

Evaluation of marine response tools: Microwave sterilisation

MAF Biosecurity New Zealand Technical Paper No: 2010/18

Prepared for MAF Biosecurity New Zealand
By Sandra Hinni and Dan McClary

Golder Associates (NZ) Ltd, Takapuna, Auckland, NZ.

ISBN 978-0-478-37077-5 (print)
ISBN 978-0-478-37078-2 (online)

ISSN 1179-5832 (print)
ISSN 1179-5840 (online)

December 2008



Disclaimer

While every effort has been made to ensure that the information in this publication is accurate, the Ministry of Agriculture and Forestry does not accept any responsibility or liability for error or omission of fact, interpretation or opinion that may be present, nor for the consequences of any decisions based on this information.

Any view or opinions expressed do not necessarily represent the official view of the Ministry of Agriculture and Forestry.

The information in this report and any accompanying documentation is accurate to the best of the knowledge and belief of Golder Associates (NZ) Ltd acting on behalf of the Ministry of Agriculture and Forestry. While Golder Associates (NZ) Ltd has exercised all reasonable skill and care in the preparation of information in this report, neither Golder Associates (NZ) Ltd nor the Ministry of Agriculture and Forestry accept any liability in contract, tort or otherwise for any loss, damage, injury or expense, whether direct, indirect or consequential, arising out of the provision of information in this report.

Requests for further copies should be directed to:

Post Border Directorate
MAF Biosecurity New Zealand
PO Box 2526
WELLINGTON

Telephone: 0800 008 333
Facsimile: 04-894 0300

This publication is also available on the MAF website at
<http://www.biosecurity.govt.nz/about-us/our-publications/technical-papers>

© Crown Copyright - Ministry of Agriculture and Forestry

Abstract

Commercial-grade microwave generators were tested for use in eradication of non-indigenous marine species living within soft sediments. We find here that at its present stage of development, microwave technology may be most suitable for eradication of shallow infaunal organisms living no deeper than 3 cm from the surface.

A series of experiments was conducted using the infaunal bivalve *Nucula* spp. to test the effectiveness of two microwave generators (operating at 0.8 kilowatt (kW) and 5 kW respectively) on a range of soft substrates (mud, sand and gravel). Individuals of *Nucula* spp. were placed at 0 and 3 cm depth in the sediment and microwave radiation was applied for 3 minutes (0.8 kW generator) or 60 secs (5 kW generator). Temperature profiles achieved in each sediment type after microwave exposure and bivalve mortality rates were determined. The highest average temperature of 94.2°C was achieved in muddy sediment using the 5 kW generator. Mortality rates recorded with the 0.8 kW generator across the 3 sediment types ranged from 0% to 90% and from 0% to 100% using the 5 kW microwave generator. Sediment moisture appeared to have no or little effect on the maximum temperature and mortality rates obtained in all sediment types.

The variable mortality rates obtained across the replicate treatments indicate that extensive trials would be necessary to develop a microwave unit for use in an eradication or control attempt in the field. Our investigations suggest that a unit of approximately 30 kW would be required to rapidly achieve consistent, high mortality rates in a field environment. We would envisage a trailer mounted microwave unit, pulled by a quad bike or mounted on an amphibious vehicle for such a large scale application. Such a high-power unit would likely require a much shorter exposure time of (~ 6 secs) and be capable of treating an area (based on the size of existing high-power generators) of approximately 18 m² per hour. Given this limitation, use of microwaves in eradication and control may be best suited to those taxa at risk of spreading through fragmentation (i.e., during attempts at physical removal).

Keywords: microwave generator, bivalve, non-indigenous, biosecurity, response tool

Abstract	i
1. Introduction	1
2. Methods	2
2.1 Overview	2
2.2 Pilot Trial	3
2.3 Preliminary Mortality Experiment	5
2.4 Efficacy Trial with 0.8 kW Microwave Generator	6
2.5 Efficacy Trial with 5 kW Microwave Generator	6
3. Results	7
3.1 Pilot Trial	7
3.2 Preliminary Mortality Experiment	9
3.3 Efficacy Trial with 0.8 kW Microwave Generator	9
3.4 Efficacy Trial with 5 kW Microwave Generator	13
4. Discussion	15
4.1 Effectiveness of Microwave Technology	15
4.2 Applicability of Microwave Technology	16
5. References	22

List of Tables

Page

Table 1: Mean temperatures (\pm one standard deviation) for the three sediment types using the 0.8 kW microwave generator ($n = 3$).	9
Table 2: Mean temperatures (\pm one standard deviation) at the centre of the microwave emitter for the three sediment types using the 0.8 kW microwave generator ($n = 3$).	11
Table 3: Mean mortality (\pm one standard deviation) of <i>Nucula</i> spp. after 3 minutes exposure to the 0.8 kW microwave generator ($n = 7 - 10$).	11
Table 4: Mean temperatures (\pm one standard deviation) at the centre of the microwave emitter for the three sediment types with the 5 kW microwave generator ($n = 3$).	14
Table 5: Mean mortality (\pm one standard deviation) of <i>Nucula</i> spp. after 60 secs exposure to the 5 kW microwave generator ($n = 5 - 10$).	15
Table 6: Summary of mean temperature increase ($\Delta T \pm$ one standard deviation) in sediments using two different microwave generators.	16

List of Figures

Page

Figure 1: a) 0.8 kW microwave generator tested on a container with gravel; b) Sandy sediment surface after 3 minutes of microwave exposure; c) 5 kW microwave generator; and d) Experimental set-up with sediment container.	3
Figure 2: Site locations for sediment collections on the North Shore in Auckland: M = Muddy sediment at Northcroft Esplanade Reserve, S = Sandy sediment at Cheltenham Beach, and G = Gravely sediment at Stanley Bay. Red square: Meola Reef, <i>Nucula</i> spp. collection site.	4
Figure 3: Collection sites for the three sediment types: a) Mud at Northcroft Esplanade, b) Gravel at Stanley Bay, c) Sand at Cheltenham Beach, and d) Sediment slabs.	5
Figure 4: Percent composition (\pm one standard deviation) of mud, sand and gravel in the three sediment types for the pilot trials ($n = 3$). Solid black line indicates moisture content (\pm one standard deviation).	7
Figure 5: Sediment temperatures reached at the centre of the microwave emitter over time in the three sediment types.	8
Figure 6: Maximum temperatures (\pm one standard deviation) achieved after 3 minutes of exposure to the 0.8 kW generator in the three sediment types ($n = 3$).	10
Figure 7: Percent composition (\pm one standard deviation) of mud, sand and gravel in the three sediment types for the 0.8 kW microwave generator trial ($n = 3$). Solid black line indicates moisture content (\pm one standard deviation).	11
Figure 8: Percent mortality (\pm one standard deviation) of <i>Nucula</i> spp. in the three sediment types after 3 minutes of exposure to the 0.8 kW microwave generator.	12
Figure 9: Percent composition (\pm one standard deviation) of mud, sand and gravel in the three sediment types for the 5 kW microwave generator trial ($n = 3$). Solid black line indicates moisture content (\pm one standard deviation).	13
Figure 10: Percent mortality (\pm one standard deviation) of <i>Nucula</i> spp. in the three sediment types after 60 seconds of exposure to the 5 kW microwave generator.	14

Abbreviations

CMA	Coastal Marine Area
GHz	Gigahertz
kW	Kilowatt
MHz	Megahertz
NIMS	non-indigenous marine species
RMA	Resource Management Act

1. Introduction

The increasing rate of non-indigenous marine species (NIMS) introductions has become a matter of global concern (Carlton 1989, Cohen & Carlton 1998, Grosholz 2005, Ruiz et al. 1997). Introduced NIMS can adversely affect natural ecosystems and biodiversity through competition with native species for resources or by habitat modification (Ruiz et al. 1997, Wallentinus & Nyberg 2007). Furthermore, NIMS can affect the economy and potentially cause substantial losses to commercial fisheries through competitive displacement (AFF-Australia 2002, Ribera & Boudouresque 1995, Ruiz et al. 1997). Lastly, human health can also be adversely affected (e.g., NIMS and toxic red tides) (Ruiz et al. 1997).

To avoid such adverse effects, prevention of NIMS introductions or eradication of organisms already present in the environment are necessary, for which effective tools are required. Successful application of a rapid response tool depends on the efficacy of the treatment against the target species and the ease of application in the environment. Documented cases of successful eradications of NIMS are limited to five species, the dreissenid mussel *Mytilopsis sallei*, the sabellid polychaete *Terebrasabella heterounicata*, the green alga *Caulerpa taxifolia*, the brown kelp *Undaria pinnatifida* and Northern Pacific seastar *Asterias amurensis* (Bax 1999, Culver & Kuris 2000, Hewitt & Campbell 2007, Woodfield & Merkel 2006, Wotton et al. 2004). Response tools used in these instances included chemical treatment, removal of potential host species and heat treatment of the ships hull infested by the organism (Wotton et al. 2004).

A major factor contributing to these successful eradications was targeted response, specifically designed to the circumstances of each situation and site. Such specific control techniques are, however, limited and are in many cases the major constraint to the eradication of NIMS (McEnulty et al. 2000). For instance, the effective treatment of burrowing NIMS living within soft sediments is particularly difficult given that the delivery of treatments into sediments using conventional methods relies on passive processes, such as diffusion, convection and conduction. The application of aqueous solutions of toxic agents relies on diffusion while the transfer of heat from the water column is by conduction. Furthermore, the chemical treatment of bivalve molluscs is hindered by valve closure, which is a common behavioural response to the presence of relatively low concentrations of most chemical agents. Microwave radiation, however, offers several advantages over conventional methods as heat is delivered directly to the target tissue through the excitation of water molecules within the organism's tissue (Oliviera & Franca 2002). Microwaves are particularly effective against organisms with calcareous tubes and shells, which are typically a barrier to thermal conduction, but are penetrated by microwaves. Microwave radiation also generates heat simultaneously throughout the volume of the treated substrate, which is conducted to interstitial organisms.

The concept of using microwave radiation as a means to kill pest species has been tested previously, particularly in the timber industry for the eradication of pest organisms in wood and wood products. Trials using a commercial 2.45 Gigahertz (GHz) microwave apparatus proved effective at killing cerabycid larvae and pine wood nematodes in red pine, as well as Asian longhorn beetles in wood packing material, thus identifying microwaves as a feasible alternative to conventional heat treatment or methyl bromide fumigation (Fleming et al. 2004, 2005).

Microwave radiation has also been trialled as a method for the sterilisation of ships ballast water, but treatment success was considered to be too variable and expensive to apply on a

commercial scale (Vinograd & Sytsma 2002). More recently, laboratory studies have resulted in the complete inactivation of microalgae, zooplankton and bivalve larvae when subjected to continuous microwave treatments at temperatures ranging from 43 - 53° C and flow rates of 1 - 2 L of water per minute (Boldor et al. 2008). Significant technological and operational challenges need to be overcome before microwave technology can be effectively used in the field in response to an incursion of benthic NIMS.

This report provides the results of a series of laboratory trials aimed at assessing the efficacy of microwave technology as a potential response tool for the eradication of NIMS. It is part of a suite of tools that are being developed and trialled for MAF Biosecurity New Zealand and that could potentially be applied to various marine situations, including different environments, species/functional groups, conditions and spatial scales. Efficacy was assessed on the ability of microwaves to penetrate sediment and destroy soft sediment benthic organisms (e.g., bivalves) or render them non-viable. The experiments presented in this study were conducted using equipment and facilities provided by Keam Holdem Associates (KHA) in Auckland, a company that designs and manufactures microwave and radio frequency heating products.

2. Methods

2.1 OVERVIEW

The two microwave generators tested are depicted in Figure 1. One generator (Figure 1a + b) operates at 2450 MHz and 0.8 kW and has a wavelength of 122 mm in air. This unit provides microwave energy to the sediment via a 100 x 50 mm rectangular opening, the waveguide. The generator is not power controlled and so operates at an undetermined power level into the sediment. Taking into account the non-conducting properties of the sediment and the resistance (impedance) presented to the generator by the waveguide aperture, the microwave power deposited into the sand was likely to be of the order of 600 W. No impedance tuning structure, to prevent microwaves from simply reflecting from the sediment, was used during the experiments.

The larger generator (Figure 1c + d) operated at 915 MHz and 5 kW and has a wavelength of 327 mm in air. It provides microwave energy to the substrate via a 280 x 160 mm rectangular waveguide. The generator was manually power controlled and the experiments were conducted with the generator producing approximately 3 kW of microwave power. At this power and wavelength some microwave reflection from the substrate was detected. To overcome this difficulty, a three stub impedance tuner was required in the waveguide section to ensure a good match between the generator and the load.

A series of experiments was conducted to assess the efficacy of the microwave system and included:

- Pilot trials to establish temperature profiles for the three different sediment types tested.
- Preliminary mortality experiments to determine lethal temperatures for *Nucula* spp.
- Efficacy trials using 0.8 kW microwave generator to establish *Nucula* spp. mortality rates.
- Efficacy trials with 5 kW microwave generator to establish *Nucula* spp. mortality rates.



a)



b)



c)



d)

Figure 1: a) 0.8 kW microwave generator tested on a container with gravel; b) Sandy sediment surface after 3 minutes of microwave exposure; c) 5 kW microwave generator; and d) Experimental set-up with sediment container.

2.2 PILOT TRIAL

The aim of the pilot trial was to establish temperature profiles at various depths of marine substrates after microwave treatment. Three types of sediment were collected at low tide from the following sites in Auckland (Figures 2 and 3):

- Mud: Northcroft Esplanade Reserve, North Shore.
- Sand: Cheltenham Beach (north-western end), North Shore.
- Gravel: Stanley Bay, North Shore.



Figure 2: Site locations for sediment collections on the North Shore in Auckland: M = Muddy sediment at Northcroft Esplanade Reserve, S = Sandy sediment at Cheltenham Beach, and G = Gravely sediment at Stanley Bay. Red square: Meola Reef, *Nucula* spp. collection site.

Using a spade, slabs of sediment were collected and placed in plastic containers (300 x 300 x 300 mm) until filled, whilst ensuring the integrity of the sediment profile was maintained (Figure 3d). Lids were placed on the containers to prevent moisture loss. Three replicate containers were collected per sediment type. Triplicate sediment samples of each sediment type were collected for moisture content and textural analyses. Moisture content was calculated as the percentage difference between wet and dry matter (dried for 16 hrs at 103° C). Sediment texture was determined by sieving the dried samples through 2 mm and 63 µm mesh to partition each sample into mud (<63 µm), sand (>63 µm – <2 mm), and gravel (>2 mm) fractions.

A preliminary experiment was conducted to determine the exposure period after which maximum sediment temperatures were reached. The 0.8 kW microwave generator was placed in direct contact with the sediment surface, while ensuring no gaps were present between the apparatus and the sediment surface. It was run for 30 secs, after which time the temperature was measured at the surface and at 2 cm depth using an Atkins (Econotemp plus) temperature probe. The same area was then heated and measured again at 30 - 60 secs intervals until 5 minutes of heating was reached. Based on the results of this experiment, 3 minutes was determined to be most suitable to achieve maximum temperatures within the shortest amount of time.

To determine temperature profiles at various depths, microwave radiation was applied to the sediment surface for 3 minutes. Temperatures for each replicate container were recorded immediately before and after treatments. Measurements were taken at 0, 3, 8 and 12 cm depth and for each depth strata at 0, 2 and 4 cm distance from the centre of the microwave emitter.



a) Northcroft Esplanade



b) Stanley Bay



c) Cheltenham Beach



d) Sediment collection

Figure 3: Collection sites for the three sediment types: a) Mud at Northcroft Esplanade, b) Gravel at Stanley Bay, c) Sand at Cheltenham Beach, and d) Sediment slabs.

2.3 PRELIMINARY MORTALITY EXPERIMENT

A preliminary experiment was conducted to determine the optimal temperature and exposure period required to kill bivalves when exposed to microwaves. The target species was the native nut shell, *Nucula* spp., as it was readily available and present in substrates similar to those inhabited by invasive bivalves. Attempts were made to source live specimens of invasive species (i.e., *Limaria orientalis* and *Theora lubrica*), but these could not be reliably or readily obtained in an abundance and suitable size range as required for further experiments.

Adult *Nucula* spp. ranging in size between 5 - 8 mm were collected from the intertidal zone at Meola Reef, Auckland (Figure 2) at low tide on the day prior to the experiments.

Sediment to a depth of 5 cm was excavated using a spade and the contents were washed through a 500 µm mesh. Live *Nucula* spp. obtained were placed in a container with seawater and stored overnight. Sand was collected from Cheltenham Beach.

Ten *Nucula* spp. individuals were placed in triplicate plastic bags containing approximately 300 g of sand. Three replicate bags per treatment were prepared. Individual bags were exposed to temperatures of 40, 60, and 80° C for periods of 1, 2, 3 and 5 minutes (the latter exposure time for 80° C only) by immersion in a waterbath. The bags were removed from the water bath after treatments and mortality of *Nucula* spp. was determined. Individuals were considered dead when the valves were gaping open or did not actively close when separated with a probe.

2.4 EFFICACY TRIAL WITH 0.8 KW MICROWAVE GENERATOR

The aim of this trial was to determine the efficacy of microwave treatment on benthic bivalves (i.e., *Nucula* spp.) residing in sediment. Triplicate samples and a control sample of each substrate type, and sediment samples were collected in the same manner as indicated previously in Section 2.1. *Nucula* spp. were collected from Meola Reef on the same day as sediment collection and using methods described previously in Section 2.2. Ten *Nucula* spp. each were placed immediately below the surface at 3 cm deep and at distances of 0, 2 and 4 cm from the heat source (i.e. six triplicate treatments each of 10 bivalves). Control treatments comprised 10 *Nucula* spp. each placed immediately below the surface and at 3 cm depth. A maximum depth of 3 cm and distance of 4 cm from the centre of the microwave emitter was chosen based on the results of the pilot trials (Sections 3.1 and 3.2), which indicated that temperatures reached beyond this depth were likely to be non-lethal.

Sediment temperature was measured at 0 and 3 cm depth immediately before and after irradiation with a 0.8 kW microwave generator for 3 minutes. The control containers were treated in the same manner as the treatment containers, but were not irradiated. All *Nucula* spp. were recovered from the sediment and mortality was determined by the same criteria as indicated in Section 2.2.

2.5 EFFICACY TRIAL WITH 5 KW MICROWAVE GENERATOR

The trial conducted in the previous section was repeated with the same purpose, but this time using a more powerful 5 kW microwave generator. The method applied was similar to that applied previously, but with the following exceptions:

- Containers of the dimensions 320 x 320 x 150 mm were required to accommodate the larger waveguide.
- Ten *Nucula* spp. each were placed immediately below the surface and at approximately 3 cm depth, inside and outside the irradiated area (i.e., four triplicate treatments each of 10 bivalves).
- Sediment samples were irradiated for 60 secs.

3. Results

3.1 PILOT TRIAL

The average proportion of gravel, sand and mud substrates for the three sediment types used in the pilot trial is illustrated in Figure 4. The gravel sediment was composed of approximately equal proportions of sand (<2 mm - $\geq 63 \mu\text{m}$) and gravel (<2 mm), while mud was dominated by the <63 μm size fraction and sand was dominated by the <2 mm - $\geq 63 \mu\text{m}$ size fraction. The muddy sediment contained on average 62% water and moisture content decreased with increasing sediment coarseness. The results of the preliminary heating trial indicated that maximum sediment temperatures were generally reached after 3 minutes of heating at both depths (Figure 5).

Average temperatures obtained after 3 minutes of microwave exposure are provided in Table 1 and Figure 6. Overall, a maximum temperature of 86.5° C was achieved in sand at 3 cm depth, at 0 cm distance from the emitter. Maximum temperatures of 86.0° C and 84.0° C were obtained in mud and gravel respectively at the surface, at 0 cm distance from the emitter. Sand was the only sediment type, where temperatures remained above 70° C on the surface at up to 4 cm distance from the emitter, and at 3 cm depth for distances of 0 cm and 2 cm from the emitter. For all sediment types, temperatures declined with increasing distance from the centre of the microwave emitter and with increasing depth.

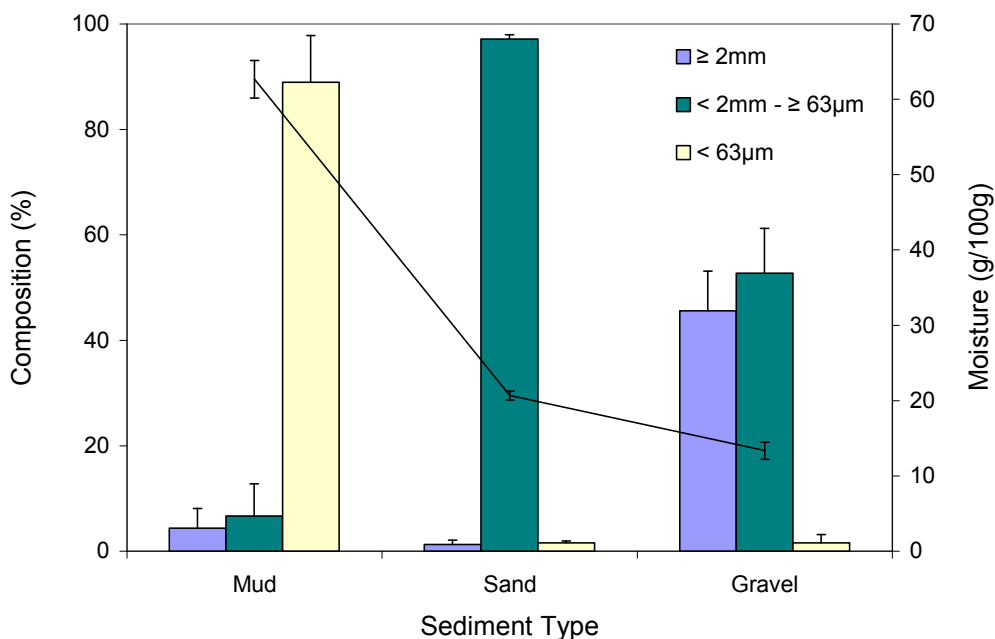


Figure 4: Percent composition (\pm one standard deviation) of mud, sand and gravel in the three sediment types for the pilot trials ($n=3$). Solid black line indicates moisture content (\pm one standard deviation).

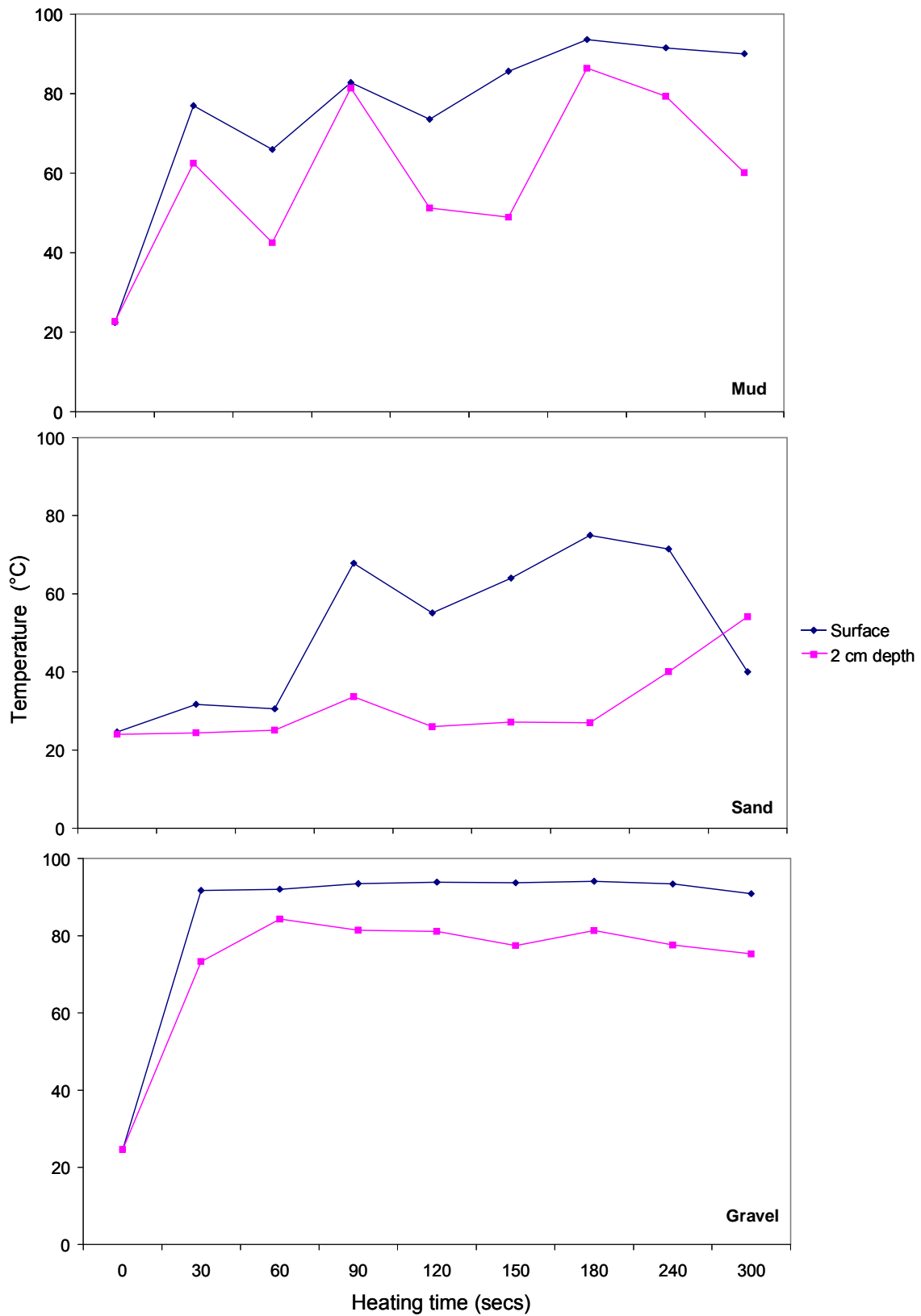


Figure 5: Sediment temperatures reached at the centre of the microwave emitter over time in the three sediment types.

Table 1: Mean temperatures (\pm one standard deviation) for the three sediment types using the 0.8 kW microwave generator ($n = 3$).

Sediment type	Distance to centre of emitter (cm)	Sediment Temperatures ($^{\circ}$ C) at depths \pm SD			
		Surface	3 cm	8 cm	12 cm
Mud	0	86.0 \pm 2.5	46.1 \pm 9.9	21.7 \pm 0.6	18.4 \pm 1.0
	2	69.6 \pm 7.9	37.2 \pm 13.7	20.3 \pm 1.0	18.1 \pm 1.2
	4	47.1 \pm 6.6	27.0 \pm 7.1	19.5 \pm 1.2	17.8 \pm 0.7
Sand	0	81.3 \pm 11.5	86.5 \pm 7.9	38.2 \pm 1.7	22.5 \pm 3.6
	2	82.4 \pm 10.3	83.1 \pm 6.3	35.4 \pm 2.3	22.7 \pm 4.1
	4	71.1 \pm 7.7	67.4 \pm 6.0	30.2 \pm 3.5	21.9 \pm 3.0
Gravel	0	84.0 \pm 1.8	51.5 \pm 18.04	23.7 \pm 0.4	20.8 \pm 0.2
	2	73.2 \pm 10.0	38.2 \pm 7.1	22.5 \pm 0.7	20.4 \pm 0.6
	4	53.5 \pm 14.4	30.6 \pm 1.4	21.4 \pm 0.5	20.1 \pm 0.3

3.2 PRELIMINARY MORTALITY EXPERIMENT

The results of the preliminary experiment indicated that *Nucula* spp. has a high tolerance to heat with 0% mortality after exposure for 1 - 3 minutes to temperatures of 40, 60 and 80 $^{\circ}$ C. The only dead individual was detected after being exposed to 80 $^{\circ}$ C for 5 minutes. Although no temperature measurements were taken in the bag itself, it can be assumed that the temperature of the sand was similar to that of the surrounding water due to the small amount of sand in the bag.

3.3 EFFICACY TRIAL WITH 0.8 KW MICROWAVE GENERATOR

The textural composition and moisture content of sediments used for this set of experiments is depicted in Figure 7. As the sediment originated from the same locations as for the pilot trials, textures and moisture contents were similar (refer Figure 4). The muddy sediment contained the most water with an average of 63%. Moisture content decreased with increasing sediment coarseness.

The highest temperatures were experienced in gravel both at the surface (88.4 $^{\circ}$ C) and at 3 cm depth (88.2 $^{\circ}$ C) (Table 2). Temperatures at the surface were however, higher than those occurring at a depth of 3 cm across all sediment types.

Mortality rates of *Nucula* spp. after exposure to the 0.8 kW microwave generator are displayed in Table 3 and Figure 8. All *Nucula* spp. in the three control containers survived. Among the treatments, 7 - 10 *Nucula* spp. were recovered from the sediments after each experiment. Due to the small adult size of the organisms used (5 - 8 mm), some individuals were lost. The highest mortality rates were recorded at the centre and surface (0 cm depth) of the sediment in all three substrate types. Mortality rates were, however, highly variable among the three replicate samples.

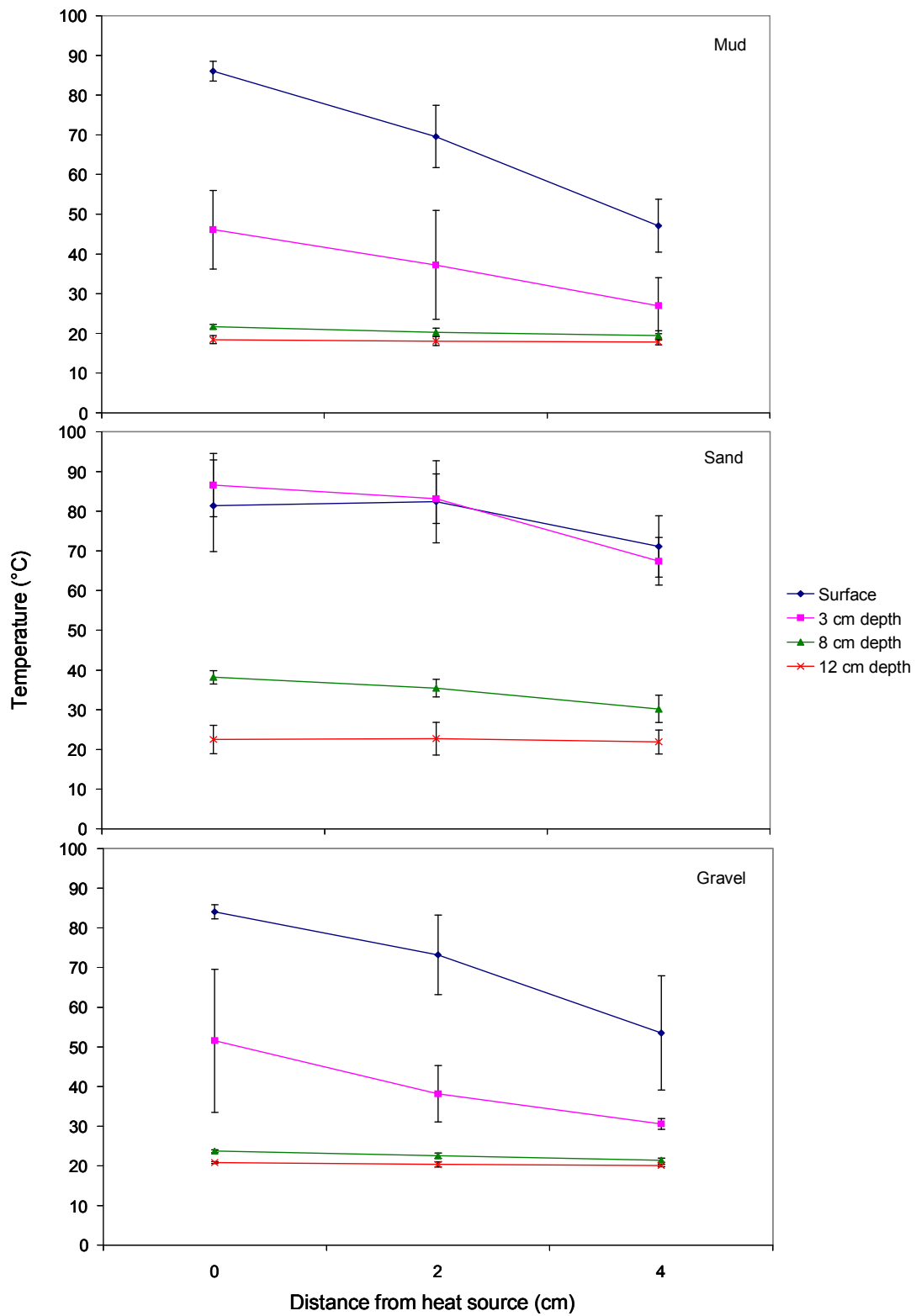


Figure 6: Maximum temperatures (\pm one standard deviation) achieved after 3 minutes of exposure to the 0.8 kW generator in the three sediment types ($n = 3$).

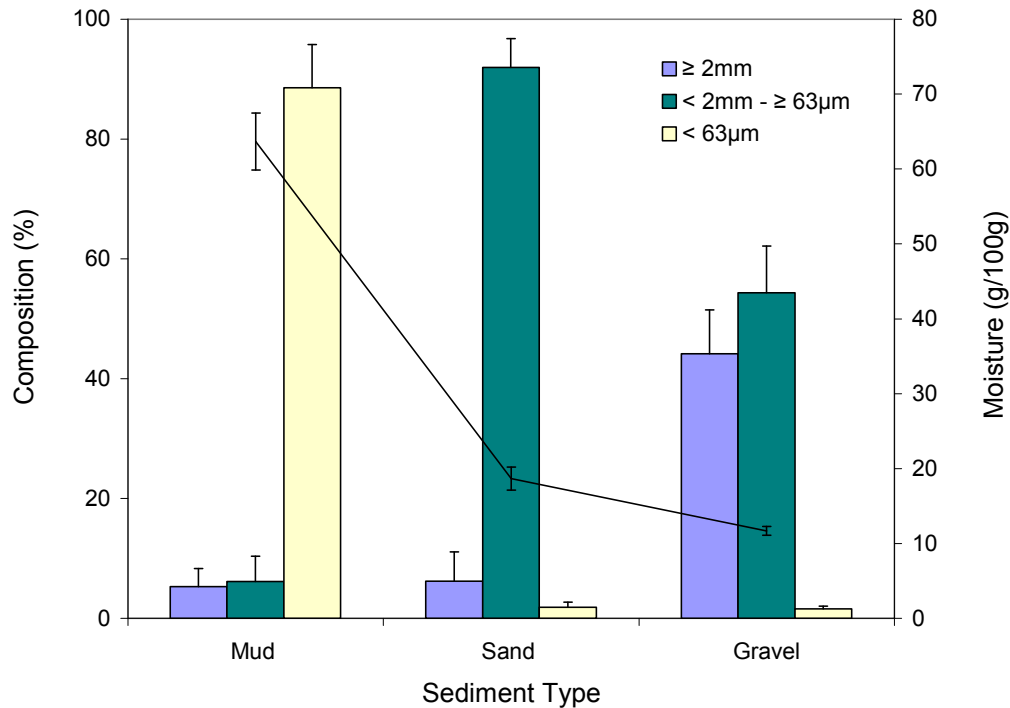


Figure 7: Percent composition (\pm one standard deviation) of mud, sand and gravel in the three sediment types for the 0.8 kW microwave generator trial ($n = 3$). Solid black line indicates moisture content (\pm one standard deviation).

Table 2: Mean temperatures (\pm one standard deviation) at the centre of the microwave emitter for the three sediment types using the 0.8 kW microwave generator ($n = 3$).

Sediment type	Average temperatures (°C) \pm SD	
	0 cm depth	3 cm depth
Mud	83.5 \pm 1.0	27.5 \pm 8.5
Sand	76.5 \pm 6.7	59.4 \pm 48.5
Gravel	88.4 \pm 6.9	88.2 \pm 8.1

Table 3: Mean mortality (\pm one standard deviation) of *Nucula* spp. after 3 minutes exposure to the 0.8 kW microwave generator ($n = 7 - 10$).

Sediment Type	Distance to centre of emitter (cm)	% Mortality \pm SD	
		0 cm depth	3 cm depth
Mud	0	90 \pm 17.3	33.67 \pm 24.2
	2	61.7 \pm 37.5	0
	4	61.9 \pm 15.9	0
Sand	0	90 \pm 10	38.3 \pm 18.9
	2	60 \pm 36.1	36.7 \pm 35.1
	4	55 \pm 48.2	19.4 \pm 10.1
Gravel	0	80 \pm 17.3	63.3 \pm 35.1
	2	30 \pm 36.1	33.3 \pm 57.7
	4	20 \pm 26.5	27.6 \pm 39.4

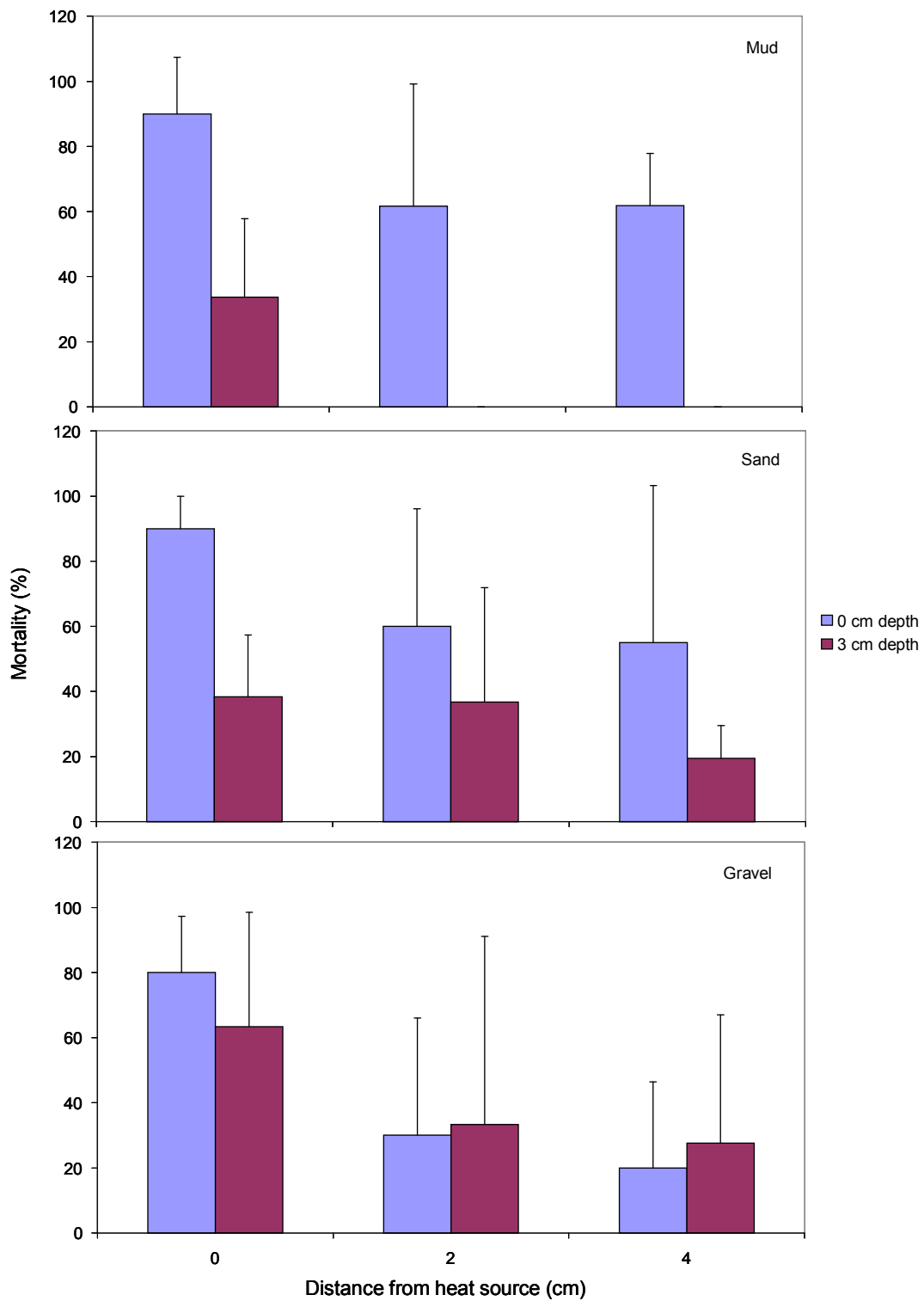


Figure 8: Percent mortality (\pm one standard deviation) of *Nucula* spp. in the three sediment types after 3 minutes of exposure to the 0.8 kW microwave generator.

3.4 EFFICACY TRIAL WITH 5 KW MICROWAVE GENERATOR

The composition of sediment used for the 5 kW microwave generator trials (Figure 9) were similar to those of the previous experiments (refer Figures 5 + 7). Mud contained the most water with an average of 60%. Temperatures attained using the 5 kW microwave generator were over 90° C at 0 cm depth in all sediment types and thus higher than those reached with the 0.8 kW generator (Table 4). Temperatures attained at 3 cm, on the other hand, were generally lower in these trials in comparison to the temperatures achieved using the 0.8 kW generator.

Mortality rates obtained after 60 secs exposure to the 5 kW microwave generator are displayed in Figure 10 and Table 5. All *Nucula* spp. in the control containers survived. Among the treatments, 5 - 10 *Nucula* spp. were recovered from the sediments after each experiment. Some organisms were lost due to their small size. The highest mortality rate was obtained in mud, where 100% of *Nucula* spp. died at the surface within the irradiated area. Microwave treatment using the 5 kW microwave generator for 60 secs was least effective in sandy sediments, where 77% mortality was achieved inside the treated area at the surface. All other recovered *Nucula* spp. in sand however, survived the treatment. In gravel, higher mortality rates were achieved inside the treated area at 3 cm depth than at the surface.

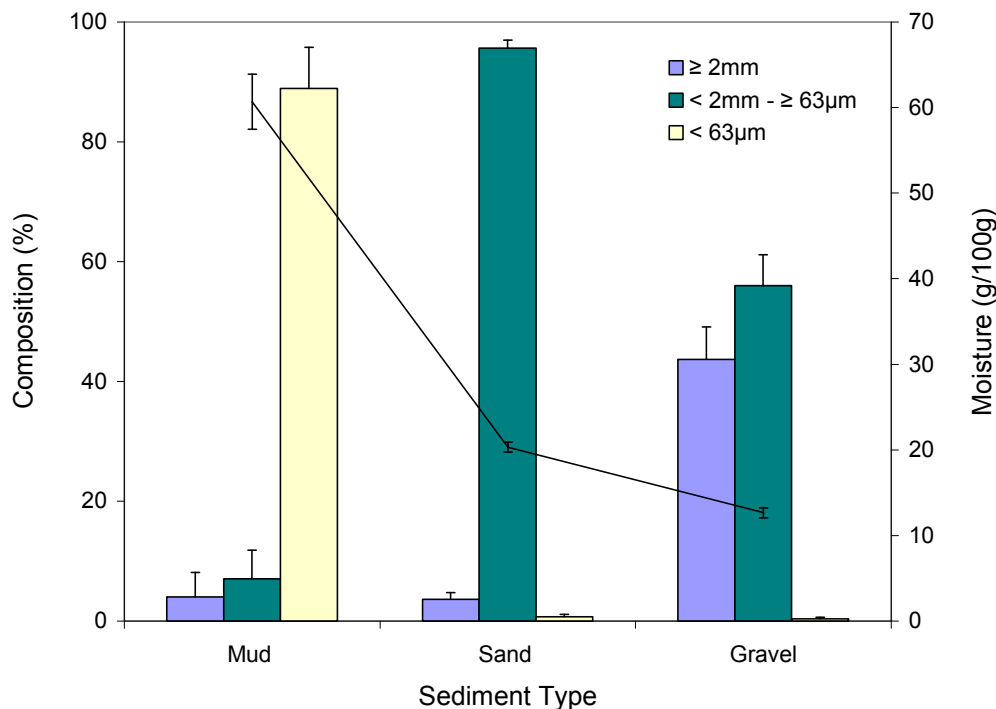


Figure 9: Percent composition (\pm one standard deviation) of mud, sand and gravel in the three sediment types for the 5 kW microwave generator trial ($n = 3$). Solid black line indicates moisture content (\pm one standard deviation).

Table 4: Mean temperatures (\pm one standard deviation) at the centre of the microwave emitter for the three sediment types with the 5 kW microwave generator ($n = 3$).

Sediment type	Average temperatures ($^{\circ}\text{C}$) \pm SD	
	0 cm depth	3 cm depth
Mud	91.5 \pm 3.9	45.2 \pm 26.5
Sand	93.0 \pm 4.0	55.4 \pm 8.8
Gravel	94.2 \pm 4.6	82.5 \pm 8.9

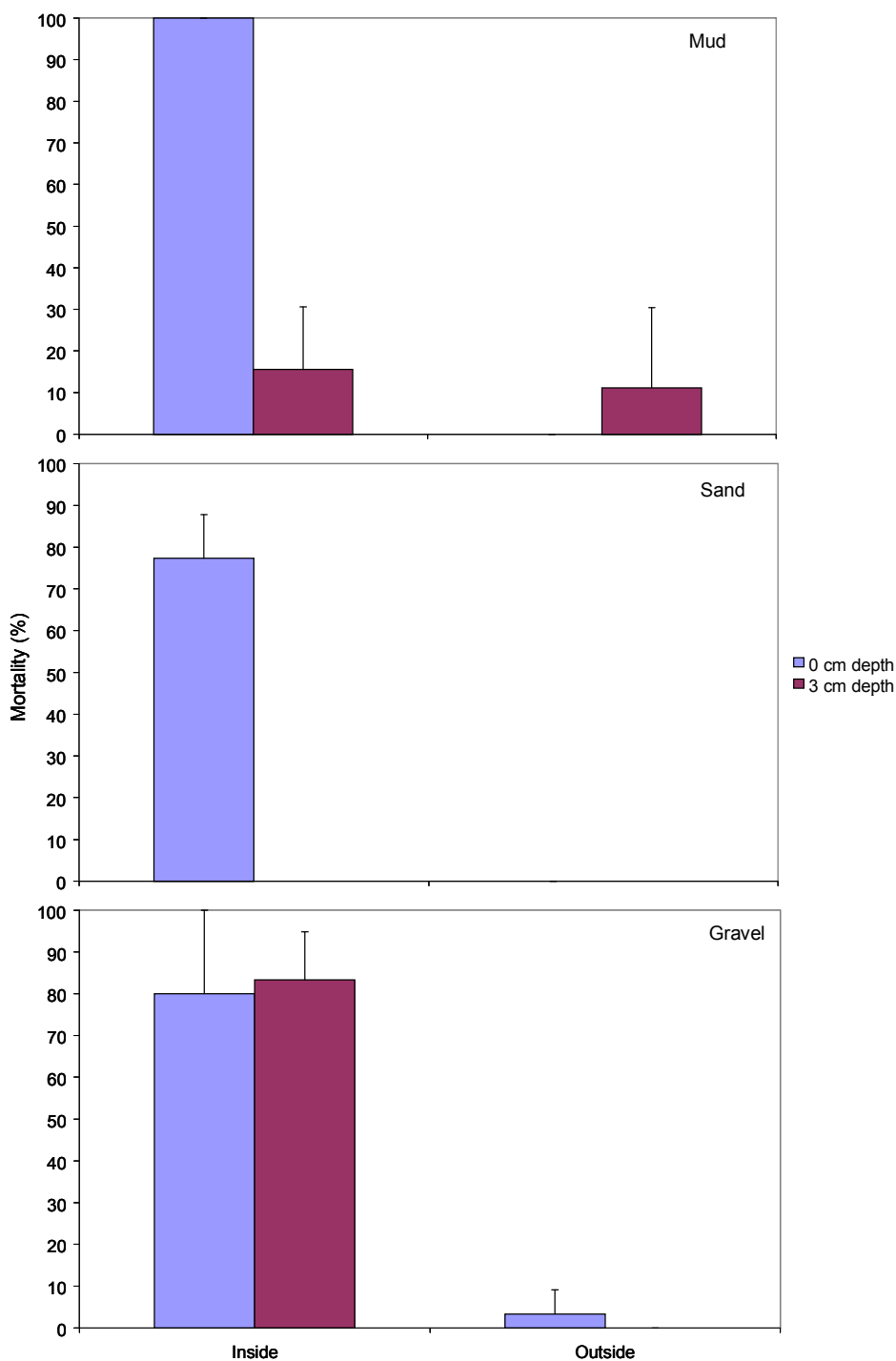


Figure 10: Percent mortality (\pm one standard deviation) of *Nucula* spp. in the three sediment types after 60 seconds of exposure to the 5 kW microwave generator.

Table 5: Mean mortality (\pm one standard deviation) of *Nucula* spp. after 60 secs exposure to the 5 kW microwave generator ($n = 5 - 10$).

Sediment Type	Area respective to microwave emitter	% Mortality \pm SD	
		0cm depth	3 cm depth
Mud	Inside	100 \pm 0	14.6 \pm 13.8
	Outside	0	11.1 \pm 19.3
Sand	Inside	77.3 \pm 10.4	0
	Outside	0	0
Gravel	Inside	75.6 \pm 21.4	83.3 \pm 11.6
	Outside	3.3 \pm 5.7	0

4. Discussion

4.1 EFFECTIVENESS OF MICROWAVE TECHNOLOGY

Microwave radiation appears to penetrate all mud, sand and gravel substrates to an effective depth of approximately 3 cm; increase in temperatures beyond this depth was only slight and likely attributed to thermal conduction from the overlying sediment rather than direct heating. Maximum temperatures attained after microwave exposure were however, relatively high and reached up to 94.1° C in the pilot trials.

As microwaves heat materials directly rather than via thermal conduction, these temperatures would be sufficient to induce bivalve mortality over a short exposure time (e.g., Azanza et al. 2005, Wilson 2006, unpub. data). For example, heat shocking experiments, using the green mussel *Perna viridis* indicated exposure times of 3 - 5 minutes and 0.33 - 1 minute to 60° C and 100° C water baths respectively, to be sufficient to induce a gaping response in treated mussels (Azanza et al. 2005).

In this study experiments using two types of microwave generators were conducted to assess the efficacy of microwave technology in obtaining temperatures that were lethal to the infaunal bivalve *Nucula* spp. in a laboratory environment. Mortality rates in all three substrate types tested were generally highest at the sediment surface and centre of microwave emission. Mortality rates decreased with increasing horizontal and vertical distance from the centre. Highest mortality rates overall were obtained in mud and sand, while mortality at 3 cm depth was highest in gravelly substrates.

Microwave radiation appeared to be more effective at inducing bivalve mortality than by simply heating the organisms in a water bath. While heating *Nucula* spp. in a bag containing sand for 3 minutes at 80° C resulted in 0% mortality, exposure to microwaves for the same amount of time induced 71.2% mortality (average across 0 - 4 cm distance at 0 cm depth) at a temperature of 76.5° C (in sand). This is explained by the difference in the mechanisms of heat transfer. In the water bath heat transfer occurs via thermal conduction, where the shell of the bivalve is heated before the heat reaches the tissue of the bivalve. Conversely, when using microwaves, electromagnetic waves penetrate the shell and oscillate the water molecules in the flesh, thus generating heat directly in the tissue. Since for all experiments adult (5 - 8 mm) *Nucula* spp. were collected at the same location, difference in sensitivity related to size or environmental adaptations can be excluded as cause for the observed differences in mortalities between the two types of heating.

Bivalves with thicker shells are considered to be more tolerant to exposure to high temperatures than those with thinner shells (e.g., dreissenids). For instance, lethal water temperatures for *Dreissena polymorpha* (>36° C for 15 minutes) are around 4° C lower than those required to kill other marine mussels (Jenner & Janssen-Mommen, 1993). The use of microwave technology can overcome this problem as bivalve shells, being transparent to microwaves, have no effect on their penetration (R. Keam 2008, pers. comm.).

Sediment moisture appeared not to have the greatest effect on the maximum temperature and mortality rates obtained in all sediment types. Mud and gravel reached similar temperature profiles and yet they were the wettest and driest sediment types respectively, and reached higher overall mortality rates than sand. Furthermore, sand reached the highest sediment temperatures, but was only slightly wetter than gravel. This suggests that factors other than sediment moisture contents might influence microwave penetration and thus, maximum temperatures and achieved mortality rates.

Boldor et al. (2008) utilized a 5 kW, 915 MHz microwave generator in their experiments to examine use of microwaves for ballast water treatment. In a flow-through system simulating ballast water transfer, the authors treated artificial ballast water inoculated with different test organisms (microalgae, zooplankton) at different power outputs (2.5 kW and 4.5 kW) and at different flow rates (~ exposure times). They found that increasing power output by 80% (i.e., from 2.5 to 4.5 kW) resulted in a 57% to 127% increase (approximately) in temperature. Similarly, in our experiments we found that the 5 kW generator having an effective operating power of 3 kW (taking into account the non-conductive properties of sediment) generally (though not consistently; see Table 6) raised sediment temperature to both a higher level and more rapidly than did the 0.8 kW generator (effective operating power of 0.6 kW).

Overall, this investigation has demonstrated that microwave technology has the potential to be used in an incursion response capacity. Additional research and development is, however, required in order to operationalise the technology for use in the marine environment.

Table 6: Summary of mean temperature increase ($\Delta T \pm$ one standard deviation) in sediments using two different microwave generators.

Sediment Type	Depth	$\Delta T \pm 1$ S.D. (°C)	
		0.8 kW, 2450 MHz, 180s	5 kW, 915 MHz, 60s
Mud	0	65.7 \pm 1.1	70.8 \pm 4.0
	3	9.2 \pm 8.6	24.3 \pm 26.4
Sand	0	57.6 \pm 7.4	72.1 \pm 3.8
	3	40.3 \pm 48.5	34.2 \pm 8.7
Gravel	0	68.4 \pm 7.4	74.1 \pm 4.7
	3	68.8 \pm 8.5	61.7 \pm 8.6

4.2 APPLICABILITY OF MICROWAVE TECHNOLOGY

4.2.1 Operational and Logistical Requirements

Key operational constraints for developing an incursion response tool for use in the coastal marine area ('CMA') are deployment time (which must be kept low in order to treat areas relatively quickly) and a requirement for a tool to be relatively mobile. Other factors for

further investigation include improving mortality rates in target organisms and the efficacy of treatment of non-homogenous substrates.

The results of our experiments suggest that a relatively small and portable microwave generator as tested would not be suitable for use as a rapid response tool. A generator capable of operating at considerably higher power would be required to achieve a high rate of mortality to required depths in a short time. Given the 5 kW microwave processor was operating at around 3 kW for 60 secs, a 3-fold increase in power to around 10kW would reduce the exposure time to a third (i.e., 20 secs). From an operational perspective, the highest power output that could be achieved on a mobile unit would be approximately 30 kW, resulting in an exposure time of approximately 6 secs.

A 30 kW generator would be approximately 1.2 m wide and 0.8 m long and the unit alone would weigh approximately 500 kg. It would thus most practically be placed on a trailer, which is towed by a quad bike, small utility vehicle or amphibious vehicle with rubber tracks (e.g., ARGO NZ 2008). The unit would be easily transportable in a 20 ft container or smaller, and the set-up time would be in the order of one hour. Equipment based around a 30 kW generator would require a 230 VAC 3-phase power supply of 50 Amps per phase, which could be provided by a diesel generator. Operation of equipment will require two people: one person to drive the quad bike and a second person to monitor the equipment. All personnel involved in the operation of the equipment would require thorough health and safety training.

The use of the microwave processor and quad bike and its associated weight would restrict the large scale treatment to relatively accessible beaches and suitable substrates. A ramp would be necessary to allow beach access and, due to the weight of the equipment, application would be more suited for firm substrates like gravel and sand. Mud, on the other hand, appears to be unsuitable to support the weight of the equipment using a quad bike. Instead, an amphibious vehicle with rubber tracks is likely to be more suitable for this environment. Preliminary field trials would be necessary to test and maximise efficiency of the equipment with respect to mortality rates achieved and also to optimise operating costs.

To treat the substrate, the generator could simply be rolled over the treatable area and microwave radiation applied for the desired amount of time. As indicated above, the necessary exposure time of the treatment area to a 30 kW microwave generator would be approximately 6 secs. Assuming an exposure area of 0.05 m² (i.e. 0.2 m x 0.25 m), 6 secs exposure time and 4 secs for moving to the next treatable area, a surface area of approximately 18 m² could be treated per hour. The laboratory results using the 0.8 and 5 kW generators indicated relatively variable mortality rates among the three replicates and it would thus be recommended to expose each area at least twice to microwave radiation.

A large, trailer mounted unit will be suitable for treating relatively flat and open areas of the intertidal zone. For steeper and more inaccessible sites, a small mobile unit can be used that is mountable on a backpack. Exposure time for such a unit, however, would be longer.

To test the effectiveness of the treatment and determine eradication success, post-treatment monitoring would have to be conducted. A suitable monitoring program would entail sample collections along a transect perpendicular to the water line. Sediment to a depth of approximately 5 cm should be excavated and numbers of live and dead target organisms counted to determine mortality rates. This sampling regime would ideally be conducted immediately after the treatment application, and 2 and 4 weeks post-treatment.

4.2.2 Permitting and Legal Considerations

The following permitting and legal considerations would have to be taken into account for conducting microwave treatment in a field environment.

4.2.2.1 Resource Management Act 1991

In accordance with the Resource Management Act 1991 ('RMA') the radiation discharged from the microwave steriliser, when used in the CMA of New Zealand, is considered to be a contaminant as defined in Part 1, section (2)(1) of the RMA. A contaminant is defined as:

"... any substance (including gases, odorous compounds, liquids, solids, and micro organisms) or energy (excluding noise) or heat, that either by itself or in combination with the same, similar, or other substances, energy, or heat –

- a) when discharged into water, changes or is likely to change the physical, chemical, or biological condition of water; or*
- b) when discharged onto or into land or into air, changes or is likely to change the physical, chemical, or biological condition of the land or air onto or into which it is discharged".*

Furthermore, section 15 of the RMA provides for the discharge of contaminants into the environment. The discharge of energy radiation from the microwave generator, when used in the CMA including intertidal environments therefore requires authorization pursuant to section 15(1) as follows:

"15 Discharge of contaminants into environment

(1) No person may discharge any –

- a) Contaminant or water into water; or*
- b) Contaminant onto or into land in circumstances which may result in that contaminant (or any other contaminant emanating as a result of natural processes from that contaminant) entering water; or*
- c) Contaminant from any industrial or trade premises into air; or*
- d) Contaminant from any industrial or trade premises onto or into land – unless the discharge is expressly allowed by a rule in a regional plan and in any relevant proposed regional plan, a resource consent, or regulations".*

4.2.2.2 Regional Plans

The Auckland Region has been assessed as an example to determine the likely activity status of the discharge from the microwave generator. It is anticipated that other regional plans may include similar rules to those in the Auckland Regional Plan: Coastal (Operative in Part 2004) ('the Coastal Plan') in relation to this activity, however this has not been investigated.

Within the Auckland Region, it is considered that a microwave steriliser in CMA's is a 'discharge of contaminants' as provided for in Chapter 20 of the Coastal Plan. Chapter 20 sets out the objectives, policies and rules relating to the discharge of contaminants to the CMA not addressed elsewhere in the Plan.

In accordance with Chapter 20, it is considered that the discharge of energy associated with the proposed microwave generator when used in the CMA is a discretionary activity pursuant to Rule 20.5.6. This rule is set out below.

Discretionary Activities

Rule 20.5.6

“Any discharge of contaminants except:

- i) the discharge of sewage from vessels; and,*
- ii) discharges authorised by any other rule in this chapter including any rules in the operative or proposed Auckland Regional Plan: Air, Land and Water listed in Rules 20.5.13 to 20.5.16”.*

The proposal does not involve the discharge of sewage and therefore the exception in i) does not apply.

In relation to ii), a review of the Proposed Auckland Regional Plan: Air, Land and Water (2001, Appeals version 2005) has determined that the proposed discharge of energy associated with the microwave generator is not authorised for by any other rule. Therefore, the exception in ii) does not apply and resource consent would be required for a discretionary activity.

There are no set assessment criteria listed for discretionary activities in the Coastal Plan, therefore it is considered that Auckland Regional Council (‘Council’) would, when assessing an application for a discretionary activity, rely on the objectives and policies of Chapter 20 of the Coastal Plan. It is noted that the objectives and policies of Chapter 20 of the Coastal Plan are consistent with the New Zealand Coastal Policy Statement 1994 (‘NZCPS’) and the Auckland Regional Policy Statement 1999 (‘ARPS’). Given these consistencies, the specific policy framework of the NZCPS and ARPS has not been considered. Instead the assessment has focussed on the relevant policy framework of the Coastal Plan.

It is noted that the Proposed New Zealand Coastal Policy Statement 2008 (‘PNZCPS’) was notified in March 2008, and submissions closed in May 2008. A hearing of submitters commenced in October 2008 and is ongoing at the time of preparation of this report. A detailed review of the PNZCPS has not been undertaken for the purposes of this report; however it would need to be considered in relation to any application for resource consent for the proposed activity.

The key objectives and policies that apply to this assessment generally seek to:

- Provide for appropriate discharges in the CMA while remedying or mitigating adverse environmental effects.
- Preserve the natural character of the coastal environment by providing for the appropriate use of the CMA.
- Maintain an appropriate water and sediment quality in the CMA.

Overall, it is considered that the proposal is not contrary to the relevant objectives and policies of the Coastal Plan for the following reasons:

- The proposed discharge will not cause more than minor adverse effects on the receiving waters and the marine environment;
- The discharge of energy associated with the microwave steriliser will not involve the disturbance of the foreshore and seabed that cannot be remedied by natural processes;
- As a result of treatment all organisms, including native organisms, to a depth of approximately 3 cm present in the intertidal area of the treated beach would be killed. It

is, however, considered that the proportion of the CMA affected would be small in scale and re-colonisation to those areas would occur and therefore, the effects associated with the discharge would be remedied by natural processes;

- During treatment, the proposed activity will temporarily restrict public access to the CMA and in particular, the intertidal area of the treated beach due to Health and Safety reasons;
- The discharge will not modify, damage or destroy any site, building, place or area scheduled for preservation or protection in Cultural Schedules 1 and 2; and,
- No human sewage or hazardous substances are proposed to be discharged into the CMA when utilizing this technology.

A review of other regional coastal plans within New Zealand has not been undertaken for the purposes of this report. It is anticipated, however, that other regional coastal plans will be consistent with the RMA and contain similar discretionary activity rules. It is recommended that those proposing to undertake microwave sterilisation in New Zealand CMA's check the activity status under the relevant regional plans and apply for a resource consent from the respective regional council(s) pursuant to section 88 of the RMA where necessary. In the event that the discharge is provided for by an operative regional plan as a permitted activity, it is recommended that an application for a certificate of compliance be submitted to the respective regional council(s) pursuant to section 139 of the RMA, to confirm that the use of microwave sterilisation in the CMA is a permitted activity and does not require a resource consent pursuant to section 88 of the RMA.

4.2.3 Environmental Applications

The above experiments have indicated that microwave technology could be applicable to a range of soft-sediment infaunal organisms. Successful application, however, would likely be restricted to those organisms living in the top 3 cm of the sediment. Likely taxonomic groups suitable for microwave treatment could be infaunal polychaetes, including those which inhabit calcareous tubes (as these are transparent to microwave radiation). A variety of surface and near-surface dwelling molluscs could also be treated, in particular shallow burrowing bivalves such as the invasive bivalve *Potamocorbula amurensis*, which is located near/at the sediment water interface (Carlton et al. 1990). In addition, gastropods, small soft sediment decapods, similar to our native crab *Helice crassa*, as well as surface dwelling seaweeds and shallow-rooting seagrasses or algae fall within the range of treatable groups. Given the non-selective nature of microwave treatment, mortality of native infaunal organisms present in the treated area would be unavoidable.

One of the key limitation with the current technology is the relatively small area which can be readily covered in a short time (e.g. between tidal cycles). It is suggested that it would therefore be most useful in controlling species that are at risk of spreading through fragmentation, which could occur during physical removal attempts.

At this stage of development, microwave technology appears to be suitable for application to the intertidal zone, including sandy and gravelly beaches and estuaries, and to a certain extent mangrove areas and muddy zones. A subtidal application could be feasible, but would face considerable technological, logistical and operational difficulties.

4.2.4 Conclusions & Future Directions

Of itself, this investigation has considerable merit in representing the first documented attempt of testing microwave technology on natural substrates as a potential biosecurity incursion response tool. The 'whole, natural sediment' approach has permitted identification of key operational and technical constraints on applying microwaves as an eradication tool and has revealed that environmental variability is the largest single factor affecting efficacy. The variability noted in the results is considered to be related to the variable dielectric properties of the substrates (sediments and organisms) tested. We recommend that further research should include examination of artificially prepared substrates, controlling for material type, texture and moisture content, in order to identify the nature of the confounding variables.

The results of the laboratory experiments were nonetheless promising, as relatively high mortality rates were achieved in all three sediment types. Using currently available 'off-the-shelf' technology, microwave penetration was restricted to the uppermost 3 cm of the sediment. While the technology at this stage would thus be restricted to treatment of shallow living infaunal organisms, there is scope to utilise higher-power generators to achieve more rapid and consistent heating to greater depths. Application of such technology for use in the natural environment would necessitate additional research and development.

5. References

- Agriculture, Fisheries and Forestry – Australia., 2002. Introduced marine pests – Scoping the socio-economic impacts. Reports prepared by Hassall & Associates Pty Ltd. For Fisheries and Aquaculture Branch of Aquaculture, Fisheries and Forestry – Australia. 75 pp.
- ARGO NZ, 2008. ARGO Amphibious Vehicles. <http://www.argo-atv.co.nz/product-intro.html> (accessed 21/05/2008).
- Azanza, M.P.V., Azanza, R.V., Ventura, S.R., 2005. Heat shocking of Philippine green mussels, *Perna viridis*. International Journal of Food Science and Technology 40, 689-694.
- Bax, N.J., 1999. Eradicating a dreissenid from Australia. Dreissenia! 10, 1-5.
- Boldor, D, Balasubramanian, S, Purohit, S, Rusch, K.A., 2008. Design and implementation of a continuous microwave heating system for ballast water treatment. Environmental Science and Technology 42 (11), 4121-4127.
- Carlton, J.T., 1989. Man's role in changing the face of the oceans: Biological invasions and implications for conservation of near-shore environments. Conservation Biology 3, 265-273.
- Carlton, J.T., Thompson, J.K., Schemel, L.E., Nichols, F.H., 1990. Remarkable invasion of San Francisco Bay (California, USA) by the Asian clam *Potamocorbula amurensis*. I. Introduction and dispersal. Marine Ecology Progress Series 66, 81-94.
- Cohen, A.N, Carlton, J.T., 1998. Accelerating invasion rate in a highly invaded estuary. Science 279, 555-558.
- Culver, C.S., Kuris, A.M., 2000. The apparent eradication of a locally established introduced marine pest. Biological Invasions 2, 245-253.
- Fleming, R.M., Janowiak, J.J., Kearns, J., Shield, J.E., Rou, R., Agrawal, D.K., Bauer, L.S., Miller, D.L., Hoover, K., 2004. Parameters for scale-up of lethal microwave treatment to eradicate cerambycid larvae infesting solid wood packing materials. Forest Products Journal 54(7/8), 80-84.
- Fleming, R.M., Janowiak, J.J., Kimmel, J.D., Halbrendt, J. M., Bauer, L.S., Miller, D.L., Hoover, K., 2005. Efficacy of commercial microwave equipment for eradication of pine wood nematodes and cerambycid larvae infesting red pine. Forest Products Journal 55(12), 226-232.
- Grosholz, E.D., 2005. Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. Proceedings of the National Academy of Sciences 102, 1088-1091.
- Hewitt, C.L., Campbell, M.L., 2007. Mechanisms for the prevention of marine bioinvasions for better biosecurity. Marine Pollution Bulletin 55, 395-401.

- Jenner, H.A., J.P.M. Janssen-Mommen., 1993. Monitoring and control of *Dreissena polymorpha* and other macrofouling bivalves in the Netherlands. In Zebra mussels, biology, impact and control. T.F. Nalepa and D.W. Schloesser, editors. Lewis Publishers, Boca Raton, Florida. 537-554.
- McEnnulty, F. R.; Bax, N. J.; Schaffelke, B.; Campbell, M.L., 2000. A review of rapid response options for the control of ABWMAC listed introduced marine pest species and related taxa in Australian waters. CRIMP Technical Report Number 23. Hobart, Australia, CSIRO Marine Research. 101 p.
- Oliveira, M.E.C., Franca, A.S., 2002. Microwave heating of foodstuffs. Journal of Food Engineering 53, 347-359.
- Ribera, M.A, Boudouresque, C-F., 1995. Introduced marine plants, with species reference to macroalgae: mechanisms and impacts. Pp 217-268 In: Round, F.E., Chapman, D.J (eds.) Progress in Phycological Research, Volume 11. Biopress Ltd. Bristol. 370 pp
- Ruiz, G.M., Carlton, J.T., Grosholz, E.D., Hines, A.H., 1997. Global invasions of marine and estuarine habitats by non-indigenous species: Mechanisms, extent and consequences. American Zoology 37, 621-632.
- Vinograd, J., Sytsma, M., 2002. Report on the Oregon Ballast Water Management Program in 2002. Produced for the Oregon State Legislature in cooperation with The Oregon Ballast Water Task Force. Portland.
- Wallentinus, I., Nyberg, C.D., 2007. Introduced marine organisms as habitat modifiers. Marine Pollution Bulletin 55, 323-332.
- Wilson, C., 2006. Small scale mussel experiments: Preliminary Findings.
- Woodfield, R., Merkel, A., 2006. Eradication and Surveillance of *Caulerpa taxifolia* within Agua Hedionda Lagoon, Carlsbad, California – Fifth Year Report. March 2006. 13 pp. <http://swr.nmfs.noaa.gov/hcd/caulerpa/Year5StatusReport.pdf> (accessed 22/04/08).
- Wotton, D. M., O'Brien, C., Stuart, M. D. and Fergus, D. J., 2004. Eradication success down under: heat treatment of a sunken trawler to kill the invasive seaweed *Undaria pinnatifida*. Marine Pollution Bulletin 49, 844-849.