482, Cairns, Qld. 4870
Phone:(07) 4035 4622 Fax:(07) 4035 4932
EMAIL: norchem@ozemail.com.au

I Identification

PRODUCT NAME **QUATSAN**

D.G. CLASS:	N/A	UN NUMBER:	N/A
HAZCHEM CODE:	N/A	PACKAGING GROUP:	N/A
POISON SCHEDULE:	N/A		

Non Hazardous according to criteria of Worksafe Australia.

Physical Description/Properties

APPEARANCE: Clear liquid - slightly viscous
VAPOUR PRESSURE: N/A
FLASH POINT: Not Combustible.
FLAMMABILITY LIMITS: Non Flammable
SPECIFIC GRAVITY 1:1 (Water=1,0)
BOILING POINT:
SOLUBILITY IN WATER: Total
pH.8

(N.B. Physical data based on material tested. May vary from sample to sample.)

Ingredients

CHEMICAL ENTITY	CAS No.		PROPORTION
Quaternary Ammonium Compound	63449-41-2	ADBAC	M
Benzalkonium Chloride	68989-00-4	ADBAC	L
Nonionic Surfactants	9013-45-9		L
Alkaline Salts	N/A		L
	H=>60%	M=10-60%	L=<10%

II Health Hazard Information

Routes Of Exposure.

SWALLOWED:	May cause damage to mucous membranes.
EYE:	May irritate eyes in undiluted form.
SKIN:	May irritate sensitive skin in undiluted form.
INHALED:	

Emergency And First Aid Procedures.

SWALLOWED:	If swallowed do not induce vomiting, give a glass of water.
EYE:	Hold eyes open, flood with water for 15 minutes and see a doctor.
SKIN:	Remove contaminated clothing and wash skin thoroughly.
INHALED:	Remove patient to fresh air
CONTACT	POISONS INFORMATION CENTRE 13 11 26

Material Safety Data Sheet.
Date Issued 13/03/2008

Product: Quatsan

III Precautions For Use

EXPOSURE LIMITS: N/A
VENTILATION: N/A

Protective Equipment.

GLOVES: Gloves may be used when mixing and applying.
EYE PROTECTION: Goggles /Safety glasses may be worn when mixing and applying.
CLOTHING :
OTHER PROTECTIVE GEAR:

Flammability & Compatibility.

FLAMMABILITY: This product is not flammable
INCOMPATIBILITY: Store away from acids or chlorine compounds.
NORMAL CONDITIONS OF USE: Add QUATSAN to water.

IV. Safe Handling Information.

Storage Transport: Store in a cool, dry place out of direct sunlight.
Reseal container when not in use.
Store away from acids or chlorine compounds.

Spills disposal: Contain spill and place in industrial waste bin.
Then hose down area where spill occurred

Fire Explosion Hazard:: Not combustible.

Extinguishing Media: N/A

Special fire fighting procedures: N/A

Unusual fire explosion hazard: N/A

Hazardous decomposition products: N/A

This material safety Data Sheet supersedes all previous issues for this product. Information contained herein is offered in good faith as being true and accurate to the best of our knowledge, but as conditions of use are beyond our control, no guarantees are either stated or implied.

Where health or safety data given discloses a risk to the user or to the environment, it is the responsibility of the Purchaser to pass on that information to employees or those who may be using the product, ensuring that adequate safety procedures are used including good industrial hygiene.

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5. CORPORATE AUTHOR

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Chief, Maritime Platforms Division

15. SECONDARY RELEASE STATEMENT OF THIS DOCUMENT

Approved for public release

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16. DELIBERATE ANNOUNCEMENT

No limitations

17. CITATION IN OTHER DOCUMENTS No

18. DSTO RESEARCH LIBRARY THESAURUS

Biofouling, Fouling Organisms, Marine Biosecurity, Internal seawater systems, Sea Chests, Mussels, Quaternary Ammonium compounds (QAC), Benzoalkonium Chloride (BAC), Toxicity

19. ABSTRACT

Mussel growth in the internal sea water systems of Navy vessels can result in significant performance issues and biosecurity concerns for affected vessels. The primary in-water treatment method for mussel fouling of the internals of Navy vessels is to flush with a 1% detergent solution containing quaternary ammonium compounds (QAC). Parameters for the application of this treatment are based on previous research; however, much of this research has been conducted at small-scales under laboratory conditions. This study examined the efficacy of two commercial QAC solutions for treating mussel biofouling under realistic field conditions using experimental sea water piping systems. The efficacy of the QAC solutions was found to be highly dependent on the size of the mussels present. All treatment solutions were effective at killing large sized mussels in the pipework and sea chest of the system following a 24 h dosing period. In contrast, small mussels appeared resilient to the majority of treatment regimes tested. Changes in water temperature and increased exposure time to treatment chemicals did not enhance efficacy of treatment.