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# Effect of ultrasonic cavitation on small and large organisms for water disinfection during fish transport

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## Abstract

Motivated by the need for additional tools to disinfect discharge water from well boats, and to prevent distribution of salmon lice, the effect of ultrasonic cavitation on the planktonic stages of the salmon louse, nauplii and copepodids, as well as marine heterotrophic bacteria, and the marine green microalgae *Tetraselmis suecica*, has been investigated. Survival and morphology were registered after different exposure times. Efficacy of the ultrasonic cavitation treatments varied with exposure time. A reduction in survival was registered even for the shortest exposure time (5 seconds) for both naupliar and copepodid stages of the salmon louse (36.7 ± 11.5 and 67.20 ± 7.2% survival respectively). Survival reached zero after exposure times of 20 and 60 seconds for the nauplii and copepodid stages, respectively. A reduction in 70% was observed for bacteria at all exposure times (5 to 300 s), while a reduction of 95% was observed after 300 s for algal cells. The logged energy transfer to the samples was on average 17.5 J/s. In conclusion, cavitation treatment is destructive for the planktonic stages of salmon lice, and may contribute to reduce discharge of pathogens and parasites from well boats when adapted for this purpose and combined with existing water disinfection methods.

**KEYWORDS**
cavitation, control strategy, fish transport, salmon aquaculture, sea lice, well boat

## 1 | INTRODUCTION

According to the Fish Health Report from 2015 (Hjeltnes, Walde, Bang, & Haukaas, 2015), the most common fish diseases identified and registered in the Norwegian salmon and rainbow trout fish farms include: winter ulcers caused by the bacteria *Moritella viscosa*, infectious pancreatic necrosis (IPN) caused by the IPN virus (IPNV), and salmon louse infections caused by the parasitic salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837). Salmon lice are a major animal welfare concern on large-scale salmon production sites, and are combated, using a variety of methods. Preventive measures have included the use of physical tarpaulin shields, cleaner fish, feed additives and chemical or fresh water treatment baths using closed tarpaulins, or well boats. In Norway, each farmed fish is transferred to and from a well boat at least 4–6 times for distribution to and from production sites, and for treatment against lice or disease. The water used in well boats for either fish transport or delousing has to be disinfected in order to prevent pathogen and parasite transmission to recipient waters. Disinfection methods approved by the Norwegian Veterinary Institute (NVI), comprises of UV irradiation and/or ozonation following filtration. Because ozone is toxic for fish even in low doses (Wedemeyer, Nelson, & Yasutake, 1979), it cannot be

used when fish are present in the water without accepting a high risk of elevated fish mortality. UV irradiation is therefore the disinfection method of choice during fish transport and treatment. The minimum UV dose required by NVI is 25 mJ/cm$^2$ (Loncarevic, 2014), which alone is insufficient to inactivate the most robust viruses such as IPNV. To achieve 99.9% inactivation of the IPNV, a minimum UV dose of approximately 200 mJ/cm$^2$ may be required (Munro & Midtlyng, 2011). The relatively new Norwegian regulation for fish transport (Norwegian Industry and Fisheries Ministry, 2014) requires reduction in pathogens such as *Aeromonas salmonicida*, *subsp. salmonicida* bacteria and ILA-virus by 99.9% prior to release of water back into the sea, with a pre-filtration step having a minimum pore size of 100 μm. Ship owners are therefore looking for improved water treatment methods to reduce and control potentially contagious water discharge of not only bacteria and viruses but also larger organisms such as the planktonic stages of *L. salmonis* to open waters.

Cavitation is instigated by the formation of microbubbles, and more generally, vapour cavities in a fluid caused by a reduction in pressure due to increased velocity gradients in a control valve or across the blades of a propeller. If these velocity gradients are sufficiently high, pressure drops below the local vapour pressure and vapour bubbles, that is, cavitation bubbles, will form. When these cavitation bubbles collapse as pressure increases following a reduction in velocity, energy is released in the form of local heat and pressure waves, which can reach magnitudes of several thousand bars at the point of collapse (Kalumuck, Hsiao, Chahine, & Choet, 2003). This has an erosive effect, which makes cavitation an unwanted phenomenon in most contexts. Cavitation can, however, be used for disinfection of fluids by deliberately inducing cavitation in a controlled manner since the temperatures and forces resulting from imploding cavitation bubbles are sufficient to cause lethal cellular damage. The most conspicuous effect when applying cavitation with the purpose of water treatment is the physical effect on a target organism caused by the force in the pressure shock generated by the implosion (Gogate, 2007; Gogate & Pandit, 2008; Jyoti & Pandit, 2001; Koval, Shevchuk, & Starchevskyy, 2011; Mahulkar & Pandit, 2010). For this effect to be maximized, it follows that the distance between the imploding bubble and the target organism should be minimized (Ross, 1987). In addition, the size of the bubble is of paramount importance. Another potential associated effect may be caused by the pressure variations themselves, for example, uptake of dissolved gas under circumstances where pressure changes. Didenko & Suslick (2002) refer to physical-chemical effects generated by cavitation (ultrasonic/hydrodynamic), including the generation of oxidant radicals such as hydroxyl radicals. While a number of studies have been published on the effect of cavitation on bacteria, algae and crustaceans (Jyoti & Pandit, 2001; Lee, Nakano & Matsumara, 2010; Guo, Khoo, Teo, & Lee, 2013; Karamah & Sunarko, 2013), no evidence demonstrating the effect of cavitation on the planktonic stages of the salmon louse are known.

The effect of cavitation on organisms causing fish disease or mortality depends on their resistance to the forces (or pressure variations), resulting from the collapsing cavitation microbubbles. Usually, the smaller the organism the greater its resistance. The most relevant fish pathogens (virus, bacteria and parasites) in Norwegian aquaculture represent three different size groups: The infectious pancreatic necrosis virus (IPNV, size in nanometres), the bacteria *Moritella viscosa* (size in micrometres) and the parasite *L. salmonis* (size in millimetres). A literature study has shown that marine heterotrophic bacteria are as robust as *Moritella viscosa* (Liltved, Bomo, Handeland, & Kristensen, 2008; Liltved & Cripps, 1999), and that the marine green algae *Tetraselmis suecica* is as robust as the most robust virus, that is, IPNV, against UV treatment (Liltved, Hektoen, & Efraimsen, 1995; Liltved, Tobiesen, Delacroix, Heiaas, & Tryland, 2012; Liltved, Vogelsang, Modahl, & Dannevig, 2006; Øye & Rimstad, 2001; Sako & Sorimachi, 1985; Yoshimizu, Takizawa, & Kimura, 1986). This indicates that the easily cultured marine heterotrophic bacteria and *Tetraselmis suecica* can be used as representative test organisms in order to avoid, using pathogenic test organisms.

There is an apparent curiosity regarding cavitation as a disinfection method in aquaculture. This can be seen from commercial efforts such as those of Aqua Farming Solutions (Aqua Farming Solutions, 2017), and the work of USonic (Prado, 2016). The efficacy of these systems however, remain uncertain and to the best of our knowledge, experimentally undocumented.

Motivated by the need for better disinfection technologies and the lack of knowledge regarding the susceptibility of salmon louses to cavitation, the effect of ultrasonic cavitation on three planktonic test organisms representing three different fish pathogen size groups; the microalgae *Tetraselmis suecica* as a robust virus representative, heterotrophic bacteria for the bacteria group, and *L. salmonis* as the parasite, has been investigated. The aim of this research was to investigate whether cavitation is a candidate disinfection method with the potential to be utilized as an additional tool for improved disinfection of discharge water from well boats with respect to viruses, bacteria and parasites.

## 2 | EXPERIMENTAL SETUP

### 2.1 | Treatment equipment

In order to investigate whether or not the planktonic stages of the salmon louse is susceptible to cavitation, a Hielscher UP200Ht (Hielscher Ultrasonics gmbh, Teltow Germany) ultrasonic homogenizer (Figure 1, left) (Hielscher, 2013)[1] was selected. The UP200Ht is a handheld ultrasonic processor measuring 300 mm × 190 mm × 90 mm weighing 1.4 kg. The unit induces cavitation in samples through an oscillating rod (Sonotrode) (Figure 1, right). The working frequency is 26 kHz, and the tip displacement ranges from 9 to 240 μm depending on the Sonotrode in use. The unit uses a 200 W 50 Hz AC power supply. For this experiment, Sonotrode S26d2 was selected in accordance with the UP200Ht manual (Hielscher, 2013) in order to maximize energy input to the samples. The UP200Ht unit was mounted on a standard laboratory stand so that the Sonotrode could be placed at the same stationary position in each sample.

[1]Citation of commercial product is not considered as endorsement.


**FIGURE 1** Left: Ultrasonic Processor UP200Ht from Hielscher. 1-activation trigger, 2-handhold, 3-sonotrode attachment, 4-sonotrode, 5-power cord, 6-temperature probe. Right: The Sonotrode used in the experiments (S26d2, 2 mm). Photos: Hielscher, Germany, ©www.hielscher.com, 2016 [Colour figure can be viewed at wileyonlinelibrary.com]

An example image of how cavitation around a Sonotrode looks like is given in Figure 2.

## 2.2 | Test organisms

### 2.2.1 | Salmon lice

*Lepeophtheirus salmonis* egg strings ($n$ = 5) were acquired from The Industrial and Aquatic Laboratory (ILAB, 5008 Bergen, Norway). The egg strings were placed in a flat bottom polyethylene tank (100 L) filled with filtered (sand filtered and a mesh filter bag, ø = 1 μm) seawater (10°C, 34 psu and pH 8.2), collected from the Trondheim fjord at 90 m depth for hatching. A gentle supply of atmospheric air through an air stone placed at the centre bottom of the tank ensured 100% oxygen saturation and water movement during hatching of the egg strings. The lice used in the experiments were collected by harvesting the individuals in a 120 μm (49% open area) screen mesh (SEFAR NITEX). Thereafter, the lice were transferred to test beakers by gentle flushing, using a seawater-filled spray bottle. The nauplii used for the first experiment were collected 2 days post-hatching, at which point all individuals were stage 2 nauplii. The copepodids used for the second experiment were collected 9 days post-hatching. The size of the nauplii and copepodites were measured, using an Infinity microscope camera with image analysing software (Luminera Corporation, Canada) connected to a stereo microscope (Nikon SMZ 1000, Japan). The nauplii and copepodids measured 600 × 200 μm and 800 × 220 μm (length × width) respectively.

**FIGURE 2** Example image of cavitation around Sonotrode. Photo: Hielscher, Germany, ©www.hielscher.com, 2016 [Colour figure can be viewed at wileyonlinelibrary.com]

### 2.2.2 | *Tetraselmis suecica*

A 1L culture of *Tetraselmis suecica* (NIVA-3/10) was supplied by the algal culture collection of the Norwegian Institute for Water Research (NIVA) in Oslo, with a density of approximately $10^6$ cell/ml. *Tetraselmis suecica* is a robust unicellular alga with an outer shell composed of a cellulose-like material. Measurements by NIVA show that *T. suecica* has an average minimum diameter of 9.3 μm ($n$ = 25) when growing exponentially in the cultures. Coulter counter measurements give an equivalent spherical diameter of 10–11 μm.

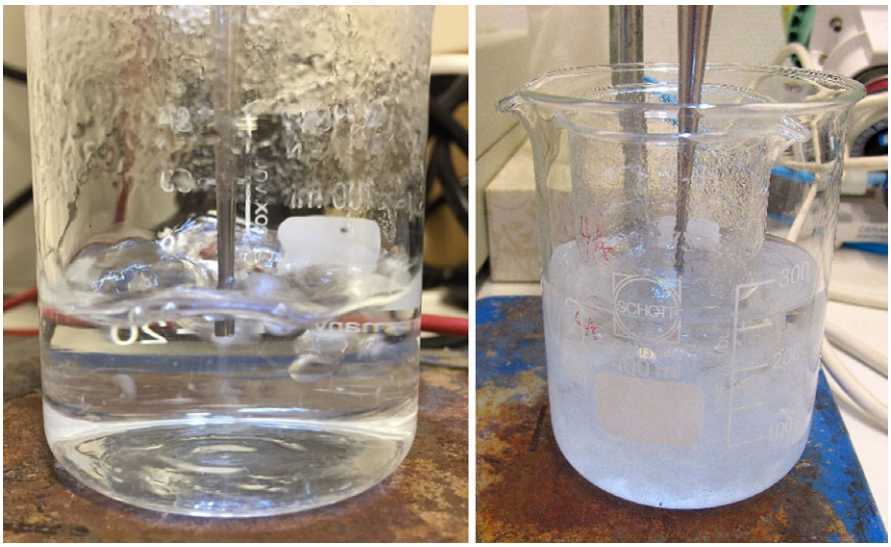

**FIGURE 3** Left: The Sonotrode placed in the middle of the cylinder, at a depth of 10 mm. Bubble ensemble underneath the Sonotrode during cavitation. This figure is an illustration photo to show the setup without obscuring the sample and Sonotrode from the ice cooling. Right: Beaker placed in ice-filled container. Parafilm removed for illustration purposes. Photos: SINTEF [Colour figure can be viewed at wileyonlinelibrary.com]

*Tetraselmis suecica* shows a high survival rate when exposed to shear forces during pumping operations, according to NIVA's 10 years of experience in ballast water treatment technology testing. In addition, *Tetraselmis suecica* shows high survival rates in salinities in the range of 15–32 PSU, and temperatures in the range of 0–27°C.

### 2.2.3 | Marine bacteria

The heterotrophic bacteria communities accompanying the cultured *T. suecica* were used as test organisms for this study. The typical size of bacteria cells was between 0.5 and 5 μm in length. A density of approximately $10^7$ Colony Forming Units (CFU) per ml was quantified in the culture of *T. suecica*.

## 2.3 | Experimental setup

The experiments were conducted at SINTEF SeaLab's laboratories in Trondheim, Norway, between November 1st and 23rd 2015.

### 2.3.1 | Salmon louse experiments

*Lepeophtheirus salmonis* nauplii and copepodites (1st and 2nd experiment respectively, $n = 20$) were placed in test beakers (60 ml volume) filled with seawater (20 ml, 10°C, 34 psu and pH 8.2) (Figure 3).

The beakers were thereafter sealed with Parafilm® (Parafilm M®, USA). The water volume was deliberately kept low in order to maximize mixing and the equipment's destructive effect (Hielscher, 2013). All tests were conducted on three identical samples (triplication). The energy transferred from the ultrasonic cavitation unit caused a rise in water temperature during the trials. In order to keep temperature levels below 20°C, a temperature that has been shown to have no negative impacts on sea lice (Samsing et al., 2016), the beakers were placed in a larger, ice-filled beaker. Control treatments were kept in glass beakers ($n = 3$) in the laboratory at room temperature (18°C) during the trials. After the individuals from each trial had been inspected and photographed, the lice for the control treatments were assessed for vitality to rule out natural death during the trials as a result bias. No mortality was recorded for the control treatments for any of the trials. During the experiments, the Sonotrode was placed in the centre of the beaker at 10 mm depth. Different exposure times were applied in triplicate to different samples of unexposed test organisms as described in Table 1.

Consistent exposure intervals were obtained, using the equipment's built-in timer function. The workflow of the experimental runs of effects of ultrasonic cavitation on salmon louse was to expose triplicate beakers containing salmon lice one at the time for a pre-selected duration to cavitation. After each test, the beakers were inspected individually, and the number of dead and live individuals registered. Consecutive experimental runs were performed with

**TABLE 1** Experimental scheme for the ultrasonic cavitation trials, showing the different exposure times and recorded water temperatures (initial–final) during the experiments for the different developmental stages of *L. salmonis*. The water temperatures recorded represent an average temperature between the triplicates during the trials

| Developmental stage | Triplicate exposure time (s) | Averaged water temperature, initial–final (°C) |
|---|---|---|
| Nauplius, stage 2 | 5 | 10–12 |
| Nauplius, stage 2 | 10 | 10–12 |
| Nauplius, stage 2 | 15 | 10–12.5 ± 0.5 |
| Nauplius, stage 2 | 20 | 10–12.5 ± 0.5 |
| Copepodid | 5 | 10–12 |
| Copepodid | 10 | 10–12 |
| Copepodid | 15 | 10–12.5 ± 0.5 |
| Copepodid | 20 | 10–12.5 ± 0.5 |
| Copepodid | 30 | 10–14 ± 1 |
| Copepodid | 40 | 10–14 ± 1 |
| Copepodid | 50 | 10–16.5 ± 0.5 |
| Copepodid | 60 | 10–16.5 ± 0.5 |

new, previously unexposed individuals. Temperature was monitored, using a thermometer (DIN12770, Glaswarenfabrik Karl Hecht GmbH & Co KG), and the frequency (kHz) was recorded continuously by the Hielscher UP200Ht unit.

The temperature column given in Table 1 indicates that the temperature registered in the beakers was not linear as would be expected. This apparent nonlinearity in temperature rise can be explained by differences in contact area between the ice and the different test beakers, and that Table 1 contains averaged temperature values for each triplicate test. The purpose of the temperature control was to keep the temperature below levels that could potentially affect survivability of the lice. Although little documentation related to the effects of high temperature on sea lice is available, temperatures below 18–20°C are shown to not have negative impact on survivability (Boxaspen, 2006; Samsing et al., 2016). The registered temperature for all experiments was well below 18°C.

#### 2.3.2 | Marine algae and marine bacteria experiments

Samples (40 ml) of *T. suecica* culture were exposed to ultrasonic cavitation for 5, 10, 60 and 300 s. The temperature was measured during each treatment. Each experiment was repeated three times. For each treatment, samples were collected for bacterial analysis by SINTEF's laboratory in Trondheim, and for algal analysis by NIVA's laboratory in Oslo within 24 hr. All equipment was autoclaved before use and the cavitation Sonotrode sterilized between each treatment to minimize cross-contamination.

### 2.4 | Analysis methods

#### 2.4.1 | Salmon louse

Total survival and morphology were registered for all exposure times, using a stereo microscope (Nikon SMZ 1000, Japan) connected to a digital camera (Lumenera INFINITY 1-3C, Canada). In total, 240 nauplii and 480 copepodites were assessed. Individuals showing apparent body damage, damage to swimming appendices or lacked swimming response when provoked (gently poked with the end of a syringe), were considered dead. Both nauplii and copepodids were inspected, counted and photographed before and after each trial in order to assess any morphological effects. Survival (%) was the response variable for all trials in this study, and the results are given as means ± standard deviations (*SD*) for the replicates ($n = 3$) unless stated otherwise.

#### 2.4.2 | *Tetraselmis suecica*

Agar plate cultivation method was used for rapid determination of the number of living *Tetraselmis suecica*. Culture medium was prepared by mixing 300 ml of Z8 medium in seawater with 200 ml of distilled water and 13 g Bactoagar (Merck, Germany) in a glass bottle (1 L). The culture medium was autoclaved at 121°C for 15 min, together with a separate glass flask with 500 ml of filtered seawater collected at 60 m depth from the Oslo fjord. After autoclaving, seawater (500 ml) was mixed with the bactoagar solution. Petri dishes (ø = 9 cm) were filled with approximately 20 ml of agar solution, closed with a lid and cooled until analysis. A quantity of 100 μl of water from each test sample was gently spread with a clean, bent, glass rod on the surface of the petri dish with agar culture medium, and incubated with constant light (20–100 $\mu M\ m^{-2}\ s^{-1}$) for 72 hr at 15–20°C. Green colonies were observed under stereo microscope with diffuse light from below at 10–20× magnification. The method has a lower detection limit of 10 cells/ml. Each sample was analysed in triplicates and diluted in autoclaved seawater (collected at 60 m depth from the Oslo fjord) by a dilution factor of 10, 100 and/or 1,000.

#### 2.4.3 | Heterotrophic bacteria

Heterotrophic bacteria were quantified according to a modified version of Norwegian Standard NS-EN 6222:1999 by spreading 100 μl of diluted water sample on Marin Agar (Difco, USA) for isolation of marine heterotrophic bacteria at a temperature of 20°C, and an incubation period of 2 and 10 days. Each sample was spread in triplicates and diluted in autoclaved seawater (collected at 90 m depth from the Trondheim fjord) by a dilution factor of 10, 100, 1,000 and 10,000. Bacteria colonies were observed under a stereo microscope with diffuse light from below.

## 3 | RESULTS

### 3.1 | Cavitation effect on salmon lice

All test subjects were intact and showed normal swimming behaviour before the onset of each trial.

The survival for the naupliar stages of the salmon lice was 36.7 ± 11.5, 16.7 ± 10.4 and 1.7 ± 2.9% after exposure times of 5, 10 and 15 s respectively (Figure 4). The survival was zero for all replicates after 20 s of ultrasonic cavitation.

The recorded survival for the copepodid stages was 67.2 ± 7.2, 66.6 ± 1.7 and 28.3 ± 7.2% after exposure times of 5, 10 and 15 s respectively (Figure 4). Hence, the copepodid stages appeared to tolerate longer exposure intervals than the naupliar stage.

For exposures between 20 and 50 s, less than 20% survival was recorded for copepodids, and 0% survival was recorded after 60 s of ultrasonic cavitation. Individuals surviving ultrasonic cavitation did not show anomalous morphology or swimming behaviour.

The damage observed for the individuals that was registered as dead, ranged from minor damage, such as broken or torn appendages, to extensive damage such as dismemberment or pulverization (Figures 5 and 6).

### 3.2 | Cavitation effect on algal cell and heterotrophic bacteria

No algal density reduction was observed after 5 s of cavitation. After 10, 60 and 300 s of exposition to cavitation, a reduction in the

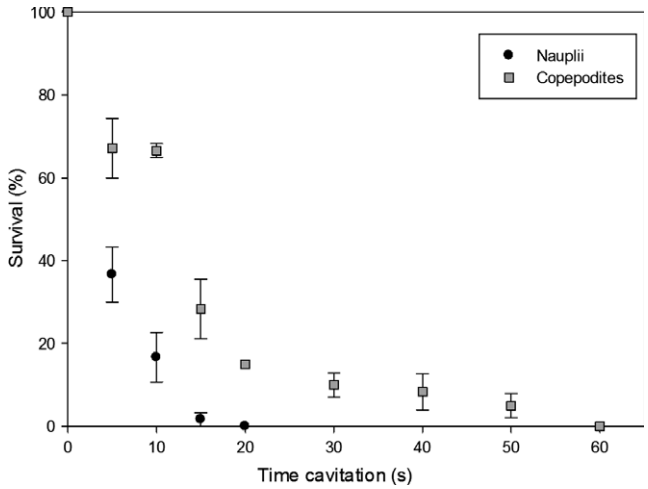


**FIGURE 4** Survival of the two development stages of salmon lice as a function of different exposure times to ultrasonic cavitation (means ± *SD*). Exposure time was set to 5, 10, 15, 20, 30, 40, 50 and 60 s. 20 lice per replicate ($n$ = 3) were included

algal density of 13, 51 and 95% respectively, was observed (Table 2 and Figure 7).

The cavitation effect on marine heterotrophic bacteria present in the algal culture was approximately the same for all exposure times (5, 10, 60 and 300 s) approximately 70 ± 6% reduction effect (Table 2); which corresponded to less than 1 log unit reduction (Figure 7).

### 3.3 | Cavitation power

The energy produced by the Hielscher Ultrasonic unit during the experiments, that is, energy output, was automatically and continuously calculated and logged by the Hielscher UP200Ht. By default, the energy was logged with the unit Joules per second (J/s). On average, the energy output from the ultrasonic cavitation unit (energy input to samples) was registered to be 17.5 J/s during all experiments. The resulting energy and power input to the samples as a function of exposure time is given in Table 3.

The transferred power from the cavitation unit to the sample's medium mainly depends on the sample's volume, distance from the Sonotrode, contact area between the Sonotrode and the medium, the viscosity of the medium, ambient pressure and user settings. In the experiments, the unit was programmed to maximize the transferred power to the samples by setting the available user settings (amplitude and duty cycle) to 100%. The Hielscher UP200Ht was equipped with a 200 W power supply which had an efficiency of 90% (Hielscher, 2013), indicating that some power is lost to heat. The power supply provided power for both the unit's internal circuitry (interface screen, Ethernet connection, central processing unit, etc.), and the Sonotrode. Therefore, although the unit was equipped with a 200 W power supply, only some of this power was input to the samples during operation due to the factors described above.

## 4 | DISCUSSION

As required by the Norwegian regulation for disinfection of aquaculture-related water, if the filter does not remove all life stages of the salmon louse, the filter should be used in combination with other disinfection methods. Therefore, the cavitation method might be an additional tool to combat sea lice, and possibly, other organisms detrimental to fish welfare. In combination with existing technologies for water quality control in aquaculture, adapted solutions for cavitation can be applied in closed containment systems where sea lice, pathogens and other unwanted organisms are present.

For salmon lice, the Norwegian Aquaculture Fish Transport Regulation (Norwegian Industry and Fisheries Ministry, 2014) requires the total removal of salmon lice through the use of a 100 μm pre-filtration step. Salmon lice in different stages can be found in water circulated in well boats, especially during delousing operations, where several hundred tonnes of infected fish are contained. In our experiments, 100% and 98.3% reduction effect was observed after 20 s and 60 s cavitation treatment on nauplii and copepodites respectively. These results imply that cavitation may prove to be an additional inactivation tool for the early, free swimming stages of sea lice, which might be too small, or presenting a too flexible body to be removed completely by the 100 μm pre-filtration step. Because the experiments indicate that nauplii and copepodites are sensitive to the destructive effect of cavitation, the requirement for removal of salmon lice can potentially be fulfilled for these stages of salmon lice, provided a feasible technological solution for efficient cavitation of large water flows can be developed.

For bacteria and algae, the Norwegian Aquaculture Fish Transport Regulation (Norwegian Industry and Fisheries Ministry, 2014) requires the reduction in pathogens as bacteria and virus by 99.9% in the outlet water after fish transport. In our experiments, the required reduction effect could not be achieved by cavitation for neither bacteria, nor algae with 70% and 95% reduction after 5 min of treatment, respectively. Poor reduction effect was also expected on virus, which are resistant to cavitation treatment due to its small size, and might be as resistant as algae to UV treatment. Therefore, cavitation treatment in combination with other treatment technologies, such as ultraviolet irradiation, may improve the efficacy of modern state of the art disinfection systems.

While UV technology has already been proven effective for inactivation of small organisms (Sommer, Haider, Cabaj, Pribil, & Lhotsky, 1998) such as bacteria and algae for drinking- or ballast water, cavitation treatment is rather effective on larger organisms, such as parasites (Guo et al., 2013). Our experiments verify that organisms' sensitivity to cavitation forces increases up to a certain size; salmon lice naupli and copepodites were more sensitive to cavitation treatment than algae, and algae were more sensitive than bacteria. However, copepodites were less sensitive than nauplii. This might be explained by the resistance difference of the body structure of these two life stages of the salmon louse. This has been also observed during NIVA's ballast water treatment testing activity over the past 10 years; with organisms in the ≥50 μm size group, such as

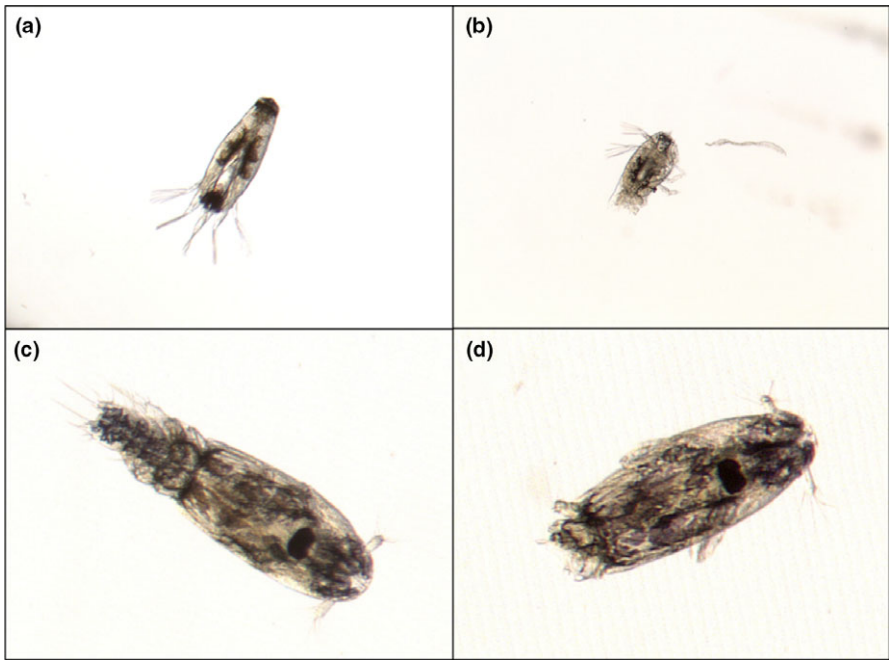


**FIGURE 5** Photos showing *L. salmonis nauplii* (a, b) and copepodites (c, d), before (a, c) and after exposure to ultrasonic cavitation (b, d). Post-exposure individuals shown here were split in half and were without self-motion. Photos: SINTEF [Colour figure can be viewed at wileyonlinelibrary.com]

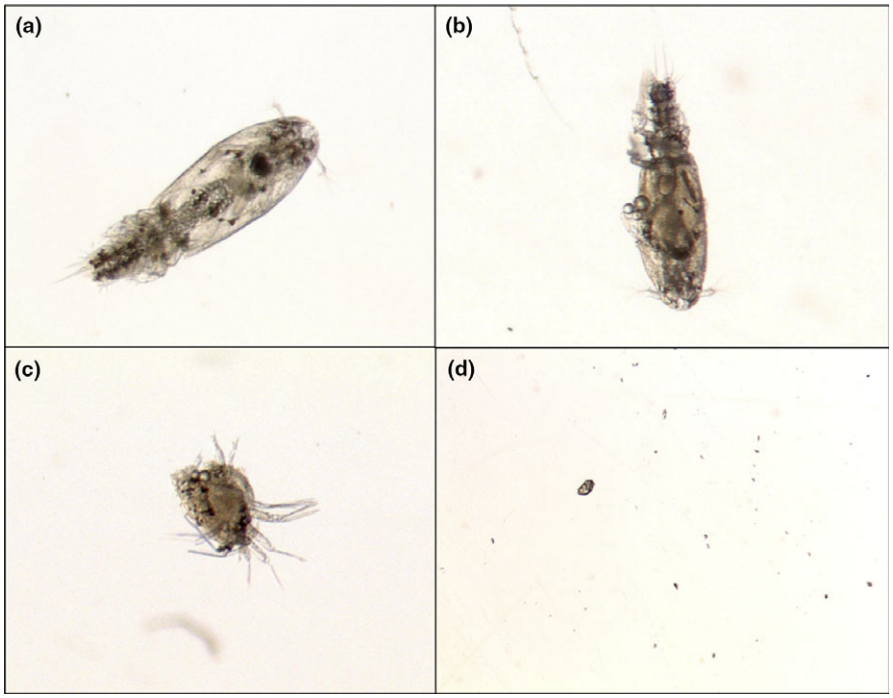


**FIGURE 6** Different types of damage observed for *L. salmonis nauplii* and copepodites after exposure to ultrasonic cavitation. (a) Torn setae on caudal ramus, (b) chapped prosome with endogenous lipid leaking, (c) burst specimen. In (d), the *L. salmonis* were pulverized and only small fragments were visible. Photos: SINTEF [Colour figure can be viewed at wileyonlinelibrary.com]

copepods, being more sensitive to cavitation forces imposed by ballast pumps or throttled valves than organisms in the ≥10–50 μm size group, such as algae, and organisms in the ≤10 μm size group, such as bacteria.

In the experiments, an energy input to the sample to be treated of 17.5 W (to be verified) was used, which was the maximum output energy the ultrasonic cavitation unit would supply with the selected Sonotrode, and a substantial energy input for a sample size of 20 ml. Disinfection of discharge water from well boats is relevant during transportation of salmon and treatment of salmon against disease or sea lice. During transportation, the well boats are usually closed, so there is no water exchange with the external environment. When administering medicinal remedies, treatment water (freshwater or seawater with added therapeutic agents) is kept segregated from the outside environment, using drainage grid systems for both fish and treatment water. In both cases, a well boat is, in effect, a closed containment system circulating and processing its contained water to ensure satisfactory water quality and/or treatment efficacy. Typically, the average internal water circulation rate is approximately 5,000 $m^3$/hr in pipes with a diameter of 20 in (1 in = 0.0254 m). In

**TABLE 2** Reduction (%) in concentration of marine bacteria and *Tetraselmis suecica* after different exposure times of cavitation. Shown data are calculated from data obtained from Figure 7

| | Reduction (%) | |
|---|---|---|
| Seconds cavitation | Marine bacteria | *Tetraselmis suecica* |
| 5 | 69 | 0 |
| 10 | 70 | 13 |
| 60 | 70 | 51 |
| 300 | 71 | 95 |

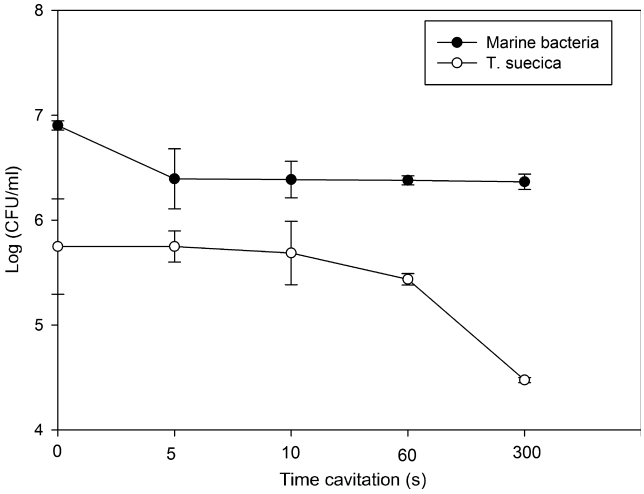


**FIGURE 7** Dose-survival curve of cavitation treated marine bacteria and *Tetraselmis suecica*, observed after four different exposure times (5, 10, 60 and 300 s). Curve showed in log scale as means ± *SD*)

**TABLE 3** Accumulated sample energy input in Joules as function of exposure time in seconds

| Seconds cavitation [s] | Accumulated sample energy input [J] | Accumulated sample power input [J/s] (Watts) |
|---|---|---|
| 5 | 87.5 | 17.5 |
| 10 | 175.0 | 17.5 |
| 60 | 1050.0 | 17.5 |
| 300 | 5250.0 | 17.5 |

order to estimate the required energy in order to achieve the same effect observed experimentally, we must first calculate the flow velocity $f_V$ through the pipe:

$$f_V = \frac{5000}{\left(\frac{20\times 0.0254}{2}\right)^2 \times \pi}\frac{m}{h} = 24669.1\frac{m}{h} = \frac{24669.1}{3600}\frac{m}{s} = 6.85\frac{m}{s} \quad (1)$$

In the experiments, 0% and 15% survival for nauplii and copepodites respectively, was registered after 20 s treatment time. Nauplii being the smaller of the two, are most likely to pass through a well boat's pre-filtration step. Therefore, 20 s was selected as an appropriate treatment time in the power calculations. Given a treatment time of 20 s, the pipe length, $L$, for the treatment volume becomes:

$$L = 6.85\frac{m}{s} \times 20s = 137\text{m} \quad (2)$$

This gives a treatment volume, $V_t$ of:

$$V_t = \left(\left(\frac{20\times 0.0254}{2}\right)^2 \times \pi\right) \times 137\ \text{m}^3 = 27.8\ \text{m}^3 = 27.8\times 10^6\ \text{ml} \quad (3)$$

The experimental power consumption was 17.5 W per 20 ml, we get the following power consumption per ml:

$$\frac{17.5}{20}\frac{W}{\text{ml}} = 0.88\frac{W}{\text{ml}} \quad (4)$$

This gives a total power estimate $P_{TOT}$ of the following:

$$P_{TOT} = 27.8\times 10^6\text{ml} \times 0.88\frac{W}{\text{ml}} = 24.5\text{MW} \quad (5)$$

Equation (5) gives the power required to disinfect 5,000 $m^3$ in 1 hr. A delousing operation, using well boats takes longer than 1 hr. The time available for disinfection is therefore longer. Results from freshwater treatments having a duration of 3 hr, indicate that this duration is insufficient (Powell, Reynolds, & Kristensen, 2015). Fish farmers also report that a typical delousing operation lasts 5–7 hr on average. The latter is therefore the time that can be spent for continuous disinfection of the contained water, using cavitation. Using 5 hr to treat 5,000 $m^3$ in our estimate gives an hourly power requirement, $P_{hr}$ of:

$$P_{hr} = \frac{P_{TOT}}{5} = \frac{24.5}{5} = 4.9\text{MW} \quad (6)$$

This power input would correspond to 0% and 15% survival after 5 hr of treatment of 5,000 $m^3$ water in a well boat for nauplii and copepodites respectively. This may be an unacceptably high power requirement. However, the effects of cavitation observed on larger organisms are most often caused by the physical effects of the implosion shockwave. When considering the physical extent of these shockwaves and the density of water, the effect of the shockwave will be dampened and eliminated by the surrounding water body. Thus, in order to achieve any effect, the organism must be located close to the origin of the implosion. In our experiment, the implosions are continuous and the effect increases as a function of time. Time will increase the probability of the organism being located sufficiently close to the implosion to be damaged. In order to maximize the physical effects of cavitation, the frequency of implosion as well as the magnitude of the generated energy in the resulting pressure released, must be addressed. Furthermore, in order to increase the probability of an organism to pass sufficiently close to an imploding vapour cavity, the geometry of the cavitation device must be carefully addressed, as must the flow-through velocity of the water containing the organisms.

There are several other ways cavitation can be introduced in the closed water circulation loop. *Microbubbles* can be created

using *ultrasonic transducers* or *cavitation nozzles* (Lecoffre, 1999). These transducers or nozzles can be distributed along the length of a circulation pipe, or in the water processing system's aerators. The principle of *mixing layer cavitation* (Lecoffre, 1999) can also be exploited by temporarily introducing a high velocity reverse flow in a pipeline, using water jets. The resulting turbulence will create a local cavitation zone the enclosed process water volume must pass through. *Vortex cavitation* is another option, where the existing water flow can be exploited. A stationary propeller like device can be fixed inside a pipe with reduced diameter. With correct dimensioning, the resulting vortex and increase in flow velocity can be expected to result in cavitation. Similarly, reversing this idea, rotating machinery optimized for creating cavitation bubbles can be developed and integrated as part of a well boat's piping system. A more novel approach includes an adaptation of *gap cavitation* (Lecoffre, 1999) which occurs when a fluid is forced in between hinged parts in a hydrofoil such as a rudder (Rhee, Lee, Lee, & Oh, 2010). The resulting high speed, low pressure flow can be a major challenge for modern ships where the rudder is placed behind the ship's propeller. Within the context of cavitating water flowing through pipes, this can be exploited through reduction in pipe diameter for increased flow velocity, and an obstruction in the pipe containing a dense grid of appropriately shaped gaps. Another cavitation technology already type approved for ballast water disinfection is based on boiling conditions created by pressure vacuum at low temperatures to eliminate the majority of the large organisms (Knutsen, 2017). Because of the amount of options available, a cost benefit analysis should be conducted for the various options so both feasibility and cost aspects can be evaluated.

## 5 | CONCLUSION

This study showed that ultrasonic cavitation is effective in killing salmon lice on a laboratory scale, and that cavitation had a significant effect at even relatively short exposure times. These preliminary results indicate that cavitation is a candidate disinfection method with the potential to be utilized as an additional tool for improved disinfection of discharge water from well boats, in particular with respect to sea lice. This method should therefore be investigated further on a larger scale to ensure an effective and as energy efficient treatment solution as possible.

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