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A Continuous Microwave System For Prevention of Invasive Species During De-Ballasting Operation – Death Kinetics

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A continuous microwave heating system was tested for its effectiveness at removing potentially invasive organisms during deballasting operations. Four different organisms, namely Nannochloropsis oculata (microalgae), Artemia nauplii, Artemia adults and Crassosstrea virginica (oyster larvae) normally found in ballast water were investigated in a controlled study to quantify their survival after continuous microwave heating of synthetic ballast water. The experiments were performed in the microwave system using a 2 x 2 factorial design with power (2.5 and 4.5 kW) and flow rate (1.0 and 2.0 lpm) and the organisms subsequently subjected to different holding times. The control treatment was performed in a water bath using the same temperatures and holding times as in the case of the microwave treatment. Overall, the results obtained indicated that the microwave system was more effective in eliminating the organisms when compared with the control treatment. In most cases there were no survivors present after the microwave treatment at holding times above 100 s, and temperatures as low as 50°C particularly for oyster larvae and Artemia adults. The results are promising, indicating that this technology has the potential to be an effective tool in controlling/preventing the introduction of invasive species into native environments.

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INTRODUCTION

Ballast water plays a critical role in maintaining the stability and maneuverability of ships during transit without cargo [Hua and Liu, 2007]. The International Maritime Organization (IMO) estimates that each year 10 billion tons of ballast water are transported and exchanged around the world [Oemcke and VanLeeuwen, 2003]. Intake of sea water in one region and its discharge into another region of the world presents a high risk of introducing non indigenous marine species in the environment [Drake *et al*., 2007; Hewitt and Campbell, 2007]. An estimated 3,000 to 10,000 different species are transported through ballast water every day [Bright, 1999] including plankton, toxic algae, waterfleas, jelly fish, mussels, clams, sea slugs and larval fish [Tibbetts, 1997].

Keywords: *Continuous microwave, ballast water, de-ballasting, invasive species, zoo plankton, oysters, microalgae, Artemia, Artemia nauplii, N. oculata, C. virginica, Artemia salina*

Once discharged into the new environment, the introduced species could become invasive as they lack natural predators and upset the local ecological balance. In the USA alone, the bio-invasion through different pathways in which ballast water discharges as an active contributor has been estimated to cost $123 billion a year [Bright, 1999].

In addition to the displacement of native flora and fauna, the organisms transported could ultimately affect human health, as, for example, *Vibrio cholera* was found to be concealed within plankton transported through ballast water [Tibbetts, 1997]. Organisms such as dinoflagellates are known to be pathogenic to shellfish and other marine organisms (oysters, shellfish, mussels and clams) by producing toxins [Burkholder, 1998]. Human consumption of contaminated marine-based food products has been linked to sickness, short-term memory loss, diarrhea and respiratory problems [Tibetts, 1997]. Hence, prevention of ecological and human risk factors through improper ballast water disposal needs to be addressed.

Different treatment methods have been proposed for safe disposal of ballast water: ballast water exchange [Burkholder *et al*., 2007; McCollin *et al*., 2007], chemical methods [Perrins *et al*., 2006; Gregg and Hallegraeff, 2007; Hua and Liu, 2007], heat treatment [Rigby *et al*., 1999], use of ultraviolet (UV) radiation [Waite *et al*., 2003], and filtration [Tang *et al*., 2006; Cangelosi *et al*., 2007]. Novel techniques such as ultrasonic, magnetic methods and use of electrical pulses [Chase *et al*., 2001] have also been investigated for their treatment efficacy.

Each of the methods mentioned above has its own advantages and disadvantages. For example, chemical treatments (using ozone, pesticides, gluteraldehyde, etc.) are considered to be effective, but they also cause corrosion of the tanks, introduce toxic chemicals and sometimes generate undesirable byproducts. Heat treatments are considered a good option, as there is no formation of by-products and the coolant water can be easily discharged into the ocean if not used, but cold weather conditions reduce its effectiveness, often require additional piping to convey water and installation of large boilers, all of which increase treatment costs. Attempts have been made to utilize the waste heat from the ships engines to heat the ballast water [Rigby *et al*., 1999], but the heat generated might not be enough to substantially inactivate all of the organisms present. The use of microwaves for ballast water treatment might be a suitable alternate to the available treatment methods. This technology has high heating rates (due to its short heating and exposure span) when compared to conventional heating, is less expensive to operate and requires fewer accessories to install (pipes, other conduits, boilers, etc.).

Incident electromagnetic waves (in the microwave region) rapidly heat dielectric material volumetrically (ballast water in this case) through two major mechanisms, polar rotation (of mostly water molecules) and oscillation of charged ions generating inter- and intra-molecular friction [Metaxas and Meredith, 1983]. The extent of temperature rise produced depends on the dielectric properties of the liquid [Nelson, 1980; Boldor *et al*., 2004; Boldor *et al*., 2007]. Microwaves have been used extensively in the past to successfully inactivate enzymes and bacterial cells present in foods [Kozempel *et al*., 1997; Tajchakavit and Ramaswamy, 1997; Kozempel *et al*., 1998; Canumir *et al*., 2002; Yaghmaee and Durance, 2005; Huang *et al*., 2007].

Thermal and non thermal effects of microwaves on the bacterial cells are believed to be the cause of inactivation. Traditionally, it was considered that microwave killing of biological entities was solely due to the heat generated [Goldblith and Wang, 1967; Vela and Wu, 1979], but more recent studies involving the use of advanced analytical equipment have lead researchers to hypothesize the existence of a non-thermal mechanism increasing the lethal effect of microwaves [Porcelli *et al*., 1997; Shin

and Pyun, 1997; Woo *et al*., 2000; Koutchma *et al*., 2001]. Several theories have been proposed to explain non-heat microwave effects, including the irreversible inactivation of otherwise thermophilic and thermostable enzymes due to internal protein structural changes occurring upon exposure to microwaves [Porcelli *et al*., 1997].

This study was undertaken as part of a larger research effort to understand the effectiveness of microwaves in preventing invasive species introduction during the de-ballasting process. A continuous microwave heating system was designed to process the ballast water, and the system effectiveness was ascertained by its ability to inactivate various organisms such as microalgae (*Nannochloropsis oculata*), zooplankton at two different growth stages (newly hatched brine shrimp-*Artemia* nauplii and adult *Artemia*), and oyster larvae (*Crassosstrea virginica*).

MATERIALS AND METHODS

Preparation of Synthetic Ballast Water

Sterilized tap water was mixed with autoclaved synthetic sea salt (Crystal Sea MarineMix, Marine Enterprises Int., Baltimore, MD, USA) to form salt solutions at two concentrations; one at 30 parts per thousand (ppt) - (3%) and the other at 20 ppt (2%). The salinity of the ballast water prepared was verified with a salt water refractometer (Aquatic Eco-Systems, Apopka, FL). The mixture was allowed to equilibrate for 48 h prior to inoculation with the organisms.

Preparation of Test Cultures

<u>*Nannochloropsis oculata (microalgae)*</u>
One mL of *Nannochloropsis oculata* stock culture under sterile conditions was transferred into a sterile 250 mL airtight flask containing the nutrient mixture, placed on well lighted shelves, and shaken twice daily at fixed times. Two to three days were required for the clear solution to turn light green in color and another 3-4 days for the solution to become bright green. Then, the contents of the flask were transferred into a sterile 11.35 liter (3 gallon) carboy containing 2% salt concentration ballast water with nutrients, sealed with foam stoppers and aerated using a pump. The carboys were placed on shelves under fluorescent lamps until turning green in color (approximately 1 week), then again transferred into sterile 75.71 liter (20 gallon) barrels filled to 80% capacity with sterile 2% salt concentration ballast water. The aerating and lighting condition were the same as previously described.

The survival of the algae was determined using two different methods: fluorescence microscopy and re-growth studies. For microscopy, samples were initially preserved in Lugol solution (Iodine solution, Cole-Parmer Instrument Company, Vernon Hills, IL), then mixed and filtered (47 mm diameter membrane filter, vacuum of 16 to 10 kPa). Two to five ml aqueous acid fuchsin solution was added to the filter which was allowed to stand for 20 min, after which the sample was washed briefly and re-filtered. Successive rinses with 50, 90 and 100% propanol while filtering were performed, then the sample was soaked for 2 min in a 100% propanol wash, filtered, added xylene and soaked for additional 10 min before filtering. Finally, the xylene-soaked filter was trimmed and placed on a microscope slide with mounting medium, covered with additional drops of mounting medium, and the glass slide was gently squeezed to remove excess medium. The edges of the cover glass were lacquered and the slide was imaged under microscope, revealing red staining of live microalgae. For re-growth studies, culture tubes with nutrient media were seeded with a small amount of sample, and stored in optimum growth conditions for extended periods of time.

The number of live microalgae present per mL in images was calculated from:

$$N_L = \frac{CA_t}{A_c V} \qquad (1)$$

Where, N_L = Number of live microalgal cells/mL; C = number of organisms counted; A_t = total area of effective filter before mounting on the slide; A_c = area counted; V = volume of sample filtered, mL.

Artemia nauplii

Two to three grams of *Artemia* cysts was added to a 208.2 liter (55 gallon barrel) of synthetic ballast water (3% salinity, 8.5-9.0 pH, 26-28°C). A constant supply of air was maintained using a small air pump. The aeration procedure was carefully controlled to ensure that the cysts were suspended in the ballast water and did not stick to the sides of the barrel. This mixture was illuminated with sunlight until the *Artemia* hatched (24-48 h), then the barrel was moved to lower light conditions. For enumeration, 2-3 mL of the *Artemia* solution was sampled using a pipette and placed on a filter paper so that it was evenly distributed. After draining, the filter paper was placed on a slide and the number of *Artemia* nauplii hatched were carefully counted. The concentration of *Artemia* nauplii at the start of the experiment was 10^2-10^3 counts per liter of solution. After the microwave experiments, the surviving *A*. nauplii were enumerated in the same way as mentioned above.

Artemia adults

The adult *Artemia* was cultured in a similar manner as the *Artemia* nauplii cells with exception that these organisms were fed for an additional period of time to allow for further growth (about 7-10 days), and the concentration was reduced to about 75 organisms per liter of solution. Total numbers and live adult *Artemia* were visually determined by enumerating swimming and non swimming individuals.

Crassosstrea virginica (oyster larvae)

A total of four million oyster larvae 6 days old (swimmers) were obtained from the Coast Seafood's Co. hatchery in Quilcene, WA and were used in this study. The larvae were suspended in the tank containing 3% synthetic sea water after temperature acclimation at the rate of 2.5°C per hour. The initial target larvae concentration was 9-10 larvae/ml. The actual initial concentrations changed slightly with the larvae batch size and due to some larvae settling that occurred during the experiment. Samples were collected in the feed tank and in seven sampling points after the heating. The larvae (both surviving and dead) were counted under a microscope in a Sedgewick-Rafter counting chamber in duplicate for each sample.

Continuous Microwave System

The experiments were conducted using a 5 kW, 915 MHz continuous flow microwave unit (Industrial Microwave Systems, LLC, Morrisville, NC). This unit is comprised of a power generation unit, wave guides to transport the generated microwaves, circulator, water load, power coupler, tuning coupler, elliptical focusing cavity that holds the applicator tube through which the process fluid flows (Figure 1(a)) and a cooling system to cool down the excess heat generated in the magnetron. The entire processing system consisted of the feed-tank, microwave processing unit, insulated holding tubes, cooling water circulation systems and positive displacement pumps to circulate the fluid through the system (Figure 1(b)). The elliptical cavity dimensions were 15.7 inch (39.878 cm) major axis, 12 inch (30.48 cm) minor axis, and 10 inch (25.4 cm) height. The system was operated in TE_{10} mode, and the tuning stubs for matching were set at depths of 4.2 cm (the stub closest to the generator), 4.6 cm (middle stub), and 6 cm (closest to the applicator), respectively.

In Figure 1(b) the inlet and outlet temperatures of the product entering and leaving the microwave unit are denoted by T_{in} and T_{out}, respectively. There were four sampling locations (ports) on the holding tube (denoted by h_1, h_2, h_3 and h_4) at equidistance of 1.5 m from each other, selected such that the

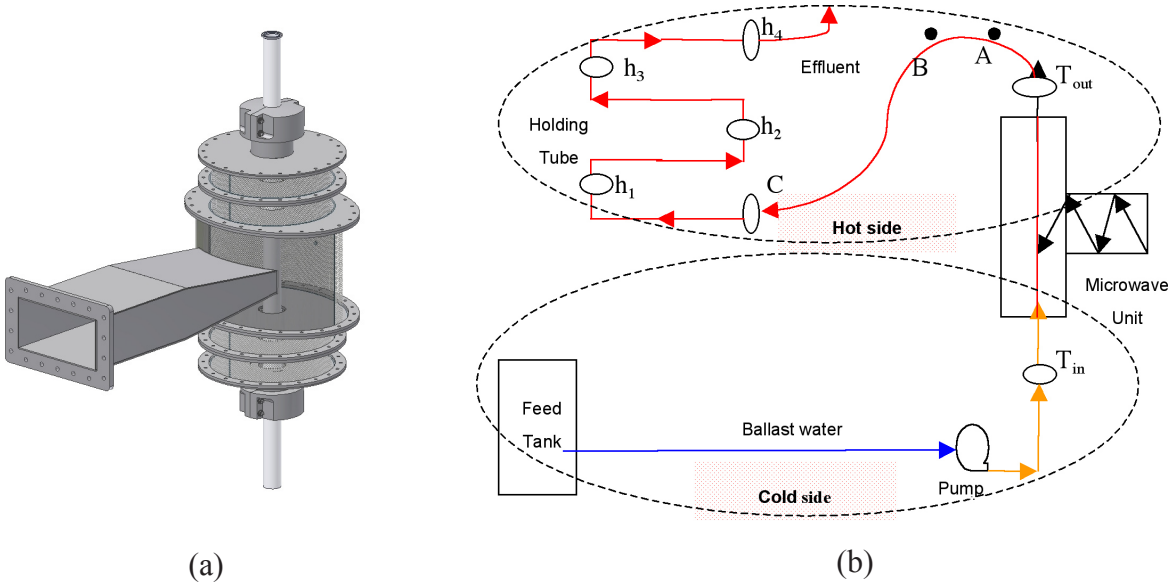


Figure 1. *a) Autodesk Inventor drawing of the microwave cavity; b) Schematics of the continuous microwave heating system with the sampling sites.*

residence times of the ballast water reached a pre-determined value at a particular flow-rate. The number of sampling sites was increased to 6 for adult *Artemia* and 7 for the oyster larvae studies in an attempt to quantify the organisms' survival after shorter residence times, especially for these latter less heat-resistant organisms. The added sampling ports were noted with letters (A, B, and C) to distinguish them from the ports used in the longer holding tubes. For the current experimental protocol, two flow-rates for the ballast water treatment were selected: 1 and 2 l pm. The residence times at each sampling location for the selected flow rates of the ballast water are given in Table 1.

The process fluid was pumped through the microwave unit using a progressing cavity pump (model # 1P610, Dayton Electric Manufacturing Co., Lake Forest, IL, USA) controlled by a variable speed DC electric motor (model # 4Z528B, Dayton Electric Manufacturing Co., Lake Forest, IL, USA). The fluid to be processed by the microwaves then reaches the applicator tube, made out of PTFE (polytetrafluoroethylene) with a height of 100 cm and a diameter of 3.81 cm. This applicator tube was housed inside the elliptical focusing cavity chamber of the microwave unit and was fixed in place using aluminum fittings at the entrance and exit of the cavity. The focusing cavity ensures that the maximum amount of microwave radiation generated gets concentrated onto the fluid to be processed, with an electric field distribution that is maximum in the tube center and minimum near the walls as illustrated by a finite element solution to Maxwell's equations (Figure 2). This distribution is similar to the velocity flow profiles, thus increasing the process efficiency of the unit and ensuring a uniform temperature at the exit.

The holding tubes (304 SS, 1.5" nominal diameter) were well insulated in order to maintain isothermal conditions throughout the tube and minimize heat loss. Samples were collected at each port to determine (micro) organism's survival. The sampling ports were made using 3/8" (0.95 cm) diameter stainless steel T-junctions connected to 3/8" (0.95 cm) diameter stainless steel ball valves at each location.

Power levels were measured using power diodes connected to the directional coupler. The net absorbed power was determined by subtracting the reflected power from the forward power.

Table 1. Residence times of the organisms for different flow rates during microwave treatment.

Location	Distance from microwave outlet, M_{out} (m)	Residence time for each flow rate (s)	
		1.0 (lpm)	2.0 (lpm)
Holding tube 1 (h_1)	3.00	207	103
Holding tube 2 (h_2)	4.50	307	154
Holding tube 3 (h_3)	6.00	408	204
Holding tube 4 (h_4)	7.50	517	258
Site A*	0.17	12	6
Site B*	0.32	22 (at flow rate of 1 lpm)	11 (at a flow rate of 2 lpm)
Site C*	1.60	112	56

The power absorbed was also calculated using the following formula [Singh and Heldman, 2001]:

$$Q = m C_p \Delta T \qquad (2)$$

Where: Q = absorbed power, W; m = mass flow rate, kg/s; C_p = specific heat capacity of salt water, J/kg-K; assumed 4000 J/kg- K; ΔT = change in temperature, K.

Experimental Procedure

The experiments were aimed at determining the effectiveness of the installed continuous microwave system in inactivating the organisms present in ballast water. Two flow rates (1 and 2 liters per minute) and two power levels (2.5 and 4.5 kW) were selected as the test parameters to form a 2 X 2 experimental design. The temperature responses during each experiment were recorded. Samples were collected at the various sampling sites (corresponding to different retention times) and the survival percentage of each organism was determined. The control experiments were carried out in a thermal water-bath with temperature control, selected depending on the processing temperature reached during the microwave treatments for each flow rate, power combination and organism. Once the control samples in the water-bath at a particular set temperature reaches the retention times of the microwave experiments, they were removed and either re-cultured (as in the case of microalgae) or counted (*Artemia* and oyster larvae). From the number of survivors ascertained, the survival percentage was calculated, and these results were compared with the survival percentage from the microwave treatment.

The inlet and outlet temperatures were measured using 10 T-type thermocouples (#OSK2K1540/PP3-60-T-116U-1-SMPW-M, Omega Engineering, Inc., CT); one at the inlet and nine at the outlet. The outlet thermocouples were arranged in groups of three at equal radial distances from each other (5 mm distance) similar to the configuration described by Coronel et al. [2003]. A data logger (model #TC-08, PICO Technologies, Cambridgeshire, UK) connected to a PC-compatible computer with Windows XP (Microsoft Corp., Redmond, WA) operating system was used to record the responses from the ten thermocouples. The captured data was processed using MS Excel (Microsoft Corporation, Seattle, WA) and Sigma Plot (Version 9.01, Systat Software, Inc., San

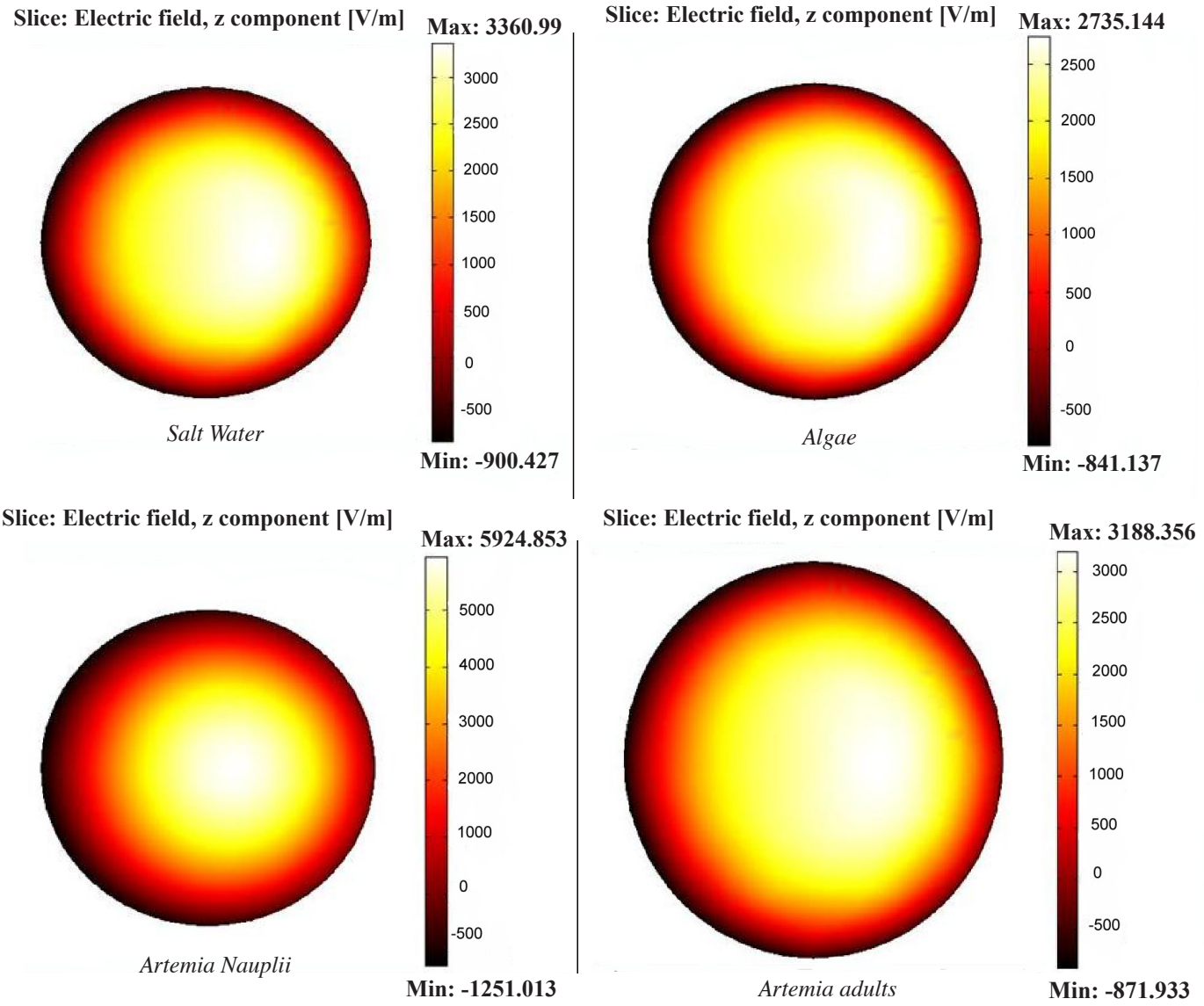


Figure 2. *Electric field distribution in the middle of the applicator tube for the different organism investigated.*

Jose, CA). All the experiments were performed in triplicate.

RESULTS AND DISCUSSION

Dielectric Heating Produced During Microwave Treatment

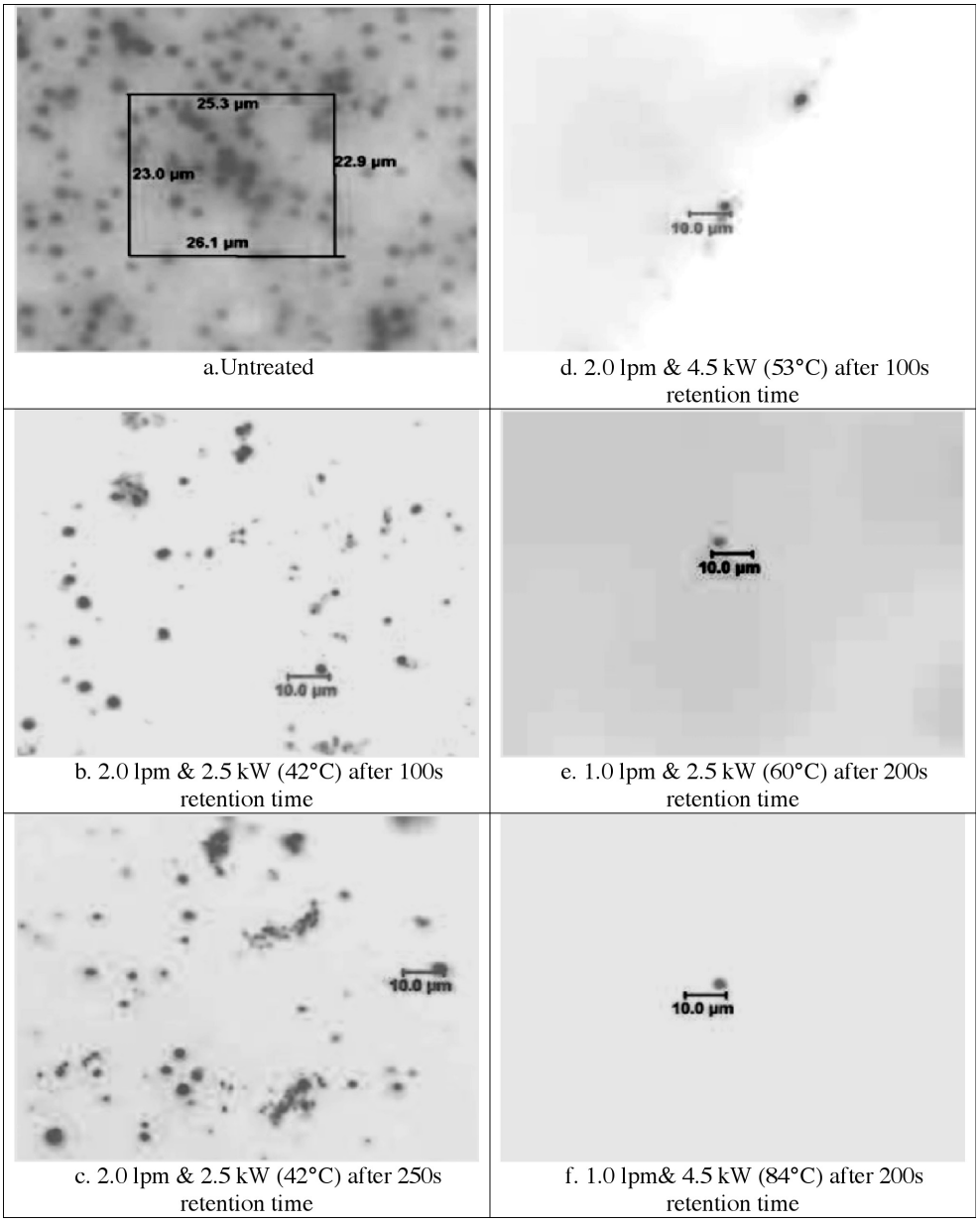
Table 2 summarizes the temperature rise occurring in the ballast water containing the various organisms observed during the microwave treatment. Previous studies on microwave heating characteristics of the ballast water containing various organisms indicated that the heating pattern follows a first order response, with outlet temperature rapidly achieving steady state (less than 30 s) [Boldor *et al*., 2008]. It can be noticed from Table 2 that the extent of heating produced due to the microwave treatment is not the same for the ballast water containing the different organisms. A maximum temperature rise of about 60°C (or greater) was observed for the ballast water containing *N. oculata*, *Artemia* adults and *C. virginica*. This highest temperature increase

Table 2. Temperature increase observed during microwave treatment of the ballast water containing the various organisms.

Organism	Parameters		Temperature (°C)		
	Flow rate (lpm)	Power (kW)	Inlet, T_{in}	Outlet, T_{out}	Difference, ΔT
N. oculata	2.0	2.5	27.91 ± 0.13	41.88 ± 0.02	13.97 ± 0.20
	2.0	4.5	27.87 ± 0.08	52.77 ± 0.01	22.89 ± 0.15
	1.0	2.5	27.45 ± 0.20	57.52 ± 0.03	30.06 ± 0.33
	1.0	4.5	27.41 ± 0.11	86.77 ± 0.02	59.35 ± 0.67
*A.*nauplii	2.0	2.5	22.02 ± 0.21	34.00 ± 0.22	11.84 ± 0.28
	2.0	4.5	22.05 ± 0.09	47.57 ± 0.22	25.52 ± 0.22
	1.0	2.5	26.25 ± 0.28	46.00 ± 0.49	19.75 ± 0.58
	1.0	4.5	26.17 ± 0.16	59.85 ± 0.29	33.68 ± 0.35
Artemia adults	2.0	2.5	24.58 ± 0.03	43.59 ± 0.20	19.00 ± 0.21
	2.0	4.5	24.42 ± 0.01	58.66 ± 0.18	34.24 ± 0.18
	1.0	2.5	27.77 ± 0.00	64.89 ± 0.18	37.12 ± 0.18
	1.0	4.5	27.37 ± 0.01	88.42 ± 0.24	61.04 ± 0.24
C. viginica	2.0	2.5	26.50 ± 0.11	39.37 ± 0.30	12.87 ± 0.31
	2.0	4.5	26.37 ± 0.07	48.26 ± 0.27	21.91 ± 0.27
	1.0	2.5	23.36 ± 0.08	57.28 ± 0.39	33.92 ± 0.42
	1.0	4.5	23.36 ± 0.09	88.26 ± 0.83	64.91 ± 0.77

was observed for maximum power input (4.5 kW) and low flow rate (1 lpm). The temperature differences between different organisms at the same operating parameters can be attributed to the changes in the dielectric properties of the medium [Boldor *et al*., 2007]. The variation of dielectric properties of water and various biological materials with compositions (carbohydrates, proteins, fats, etc.) influence the amount of microwave energy converted into heat by these materials [Nelson, 1980; Boldor *et al*., 2004; Bento *et al*., 2006; Zhang *et al*., 2006; Coronel *et al*., 2007]. The maximum amount of heating observed for the ballast water containing *Artemia* nauplii was only 33.68°C compared to 61°C for adult *Artemia*. Hence, it can be stated that for the same organism present at different growth stages, different heating profiles will be obtained. The young *Artemia* immediately after hatching is rich in proteins, carbohydrates, lipids and fatty acids [Treece, 2000]. The percentage of these nutrients reduces as the *Artemia* grows and reaches adulthood in about eight days after hatching. The presence of these nutrients in large percentages at the earlier stages of growth could have had an influence on the dielectric properties and on the variation of heating rates produced during the microwave treatments [Boldor *et al*., 2007] of *Artemia* nauplii and *Artemia* adults. The concentration of *Artemia* adults was also smaller by counts. Due to this variation in dielectric heating observed, care should be taken while designing a microwave system for treating medium containing different (micro) organism or organisms of the same species but at different growth stages.

For oyster larvae it can be summarized that for the same power level, the temperature in-



a. Untreated

d. 2.0 lpm & 4.5 kW (53°C) after 100s retention time

b. 2.0 lpm & 2.5 kW (42°C) after 100s retention time

e. 1.0 lpm & 2.5 kW (60°C) after 200s retention time

c. 2.0 lpm & 2.5 kW (42°C) after 250s retention time

f. 1.0 lpm& 4.5 kW (84°C) after 200s retention time

Figure 3. *Survival of microalgae at various treatment parameters*.

crease produced at the lower flow rate was nearly three times the temperature increase produced at the higher flow rate. In contrast, the temperature increase produced for the ballast water containing the other organisms were much lower at the different flow rates for the same input power (approximately twice for adult *Artemia* from high flow rate to low flow rate). The rate of increase was previously determined to be dependent only on the flow rate while being independent of power level [Boldor *et al*., 2008].

Effect of Microwaves on N. oculata

Microscopic images of the algae show the difference between the effects of the different parameters used (Figure 3). Figures 3(b) and 3(c) show the number of live cells after 100 and 250 s of retention time, respectively. Figures 3(d), 3(e) and 3(f) show the live cells for the other combinations of operating parameters after the first segment of the holding tube (~ 200 s except for Figure 3(d)). Since there are no surviving cells

Table 3. Survival of algae after microwave heating and holding (normalized %). Values are based on fluorescence microscopy measurements.

Parameters		T_{out}	ΔT	Net power (kW)		Survival at different residence times (%)			
Flow (lpm)	Power (kW)	°C	°C	Measured	Calculated*	100 s	150 s	200 s	250 s
2.0	2.5	42	17	2.31	2.27	85	76	70	65
2.0	4.5	53	28	3.96	3.73	1	0.1	0	0
						200 s	300 s	400 s	500 s
1.0	2.5	60	35	2.33	2.33	0	0	0	0
1.0	4.5	84	64	4.24	4.27	0	0	0	0

*Using Equation 1.

Table 4. Results for algae re-growth after microwave and control experiments.

Parameters	Temp.	Experiment	Sample Sites			
			S1	S2	S3	S4
2.0 lpm / 2.5 kW	42 °C	Microwave	1,1,1*	1,1,1*	1,1,1*	1,1,1*
		Control	1,1,1*	1,1,1*	1,1,1*	1,1,1*
2.0 lpm / 4.5 kW	53 °C	Microwave	0,0,0*	0,0,0*	0,0,0*	0,0,0*
		Control	1,0,1*	0,1,0*	0,0,0*	0,0,0*
1.0 lpm / 2.5 kW	60 °C	Microwave	0,0,0*	0,0,0*	0,0,0*	0,0,0*
		Control	0,0,0*	0,0,0*	0,0,0*	0,0,0*
1.0 lpm / 4.5 kW	84 °C	Microwave	0,0,0*	0,0,0*	0,0,0*	0,0,0*
		Control	0,0,0*	0,0,0*	0,0,0*	0,0,0*

*Individual replicates are shown

after the first segment (Figure 3) there should be no surviving cells at the other sampling locations with longer retention times. The results of these microscopic measurements were quantified and averaged for each sampling location and operating parameters, and they are summarized in Table 3. In general, microwave heating to temperatures higher than 53°C results in greater than 99% removal after 100 s of retention time (Table 3). This temperature was achieved with a flow rate/power combination of 2.0 lpm and 4.5 kW. At lower power level (2.5 kW) and the same flow rate, the outlet temperature was only 42 °C, insufficient to effectively remove all algae (Table 3, Figure 2(c)).

The results of the re-growth studies are summarized in Table 4 and support the results of the fluorescence microscopy measurements. In Table 4, "1" represents a positive re-growth in the tube and "0" represents no growth (negative re-growth). Algal re-growth was observed only for the 2.0 lpm/2.5 kW operating parameters

(42°C), while for the control some re-growth was also observed after processing at 53°C in addition to the 42°C study.

These results are similar with data available in literature. Bosch and Hallegraeff [1993], Rigby [1994], and Hallegraeff [1998] identified that most phytoplankton algae including diatom *Skeletonema costatum*, dinoflagellates *Amphidinium carterae*, *Gymnodinium catenatum* and *Alexandrium catenella*, and the golden brown flagellate *Heterosigma akashiwo*, in their vegetative stages could be readily killed at temperatures as low as 35ºC and treatment times in the range of 30 min to several hours by traditional heating methods. In addition, significant mortality was also achieved with *Gymnodinium catenatum* and *Alexandrium catenella* cysts using longer incubation times (several hours) at temperatures as low as 35 to 37.5ºC, with total mortality achieved at 38°C after 4.5 hours. Mountfort *et al*. [1999] investigated free-swimming or dispersive forms of the model organisms (seaweed *Undaria pinnatifida*, mollusc *Crassostrea gigas* and starfish *Coscinasterias calamaria*), which were considered most likely to be spread through the entirety of a ballast tank. That study concluded that effective treatment would be one that is either long (≥ 16 h at ≤ 36 °C), medium (10 min to 16h at 36-45 °C) or short duration (≤10min at ≥ 46°C). By comparison, total mortality is achieved in the microwave heating system in a matter of seconds if considering only the heating region, or minutes when also considering the holding tube.

Inactivation of Artemia nauplii

For this particular organism, continuous microwave heating seems to present a marked advantage over traditional heating (Figure 4). This method compares well with the killing rates obtained by other researchers using various technologies. Oemcke et al. [2004] reported that a more than 2-log (>99%) removal of *U. pinnatifida* (a seaweed zoospore) was achieved with a dose of 60 mWs/cm^2 of UV irradiation. Mountfort *et al*. [1999] conducted a sea trial between Wellington and Auckland where the tank temperature increased from 24°C to 42°C over a 10 h period. The results demonstrated that all of the seeded *C. calamaria* starfish larvae were killed, while the number of larvae and zooplankton in the control tank decreased to just 60% of their initial concentrations after 70 h.

One reason for observing only a marked increase in the inactivation of *Artemia* nauplii over that of traditional heating is the much lower temperatures achieved with this organism compared to all other, due to their much less suitable dielectric properties [Boldor *et al*., 2008]. As the microwave system was tuned using pure salt water with much higher dielectric loss [Boldor *et al*., 2008] at the beginning of the study, it can be expected that re-tuning the system specifically for *Artemia* nauplii would increase the efficiency of microwave absorption and therefore the temperature.

The major advantage in the case of this organism can be provided in terms of costs (considering recent spikes in gas and oil prices) and in the speed with which these lethal temperatures can be achieved, allowing processing of a large amount of ballast water in a relatively short amount of time. Boldor *et al*. [2008] calculated the power utilization efficiency of the microwave heating system at different flow rate and power input combinations. The continuous microwave system used in that study for treating the ballast water containing *Artemia* nauplii had a power utilization efficiency of greater than 80% at all flow rate (2 and 1 lpm) and power combinations (2.5 and 4.5 kW) tested in conditions of non-optimum matching of the microwave system with the organism. In the case of real ballast water, the temperature will depend on the dielectric properties of a mixture of organisms, increasing the overall efficiency to close to unity. This underlines the energy efficiency of using a continuous microwave system for ballast water treatment.

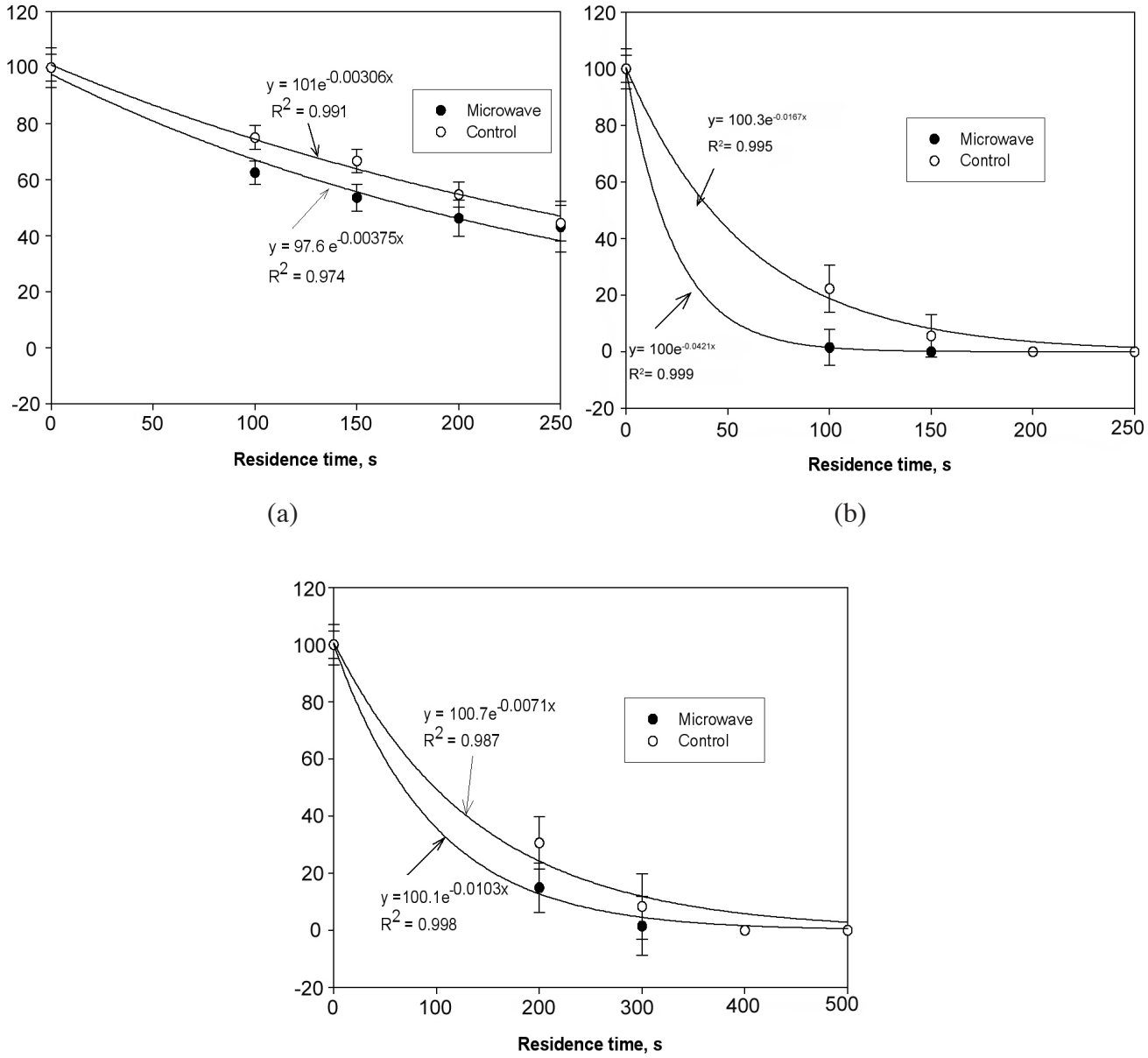


Figure 4. *Artemia nauplii survival, microwave treatment vs control at various microwave conditions. (a) Microwave at 2.5 kW, 2.0 l pm and 36°C; Control at 36°C. (b) Microwave at 4.5 kW, 2.0 l pm and 47°C; Control at 47°C. (c) Microwave at 2.5 kW, 1.0 l pm and 45°C; Control at 45°C.*

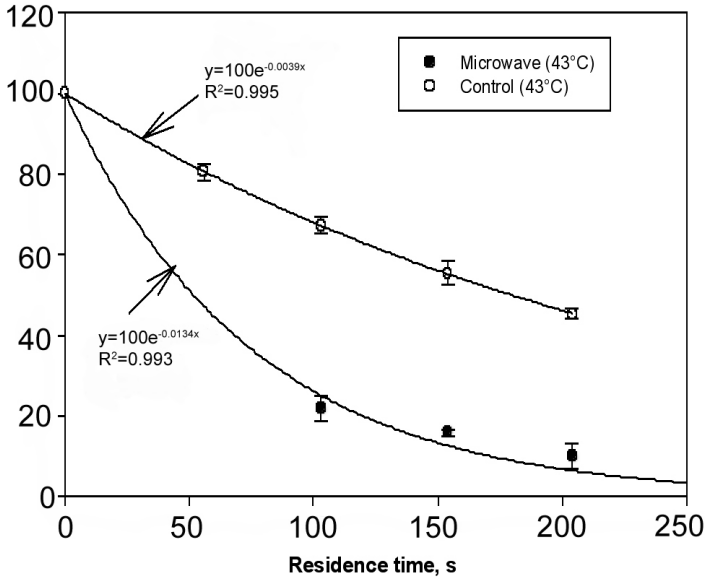


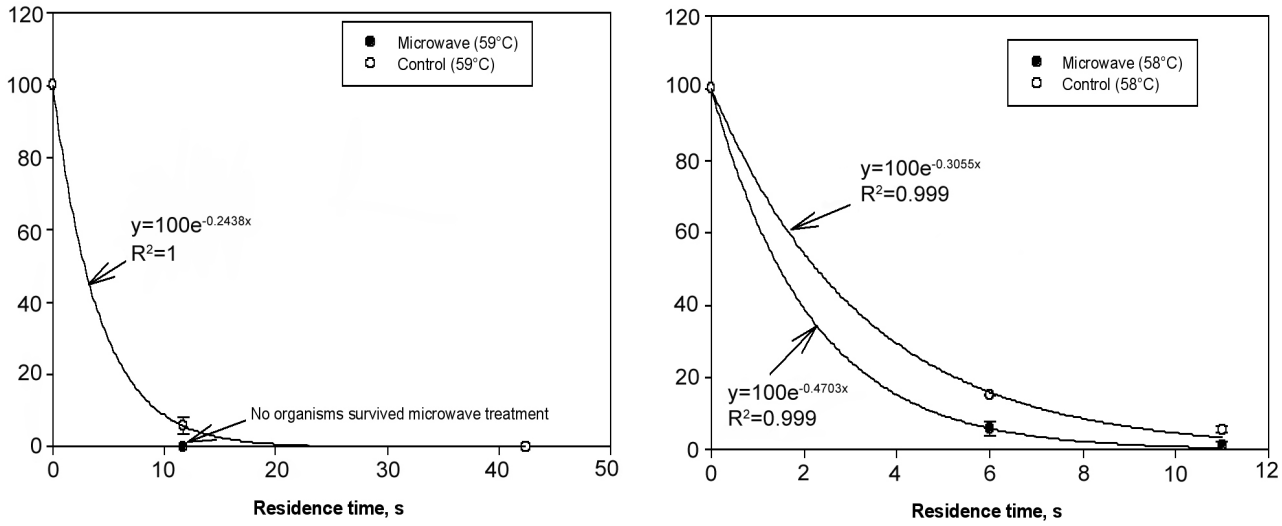
***Figure 5**. Artemia adult survival under microwave (at 2.5 kW and 2.0 l pm) and control treatment.*

Inactivation of Artemia adults

The microwave treatment proved to be extremely effective at killing adult *Artemia* even at low temperatures. For example, at 2 lpm and 2.5 kW (43°C), the microwave treatment was 100% effective after holding (Figure 5) compared to the control. For all other processing parameters (resulting in higher temperatures), the first sampling port did not yield any live organisms. Due to this reason subsequent tests were performed with sampling ports placed much closer to the microwave exit (Sites A, B and C in Table 1). In all cases, microwaves proved more efficient at killing *Artemia* than the controls. Figure 6 shows the survival curves obtained for the *Artemia* adults during microwave treatments and control treatments at lower residence times. From the figure it is evident that the microwave treatment was effective in eliminating virtually all the *Artemia* present even at these lower residence times. The dielectric properties of the medium which is dependent on the growth stage of the *Artemia* plays an important role in the heating observed and the subsequent inactivation of *Artemia*. *Artemia* are considered to be resilient to treatments like ozone [Tolomei *et al*., 2004], and survive at temperatures between 15-55°C [Treece, 2000]. The performance of the continuous microwave system indicates that temperatures above 55°C could be easily achieved for the ballast water containing *Artemia* adults by carefully controlling the flow rate and power input, thus creating environments hazardous/detrimental to *Artemia* survival. At the highest temperature (88°C), corresponding to 1 lpm and 4.5 kW, no *Artemia* survived in either the microwave or control experiments, even after very short residence times.

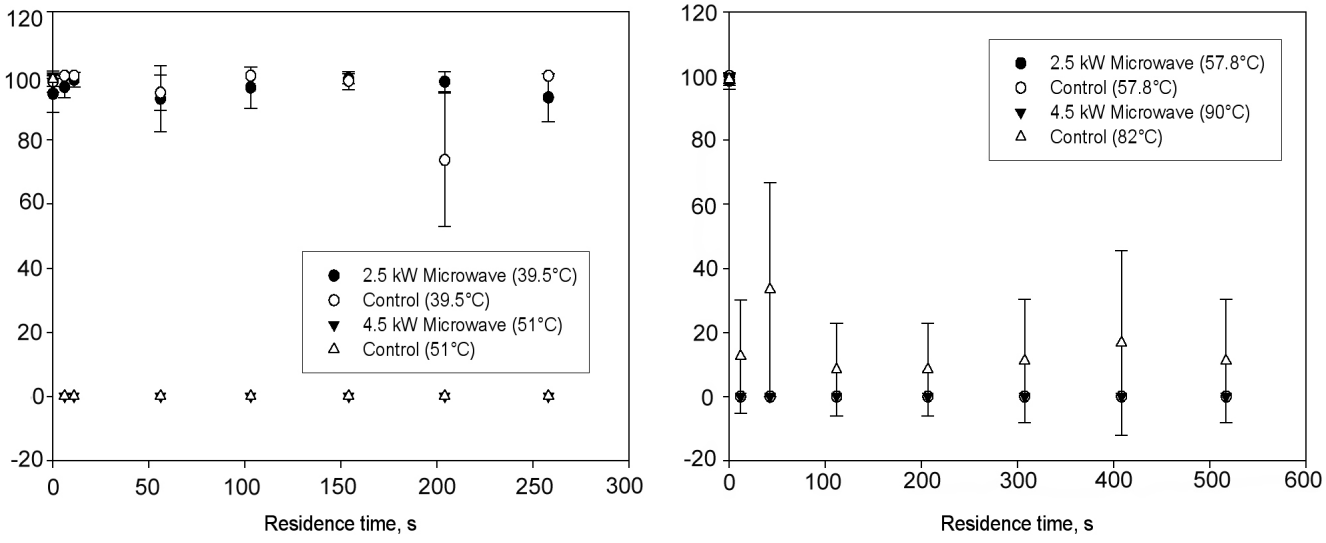
Microwave Effect on C. virginica

With the exception of the mildest treatment (2 lpm 2.5 kW at 40°C), no oyster larvae survived after microwave treatment (Figure 7), even after the shortest residence times (11 and 6 s for 1 and 2 lpm, respectively). Imaging microscopy (Figure 8) revealed the physical effects of the microwave treatment on the oyster larvae. It can be observed that at milder (2 lpm 4.5 kW at 59°C) but still lethal treatment (Figure 8, top right), the shells remained intact. At the more aggressive treatment the shells were either partially shredded (Figure 8, bottom), or even completely



(a) (b)

Figure 6. *Artemia adult survival under microwave and control treatments. (a) Microwave at 4.5 kW, 2.0 lpm. (b) Microwave at 2.5 kW and 1.0 lpm.*



(a) (b)

Figure 7. *Survival of Crassostrea virginica after microwave and control treatments at flow rate of 2 lpm (a) and 1 lpm (b).*

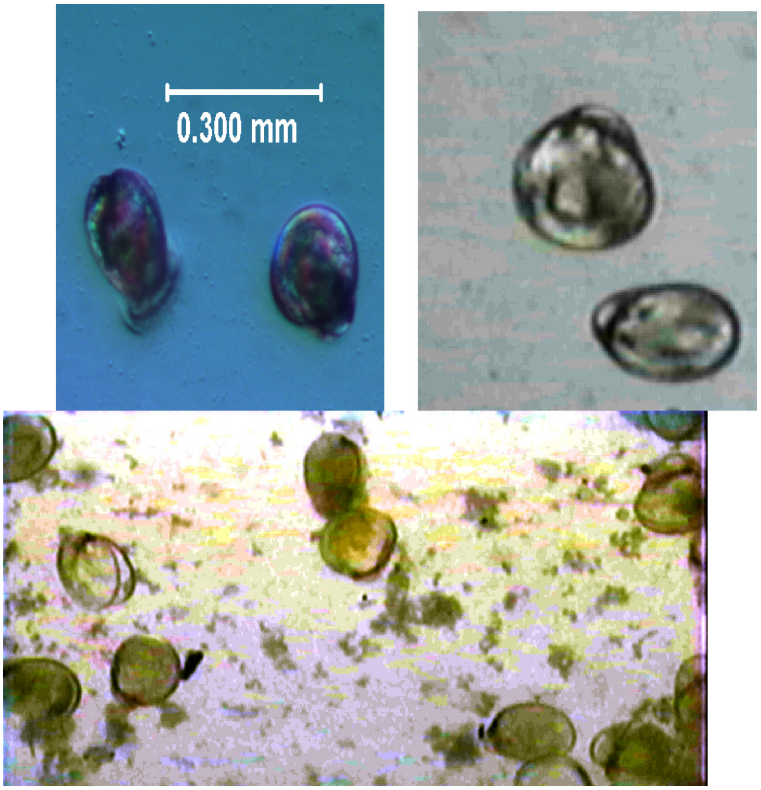


***Figure 8**. Microscopic images of oyster larvae before (top left) and after treatment (top right and bottom).*

dissolved at the highest temperatures (1 lpm 4.5 kW at 90°C; image not shown). No correlation (first order-type) between the oyster larvae survival and holding time was observed during the 2 lpm, 4.5 kW treatment (40°C). For the control at 82°C, some of the oyster larvae survived (see Figure 7), although at lower temperatures they did not. Further investigations need to be carried out to ascertain if this observation of oyster larvae survival at higher temperature and no survival at lower temperature is an anomaly or a genuine effect related to microwave/heat treatment. Experiments on the thermal tolerance of *Crassostrea gigas*, an invasive oyster, indicate that at temperatures of 42°C for 1 h complete mortality occurred [Rajagopal *et al*., 2005]. Their experiments show that the time and temperature of treatment is critical for achieving complete destruction of this invasive oyster. For example, 11 mm size oysters required 163 min at 39°C to achieve complete inactivation, while at 45°C it took only 2 min to obtain the same level of inactivation. Our experiments conducted on *C. virginica* show that there was no surviving oysters at microwave treatments above 40°C even at holding times as low as 6 s. The complete destruction of oyster larvae obtained at low residence times shows that microwave treatments could be used as an effective tool for continuous rapid treatment of ballast water containing these organisms with uniform temperature distribution at the exit of the system. Overall, the continuous microwave heating treatment achieves a greater than 4-log reduction for oyster spats (larvae), with even the mild treatments achieving a 100% killing rates.

CONCLUSIONS

A continuous microwave system was tested for its effectiveness in inactivating invasive species present in ballast water. Four different invasive species, namely *Nannochloropsis oculata*, *Artemia* nauplii, *Artemia* adults and *Crassosstrea*

Table 5. Percent survival of *Artemia* nauplii after control and microwave heating treatments at various residence times (normalized %).

Parameters		T_{out}	ΔT	Survival (Control) at different residence times (%)				Survival (Microwave) at different residence times (%)			
Flow	Power			100 s	150 s	200 s	250 s	100 s	150 s	200 s	250 s
(lpm)	(kW)	°C	°C								
2.0	2.5	36	14.5	75.00 ± 4.30	66.67 ± 4.18	54.67 ± 4.49	44.42 ± 6.28	62.50 ± 4.12	53.57 ± 4.81	46.16 ± 6.48	43.12 ± 9.08
2.0	4.5	47	24.5	22.25 ± 8.29	5.58 ± 7.57	0.0	0.0	1.52 ± 6.35	0.0	0.0	0.0
				200 s	300 s	400 s	500 s	200 s	300 s	400 s	500 s
1.0	2.5	45	20.0	30.58 ± 9.12	8.33 ± 11.42	0.0	0.0	14.91 ± 8.62	1.52 ± 10.25	0.0	0.0
1.0	4.5	59	34.3	3.778	2.287	0.0	0.0	0.0	0.0	0.0	0.0

*virginic*a were separately innoculated into synthetic ballast water, then treated at four different process conditions: combinations of 2.5 and 4.5 kW power and 1.0 and 2.0 lpm flow rate. The microwaves successfully produced dielectric heating of the ballast water increasing the temperature by nearly 60°C or more when *N. oculata*, *Artemia* adults and *C. virginica* were present. In the case of inactivating microalgae (*N. oculata*) microwave treatment performed better in eliminating microalgae when compared to the control treatment. For oyster larvae and *Artemia* adults survival was observed only for the milder microwave treatment condition (namely 2.5 kW, 2.0 lpm at about 40°C) at holding times above 100 s. Hence, new microwave experiments had to be conducted at lower residence times to observe any survivors present for these two organisms. For these two organisms microwave treatment was more effective than the control by totally eliminating these organisms. In the case of *Artemia* nauplii, the microwave treatment was slightly better than the control treatments.

Future studies will be focused on the inactivation of cysts and spores of marine organisms as well as on mixtures of different potentially invasive species present in the ballast water, and on optimizing the system for maximum power utilization to obtain better inactivation results.

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REFERENCES

Bento, L., Rein, P., Sabliov, C., Boldor, D., Coronel, P. (2006). "C Massecuite re-heating using microwaves." *Journal of American Society for Sugarcane Technologists,* 26, pp.1-13.

Boldor, D., Sanders, T. H., Simunovic, J. (2004). "Dielectric properties of in-shell and shelled peanuts at microwave frequencies." *Transactions of the ASABE,* 47(4), pp.1159-1169.

Boldor, D., Ortego, J., Rusch, K. A. (2007). "An analysis of dielectric properties of synthetic ballast water at frequencies ranging from 300 to 3000 MHz." Proceedings Book of 11th International Conference on Microwave and High Frequency Heating, September 3-6, Oradea, Romania. Edited by A.M. Silaghi and I.M. Godean: University of Oradea Press, Romani.

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), pp.4121–4127.

Bosch, C. J., Hallengraeff, G.M. (1993). "Chemical and physical treatment options to kill toxic dinoflagellate cysts in ships' ballast water." *Journal of Marine Environmental Engineering,* 1, pp.23-29.

Bright, C. (1999). "Invasive species: pathogens of globalization." *Foreign Policy,* 116, pp.50-64.

Burkholder, J. M. (1998). "Implications of harmful microalgae and heterotrophic dinoflagellates in management of sustainable marine fisheries." *Ecological Applications,* 8(1), pp.S37-S62.

Burkholder, J. M., Hallegraeff, G. M., Melia, G., Cohen, A., Bowers, H. A., Oldach, D. W., Parrow, M. W., Sullivan, M. J., Zimba, P. V., Allen, E. H., Kinder, C. A., Mallin, M. A. (2007). "Phytoplankton and bacterial assemblages in ballast water of U.S. military ships as a function of port of origin, voyage time and ocean exchange practices." *Harmful Algae* ,6, pp.486-518.

Cangelosi, A. A., Mays, N. L., Balcer, M. D., Reavie, E. D., Reid, D. M., Sturtevant, R., Gao, X. (2007). "The response of zoo plankton and phytoplankton from the North American great lakes to filtration." *Harmful Algae*, 6(4), pp.547-566.

Canumir, J. A., Celis, J. E., de Bruijn, J., Vidal, L.V. (2002). "Pasteurization of apple juice by using microwaves." *Lebensmittel Wissenschaft und Technologie,* 35(5), pp.389-392.

Chase, C., Reilly, C., Pederson, J. (2001). "Marine bioinvasions fact sheet: Ballast water treatment options. MIT Sea Grant Center for Coastal Resources, Cambridge, MA.

Coronel, P., Simunovic, J., Sandeep, K. P. (2003). "Temperature profiles within milk after heating in a continuous-flow tubular microwave system operating at 915 MHz." *Journal of Food Science,* 68(6), pp.1976-1981.

Coronel, P., Simunovic, J., Sandeep, K. P., Cartwright, G. D., Kumar, P. (2007). "Sterilization solutions for aseptic processing using a continuous flow microwave system." *Journal of Food Engineering*, 84(4), pp.528-536.

Drake, L.A., Doblin, M. A., Dobbs, F. C. (2007). "Potential microbial bioinvasions via ship's ballast water, sediment and biofilm." *Marine Pollution Bulletin,* 55(7-9), pp.333-341.

Goldblith, S. A., Wang, D. I. C. (1967). "Effect of microwaves on Escherichia coli and Bacillus subtilis." *Applied Microbiology,* 15, pp.1371-1375.

Gregg, M. D., Hallegraeff, G. M. (2007). "Efficacy of three commercially available ballast water biocides against vegetative microalgae, dinoflagellate cysts, and bacteria." *Harmful Algae,* 6(4), pp.567-584.

Hallegraeff, G. M. (1998). "Transport of toxic dinoflagellates via ships' ballast water: bioeconomic risk assessment and efficacy of possible ballast water management strategies." *Marine Ecological Progress Series,* 168, pp.297-309.

Hewitt, C. L., Campbell, M. L. (2007). "Mechanisms for the prevention of marine bioinvasions for better biosecurity." *Marine Pollution Bulletin*, 55(7-9), pp.395-401.

Hua, J., Liu, S. M. (2007). "Butyltin in ballast water of merchant ships." *Ocean Engineering,* 34, pp.1901-1907.

Huang, Y., Sheng, J., Yang, F., Hu, Q. (2007). "Effect of enzyme inactivation by microwave and oven heating on preservation quality of green tea." *Journal of Food Engineering,* 78(2), pp.687-692.

Koutchma, T., Le Bail, A., Ramaswamy, H. S. (2001). "Comparative experimental evaluation of microbial destruction in continuous-flow microwave and conventional heating systems." *Canadian Biosystems Engineering*, 43, pp.3.1-3.8.

Kozempel, M., Scullen, O. J., Cook, R., Whiting, R. (1997). "Preliminary investigation using a batch flow process to determine bacteria destruction by microwave energy at low temperature." *Lebensmittel Wissenschaft und Technologie,* 30(7), pp.691-696.

Kozempel, M. F., Annous, B. A., Cook, R. D., Scullen, O. J., Whiting, R. C. (1998). "Inactivation of microorganisms with microwaves at reduced temperatures." *Journal of Food Protection,* 61, pp.582-585.

McCollin, T., Shanks, A. M., Dunn, J. (2007). "The efficiency of regional ballast water exchange: Changes in phytoplankton abundance and diversity." *Harmful Algae,* 6(4), pp.531-546.

Metaxas, A. C., Meredith, R. J. (1983). "Industrial Microwave Heating." Peter Peregrinus Ltd., London, U.K.

Mountfort, D., Hay, C., Taylor, M., Buchanan, S., Gibbs, W. (1999). "Heat treatment of ship's ballast water: development and application of a model based on laboratory studies." *Journal of Marine Environmental Engineering,* 5, pp.193-206.

Nelson, S. O. (1980). "Microwave dielectric properties

of fresh fruits and vegetables." *Transactions of the ASAE,* 23, pp.1314-1317.

Oemcke, D., Parker, N., Mountfort, D. (2004). "Effect of UV irradiation on viability of micro scale and resistant forms of marine organisms: Implications for the treatment of ship's ballast water." *Journal of Marine Environmental Engineering,* 7, pp.153-171.

Oemcke, D. J., VanLeeuwen, J. (2003). "Chemical and physical characterization of ballast water. Part 2: Determining the efficiency of ballast exchange." *Journal of Marine Environment and Engineering,* 7(1), pp.65-76.

Perrins, J. C., Cordell, J. R., Ferm, N. C., Grocock, J. L., Herwig, R. P. (2006). "Mesocosm experiments for evaluating the biological efficacy of ozone treatment of marine ballast water." *Marine Pollution Bulletin,* 52(12), pp.1756-1767.

Porcelli, M., Cacciapuoti, G., Fusco, S., Massa, R., d'Ambrosio, G., Bertoldo, C., Rosa, M.D., Zappia, V. (1997). "Non-thermal effects of microwaves on proteins: thermophilic enzymes as model systems." *FEBS Letters*, 402, pp.102-106.

Rajagopal, S., Van der Velde, G., Jansen, J., Van der Gaag, M., Atsma, G., Janssen-Mommen, J. P. M., Polman, H., Jenner, H. A. (2005). "Thermal tolerance of the invasive oyster Crassostrea gigas: Feasibility of heat treatment as an antifouling option." *Water Research,* 39, pp.4335-4342.

Rigby, G. (1994). "Possible solutions to the ballast water problem." *Ballast Water Symposium Proceeding,* Canberra 11-13 May, pp.87-106.

Rigby, G. R., Hallegraeff, G. M., Sutton, C. (1999). "Novel ballast water heating technique offers cost-effective treatment to reduce the risk of global transport of harmful marine organisms." *Marine Ecology Progress Series,* 191, pp.289-293.

Shin, J. K., Pyun, Y. R. (1997). "Inactivation of Lactobacillus plantarum by pulsed-microwave irradiation." *Journal of Food Science,* 62, pp.163-166.

Singh, R. P., Heldman, D. R. (2001). "Introduction to Food Engineering." Academic Press, San Diego, CA.

Tajchakavit, S., Ramaswamy, H. S. (1997). "Thermal Vs. microwave inactivation kinetics of pectin methylesterase in orange juice under batch mode heating conditions." *Lebensmittel Wissenschaft und Technologie*, 30(1), pp.85-93.

Tang, Z., Butkus, M. A., Xie, Y. F. (2006). "Crumb rubber filtration: a potential technology for ballast water treatment." *Marine Environmental Research,* 61(4), pp.410-423.

Tibbetts, J. (1997). "Exotic invasion." *Environmental Health Perspectives*, 105(6), pp.590-593.

Tolomei, A., Burke, C., Crear, B., Carson, J. (2004). "Bacterial decontamination of on-grown Artemia." *Aquaculture,* 232, pp.357-371.

Treece, G. D. (2000). "Artemia production for marine larval fish culture." *Southern Regional Aquaculture Center (SRAC)* publication # 702, USDA-CSREES.

Vela, G. R., J. F. Wu. (1979). "Mechanism of lethal action of 2450-MHz radiation on microorganisms." *Applied and Environmental Microbiology*, 37, pp.550-553.

Waite, T. D., Kazumi, J., Lane, P. V. Z., Farmer, L. L., Smith, S. G., Smith, S. L., Hitchcock, G., Capo, T. R. (2003). "Removal of natural populations of marine plankton by a large-scale ballast water treatment system." *Marine Ecology Progress Series,* 258, pp.51-63.

Woo, I., Rhee, I., Park, H. (2000). "Differential damage in bacterial cells by microwave radiation on the basis of cell wall structure." *Applied and Environmental Microbiology*, 66(5), pp.2243–2247.

Yaghmaee, P., Durance, T. D. (2005). "Destruction and injury of Escherichia coli during microwave heating under vacuum." *Journal of Applied Microbiology,* 98, pp.498-506.

Zhang, M., Tang, J., Majumdar, A. S., Wang, S. (2006). "Trends in microwave-related drying of fruits and vegetables." *Trends in Food Science and Technology,* 17, pp.524-534.