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Water disinfection by ozonation has advantages over UV irradiation in a brackish water recirculation aquaculture system for Pacific white shrimp (*Litopenaeus vannamei*)

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Abstract

By keeping tropical shrimp, like *Litopenaeus vannamei*, in recirculating aquaculture systems (RAS), valuable food for human consumption can be produced sustainable. *L. vannamei* tolerates low salinities, and therefore, the systems can operate under brackish water conditions. The stabilization of the microbial community in RAS might be difficult under high organic loads, and therefore, water treatment measures like UV irradiation or ozone application are commonly used for bacterial reduction. To investigate the impact of these measures, the effects of UV irradiation and ozone application were studied in small-scale brackish water RAS with a salinity of 15‰ stocked with *L. vannamei*. UV reactors with 7 and 9 W were used, and by ozonizers with a power of 5–50 mg/hr, the redox potential in the water was adjusted to 350 mV. Ozone had a stabilizing effect on the microbial composition in the water and on biofilms of tank surfaces and shrimp carapaces, prevented an increase of nitrite and accelerated the degradation of nitrate in the water. UV irradiation led to changes in the microbial composition and was less effective in optimizing the chemical water quality. Thus, the use of ozone could be recommended for water treatment in brackish water RAS for shrimp.

KEYWORDS

disinfection, microflora, ozone, Pacific white shrimp, UV irradiation

1 | INTRODUCTION

By keeping Pacific white shrimp (*Litopenaeus vannamei*) independent from natural sea water on inland farms in recirculating aquaculture systems (RAS), the production of these animals has become possible in northern European countries. A reduced consumption of water, a low environmental impact and the possibility of high stocking densities and therefore high productivity are the primary advantages of keeping shrimp in RAS. Challenging are the maintenance of high water temperatures and appropriate salinities. Nevertheless, the costs for artificial sea salt can be reduced, as Pacific white shrimp are very tolerant against low and moderate salinity levels and RAS can be operated under brackish water conditions at 10–13‰ salinity (Bray, Lawrence, & Leungtrujillo, 1994; Jayasankar et al., 2009). Additionally, by using waste heat from biogas plants for heating the water in RAS, the production of shrimp can be a sustainable option for the local production of high-quality food and fresh marine shrimp can be offered to customers in areas far away from the sea.

Like in fish keeping facilities, also in intensive shrimp aquaculture, disease outbreaks might occur due to infections with viral, parasitic or bacterial pathogens (Austin & Zhang, 2006; Bauer et al., 2018; LeRoux et al., 2015; Lotz, 1997; Soto-Rodriguez, Gomez-Gil, & Lozano, 2010). RAS are usually stocked with specific pathogen-free post-larvae that reduces the infection risk especially for viral and parasitic pathogens. Bacteria on the other hand are always present in shrimp RAS and are even necessary for the maintenance of optimal water conditions. This applies particular to nitrifying bacteria but also heterotrophic bacteria in moderate amounts are beneficial for an aquaculture system. By mineralizing organic matter derived from uneaten feed, dead bodies and excreta of the animals they contribute to an optimal water quality and they can even be used as additional nutrition source for shrimp for example in biofloc systems (Rurangwa & Verdegem, 2015). However, under conditions of high organic loads heterotrophs can overgrow nitrifying bacteria with negative effects for the nitrification process (Rurangwa & Verdegem, 2015). Due to high stocking densities, large amounts of organic material from faeces and non-utilized feed and low water exchange rates, the load of heterotrophic bacteria can increase to a high abundance in shrimp RAS when no management strategies are in place for reduction and the numbers of bacteria might even increase further over time (Bauer et al., 2018). Bacteria in RAS are present as planktonic stages in water, but many species also attach to every possible surface, like tank walls, bio-filters and also to the surface of the animals. In shrimp, bacteria from the surrounding water usually colonize the carapaces and the gills. In fish it is known, that a dense population of bacteria on mucosal surfaces is not necessarily harmful but acts even as an important component of the external infection barrier and might protect them from infection with pathogenic bacteria (Balcazar et al., 2006). It can be assumed that this is similar in shrimp and that a stable and diverse microflora plays an important role in disease prevention for these animals. Nevertheless, although most of the bacteria within this physiological bacterial microflora have only a low pathogenic potential for shrimp, also potentially harmful organisms might occur. In marine and brackish water RAS, especially high diversity of *Vibrio* spp. can be found and among these bacteria, there are several potentially pathogenic species (Bauer et al., 2018). It is known that under suboptimal conditions, the colonization of surfaces with potentially pathogenic bacteria can be the starting point of bacterial infections (Abraham, Sharon, & Ofek, 1999). A direct correlation between the total abundance of bacteria and especially of *Vibrio* spp. populations was not only shown between water and surfaces but also between water and muscle tissue of kept finfish (Kim & Lee, 2017). It can be assumed that there is a similar correlation possible for shrimp. Therefore, the composition of the bacterial flora and the total amount of bacteria have not only an effect on animal health and welfare but also on product quality and thus on human health (Kim & Lee, 2017). The control of potentially pathogenic, mostly heterotrophic bacteria, is therefore one of the aims in RAS for fish production and different approaches are used to reduce the risk of bacterial infections and to reduce the total amount of bacteria. The aim is a general reduction of high bacterial numbers in water and additional the stabilization of a mature physiological microflora to prevent the establishment of potentially pathogenic bacteria within the mature microflora. For a bacterial reduction, especially physical methods such as ozonation and ultraviolet (UV) irradiation are used either separately or in combination (Attramadal et al., 2012; Bullock et al., 1997; Christensen, Rusch, & Malone, 2000; Gullian, Espinosa-Faller, Nunez, & Lopez-Barahona, 2012; Krumins, Ebeling, & Wheaton, 2001a, 2001b; Sharrer & Summerfelt, 2007; Sharrer, Summerfelt, Bullock, Gleason, & Taeuber, 2005; Summerfelt & Penne, 2007; Summerfelt, 2003; Summerfelt & Hochheimer, 1997; Summerfelt, Sharrer, Hollis, Gleason, & Summerfelt, 2004). UV disinfection is used in intensive aquaculture systems routinely to prevent the accumulation of coliform and heterotrophic bacteria in the water (Gullian et al., 2012). Especially in RAS, UV irradiation has been shown to inactivate microorganisms (Farkas, 1986; Sharrer et al., 2005; Zhu, Saucier, Chen, & Durfey, 2002) and to destroy dissolved O_3 (Summerfelt et al., 2004). Nevertheless, there are some limiting factors in the effectiveness and usefulness of UV irradiation. Water turbidity influences the effectiveness of UV irradiation to a great extent, and thus, turbidity should be reduced by mechanical filters to achieve an effective UV treatment (Gullian et al., 2012). Also, microorganisms can be shielded by particles in the water and it is known that some species, including specific pathogens, are tolerating high UV dosages (Huyben, Bevan, Stevenson, Zhou, & Moccia, 2018; Lazarova, Bellahcen, Manem, Stahl, & Rittmann, 1999; Liltved & Cripps, 1999). Some administered probiotic bacteria on the other hand might be reduced by UV irradiation (Garrido-Pereira, Braga, da Rocha, Sampaio, & Abreu, 2013). It could also be shown that UV irradiation fostered the formation of photoproducts like ozone, which can be harmful for animals and may for example cause cataracts in fish like cod (Björnsson, 2004).

Ozone is a powerful oxidizing agent and, in general, is effective against bacteria, parasites and viruses, and is therefore used also in RAS to control pathogens (Sugita et al., 1992; Summerfelt, 2003; Summerfelt & Hochheimer, 1997). While UV irradiation is primarily used for disinfection, the application of ozone is additionally effective for the improvement of water quality. It is able to remove not only pathogens but also organic carbon, turbidity, algae, colour, odour and taste (Goncalves & Gagnon, 2011; Owsley, 1991; Rosenthal & Otte, 1980; Summerfelt, 2003; Summerfelt & Hochheimer, 1997). As ozone can also oxidize organic matter and fine particles, its application therefore also indirectly reduces the bacterial amount in a system (Blancheton, Attramadal, Michaud, d'Orbcastel, & Vadstein, 2013a, 2013b; Martins et al., 2010). Additionally, ozone is effective in reducing nitrogen compounds like nitrite and nitrate in the water and therefore contributes not only to the maintenance of an optimal microbiological but also to an optimal chemical water quality (Honn & Chavin, 1976). The use of ozone in sea water is more effective than in freshwater due to its higher amount of bromide, which is highly reactive with ozone (Haag & Hoigne, 1983, 1984; Von Gunten, 2003a, 2003b; Von Gunten & Hoigne, 1994; Von Gunten & Oliveras, 1998). Bromide is oxidized by ozone into hypobromite that limits bacterial regrowth

and therefore contributes to an effective disinfection (Penru, Guastalli, Esplugas, & Baig, 2013). Studies on the effectiveness of ozone were mainly performed in RAS stocked with finfish, but also in the culture of European lobster (*Homarus gammarus*) water treatment using ozone was more effective and resulted in better growth and survival rates and improved general fitness compared to probiotic as water additive or UV irradiation (Middlemiss, Daniels, Urbina, & Wilson, 2015). However, ozone is toxic in low levels for the kept animals as well as for humans working with them. To remove residual oxidants, a post-treatment of the water by for example activated carbon or UV irradiation is therefore recommended (Penru et al., 2013; Sharrer et al., 2005).

Despite all advantages of water disinfection methods, it is worth to discuss if any of these methods is advisable as long as a system is well protected against invasion of pathogens from outside sources. Any disinfection method may reduce not only pathogens but also decimates beneficial microbial populations and additionally favours the proliferation of opportunistic pathogens (Blancheton, Attramadal, Michaud, d'Orbcastel, et al., 2013; Blancheton, Attramadal, Michaud, d'Orbcastel, et al., 2013). Therefore, not only bacterial reduction but also the stabilization of the physiological microflora should be considered. Microflora stabilization aims at a diverse microflora where different bacterial species occupy practically all available ecological niches so that pathogenic bacteria are impeded from asserting themselves within the system. The physiological microflora might be influenced by disinfection methods like UV light and ozone application, and those disinfection methods might have negative effects on animal health instead of being effective by optimizing the microbial water quality. Whether and how disinfection routines should be implemented in the maintenance of RAS has to be investigated further beyond this background (Blancheton, Attramadal, Michaud, d'Orbcastel, et al., 2013; Blancheton, Attramadal, Michaud, d'Orbcastel, et al., 2013). Most studies conducted on this topic were performed in RAS for finfish production, and only little information is available on the situation in shrimp RAS.

In the present study, the effect of different doses of UV light as well as the effect of ozone application on the microflora in RAS for *L. vannamei* was investigated.

2 | MATERIAL AND METHODS

2.1 | Recirculating aquaculture systems

The experiments were performed in six separated laboratory scaled RAS. Each RAS had a total water volume of 280 L and consisted of three holding tanks with 70 L volume each and one biofilter including a sedimentation tank with a total volume of 70 L. All six RAS were maintained with the addition of food for three months before shrimp were introduced into the holding tanks. Water temperature was adjusted to 30°C and water salinity to 15‰. For the experiment using UV irradiation, two holding tanks per RAS were used and for ozonization two holding tanks of only four RAS each were used.

2.2 | Shrimp

For both experiments, specific pathogen-free *L. vannamei* from a hatchery (Shrimp improvement systems, Singapore 718873, Facility Florida) were used that arrived as post-larvae (PL 12, approx. 12 days old) and were kept for acclimatization for three weeks in a separated holding tank with a water volume of 700 L equipped with a biofilter and a skimmer at a salinity of 30‰ and a temperature of 30°C. After the acclimatization period, salinity was lowered to 15‰ within seven days and the shrimp were transferred to the holding tanks of the different RAS for each experiment. At the start of the UV irradiation experiment, the shrimp were approximately 39 days old with a mean body weight of 0.79 ± 0.46 g and a mean body length of 2.94 ± 0.07 cm. Shrimp used in the ozone experiment were approximately 107 days old with a mean body weight of 5.56 ± 0.20 g and a mean body length of 9.65 ± 0.09 cm. In both experiments, the RAS were planned to be stocked with 100 shrimp per tank square metre, and therefore, in both experiments the two holding tanks of each of the six RAS were stocked with 20 shrimp specimen. Automatic feeders (Eheim GmbH & C. KG) were installed on individual tanks and allowed the shrimp to be fed on a regular basis. Before and after the start of UV irradiation and ozone application, body weights and lengths of six shrimp per tank were measured.

2.3 | UV irradiation

For ultraviolet irradiation in two RAS, UV-C reactors with a power of 7 Watt (ReeflexUV 350, Eheim GmbH & C. KG; recommended by the manufacturer for water volumes between 80 and 350 L) and UV-C reactors with a power of 9 Watt (ReeflexUV 500, Eheim GmbH & C. KG; recommended by the manufacturer for water volumes between 300 and 500 L) were installed behind the biofilter and sedimentation tank. The water was pumped from the biofilter into the sedimentation tank and then through the UV-C reactors. Water leaving the UV-C reactors was distributed by tubes to the two holding tanks of each RAS. Per hour 159.25 L of water per RAS was pumped through the UV-C reactors what corresponded to 56.88% of the total water volume once per hour. Two additional RAS were used as controls and were maintained without UV reactors. The UV-C reactors were operated 24 hr per day for 42 days. During the experiment, samples from recirculating water, tank surface and shrimp carapace were collected three days before and 7, 28 and 42 days after the start of the UV irradiation.

2.4 | Ozonation

The ozone experiment was conducted in the same aquaculture systems as the UV irradiation experiment, but then only four RAS

were used. In two RAS, ozone generators (Certizon C50, Sander, Germany) were installed in the filter tank in a sector separated from the biological part of the filter. The ozone concentration was infinitely adjustable between 5 and 50 mg ozone per hour and was regulated by adjusting the redox potential in the water of the holding tanks at a stable level of 350 mV. Before entering the biological part of the filter tank, the water was passed through a protein skimmer and an activated carbon filter to eliminate free ozone. In the holding tanks, ozone concentration was measured daily to ensure that no free ozone was present in the animal keeping part of the systems. At any time, free ozone was not detectable in the holding tanks. During the experiment, samples were collected six days before and two, nine and 31 days after starting the ozone generators.

2.5 | Experimental design

In recirculating water, the concentration of the nitrogen compounds NH_4^+, NO_2^- and NO_3, the pH value and the concentration of dissolved and total organic carbon (DOC and TOC) as well as the amount of filterable substances were measured at each of the sampling timepoints. NH_4^+, NO_2^- and NO_3 were analysed with commercial test systems for sea water (Merck) and measured photometrically. The pH values were measured by a pH meter and for measurement of DOC and TOC samples were sent to an external laboratory (GBA Gesellschaft für Bioanalytik mbH, Hildesheim, Germany) where the amounts were analysed with a certified method (DIN EN 1484[H3]). To assess the effect of UV irradiation and ozonization on the bacterial microflora in the RAS at each sampling timepoint, from each holding tank stocked with shrimps, water samples, swabs from the biofilm of the tank surface and swabs from the transition from the carapax to the abdominal segments of three shrimp specimens were collected. Additionally, swabs from the abdominal cavity of three shrimp specimens per holding tank were taken at the first and the last sampling timepoints. For this, the shrimp were killed individually with iced water at a temperature of 0 ± 1°C in a 1-L plastic aquarium. The ratio of ice to water was adjusted so that there was a clear excess of ice (approx. 3:1), but at the same time the individual shrimp was completely surrounded by iceless water. After a minimum of five minutes, shrimp were removed from the iced water, the abdominal cavity was opened with a sterile scalpel, and samples were taken with a swab.

For the determination of the total amount of bacteria in the water samples, dilution series with sterilized water of a salinity of 15‰ were prepared from undiluted water samples to a dilution level of 10^{-5} and each dilution was spread on two sheep blood agar plates containing 15‰ artificial sea salt and incubated at 25°C for 48 hr. Colony-forming units (Cfu) on the plates were counted after 12 and 48 hr of incubation, and subsequently, the amount of Cfu per mL of tank water was calculated. The abundance of morphologically different Cfu was described semi-quantitatively (low: +; up to 10 colonies/ plate, moderate: ++; 10–50 colonies/plate), high: +++; >50 colonies/plate), and all morphologically different colonies were subcultured on sheep blood agars containing 15‰ artificial sea salt. After a second 48-hr incubation period at 25°C, subcultures were stored at −80°C in 2 ml of veal infusion broth until further analysis for identification of the bacterial species.

The swab samples from tank surfaces, shrimp carapaces and the abdominal cavities of shrimp specimens were plated on blood agar plates containing 15‰ artificial sea salt. The plates were cultivated at 25°C for a total of five days. Every day, the plates were checked for bacterial growth. The number of bacterial colonies was assessed semi-quantitatively. On average, subcultures of bacteria were prepared after one day of incubation. From fast growing colonies, subcultures were already prepared after 12 hr of incubation and from slow growing colonies subcultures were taken within the five days of cultivation as soon as a distinct Cfu was visible on the plate. Afterwards, from macroscopically different colonies, one colony was picked with a loop and fractionated on a separate blood agar plate. The subcultures were checked for purity after a 24-hr incubation period and were stored at −80°C in 2 ml of veal infusion broth until further analysis for the bacterial species.

2.6 | Identification of bacteria

For species identification, pure cultures of the isolates were identified by 16S rRNA gene sequencing. For this, DNA was extracted by adding one colony per isolate to 500 µl of AF-buffer (Qiagen GmbH), incubation at 92°C for 15 min while shaking and centrifugation at 13,000 *g* for 5 min. DNA concentrations were measured using spectrophotometry (NanoDrop ND-1000 Lab, Peqlab Biotechnologie GmbH) and adjusted to a concentration of 10 ng/µL with PCR grade water (Thermo Fisher Scientific Inc.). The V1-V9 region of the 16 S rRNA–encoding gene was amplified using forward and reverse primers designed by Jiang et al. (Jiang, Gao, Xu, Ye, & Zhou, 2011). An endpoint PCR was performed with 0.2 U of hot-start KAPA 2G robust polymerase (PeqLab Biotechnologie GmbH), 1x KAPA A buffer, 200 nM of each primer, 200 µM of each dNTP, 5.0 µL of DNA samples and nuclease-free water to a final volume of 25 µL. The PCR was performed in a SensoQuest thermocycler (SensoQuest GmbH) with a PCR profile consisting of an initial denaturation step at 95°C for 5 min, five cycles at 95°C for 30 s, 63–58°C for 30 s, 72°C for 60 s (every cycle with annealing temperature 1°C lower), followed by 35 cycles at 95°C for 30 s, 57°C for 30 s, 72°C for 60 s and an extension step at 72°C for 7 min. Sequencing of PCR products was performed by LCG Genomics GmbH, Berlin, Germany.

The obtained sequences were compared to known 16S rRNA sequences using the online databases EzBioCloud (http://www.ezbiocloud.net) and Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.7 | Calculation of bacterial population diversity

The composition of the bacterial microflora was analysed using the following ecological terms:

Prevalence—number of samples in which a particular bacterial species could be found divided by the number of samples examined expressed as a percentage (%).

Mean intensity—number of a particular bacterial species found in a sample divided by the number of samples in which this particular bacterial species could be found. An arbitrary scale was used for quantifying the bacterial species: 0 = absent, 1 = low amount, 2 = moderate amount, 3 = high amount.

Mean abundance—total amount of a particular bacterial species in a particular sample divided by the number of samples examined; mean abundance is equivalent to mean intensity multiplied by prevalence.

The diversity of the bacterial community was evaluated by calculating the Shannon–Wiener index of diversity for individual samples ($H' = -\sum (p_i \ln p_i)$ where p_i is the relative intensity of bacterial amount i).

2.8 | Statistical analysis

The data were statistically analysed using the computer program SigmaPlot 12. When the data were normally distributed (tested with a Shapiro–Wilk test), an ANOVA was performed, followed by an all pairwise multiple comparison procedure. The Turkey test was used for comparing groups with an equal number of data, and the Dunn's method was used for comparing groups with an unequal number of data. When the test for normality failed, the Mann–Whitney rank sum test was used for comparing the data. Differences between tested data sets were considered significant at a probability of error of $p < .05$. Principal component analysis (PCA) was performed using the Excel Add in Analyse it for the data on the microbial community in respect to their relationship between different treatment groups and different sample types.

3 | RESULTS

3.1 | UV irradiation

3.1.1 | Animals

In the UV irradiation experiment, no clinical signs of a disease were detected in the shrimp. In all RAS, the shrimp grew and gained weight over the experimental period. No statistically significant differences in the control RAS and the RAS treated with 7 W UV light occurred, but the shrimp in the RAS treated with 9 W UV light gained less weight during the experimental period (Figure 1).

3.1.2 | Water chemistry

The pH values stayed constant at pH 7.9 ± 0.1 in the control and the UV-treated RAS. Differences were seen in the concentration of the nitrogen compounds, ammonia and nitrite in recirculating water of the control RAS and both RAS treated by UV irradiation (Figure 2). Only in the RAS treated with 9 W UV light the concentration of ammonia decreased significantly until day 42. A decrease in the concentration of nitrite could only be measured in the control RAS, whereas in the RAS treated with 7 W UV light even an increase was seen until day 42. The nitrate concentration increased in all RAS similarly. A significant decrease in the amounts of dissolved and total organic carbon (DOC and TOC) occurred only for the RAS treated with 9 W UV light but stayed high in the control RAS and in the 7 W UV light treated RAS. No differences between the three RAS were seen in the amount of filterable substances (Figure 2).

3.1.3 | Bacterial community

A total of 63 bacterial species were isolated from all examined samples. The greatest numbers of bacterial species were isolated from the carapaces of shrimp ($n = 41$) and from water samples ($n = 39$). In samples from the abdominal cavities of the shrimp, 27 different bacterial species were found and the lowest number of bacterial species was isolated from the biofilms of tank surfaces ($n = 24$) (Tables 1–4). The detected bacterial species belonged to 30 bacterial genera and most of the isolated bacteria belonged to the phylum Proteobacteria. To a lesser extent, bacteria from the phyla Firmicutes, Actinobacteria and Bacteroidetes were isolated (Tables 1–4). The relationship between samples of different origin (tank water, biofilms in the tanks, carapaces of the shrimp and abdominal cavity of the shrimp) was analysed by performing PCA. Differences in the composition of the microflora occurred in all different types of samples over time. However, when looking at the mean values for the control RAS and the four UV-treated RAS in samples from water, carapaces and

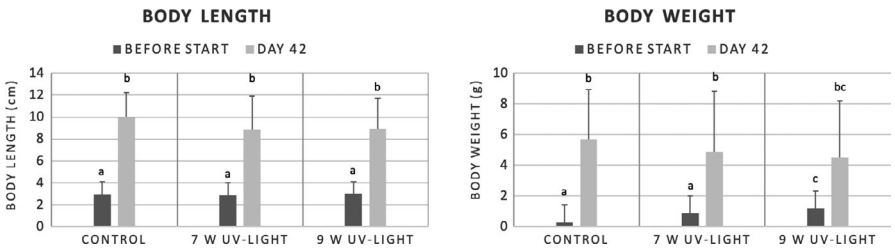

FIGURE 1 Body length (cm) and body weight (g) of *Litopenaeus vannamei* before and 42 days after treatment of the holding water with 0 W (control), 7 W and 9 W UV light. The figure is showing the mean values of two RAS with each two holding tanks for every treatment. The statistically significant differences between the treatments are marked by different letters

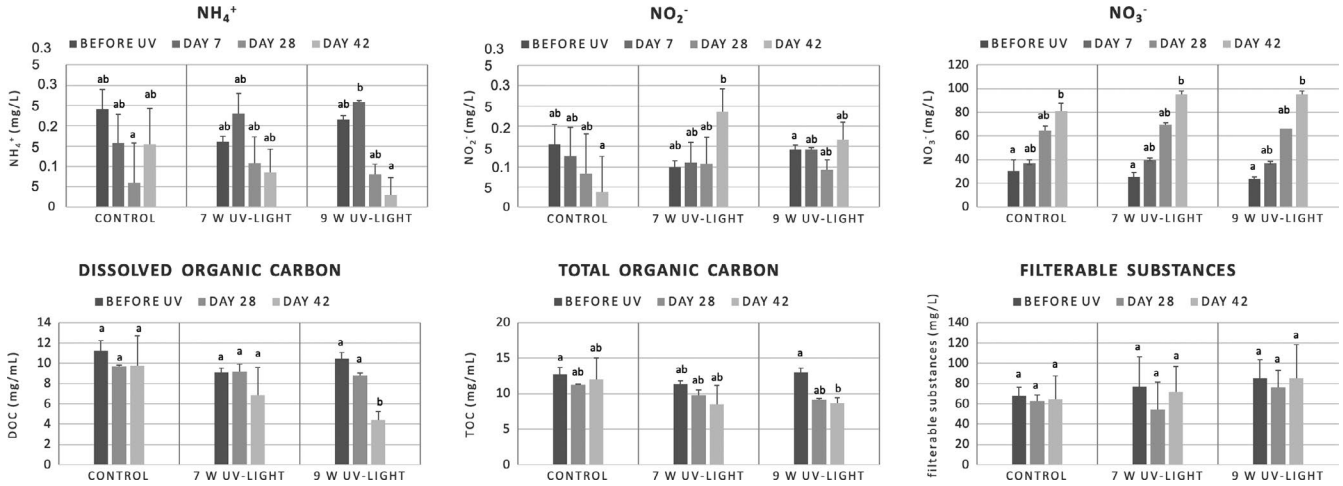

FIGURE 2 Concentration of ammonia (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), dissolved organic carbon (DOC), total organic carbon (TOC) and filterable substances in six RAS stocked with *Litopenaeus vannamei* and treated with 0 W (control), 7 W and 9 W UV light. Shown are mean values and standard deviations of measurements of NH_4^+, NO_2^- and NO_3^- before and 7, 28 and 42 days after start of a continuous UV irradiation and of DOC, TOC and filterable substances before and 28 and 42 days after start of a continuous UV irradiation. The statistically significant differences between the treatments are marked by different letters

abdominal cavities, a very close relationship was seen. Compared to this, the microbial composition of the samples from the biofilms of tank surfaces differed between the control RAS and all UV-treated RAS. Especially in samples from the RAS treated with 7 W UV light, the microbial composition differed to a great extent from those of the control RAS (Figure 3). Independent from the different treatments of the six RAS, the microflora in the abdominal cavities of shrimp differed to the greatest extent to that isolated from the water, biofilms on tank surfaces or shrimp carapaces (Figure 3). The composition of the bacterial microflora of the water was closely related to the composition of that of the biofilms of tank surfaces and the biofilms of the shrimp carapaces.

Total number of bacteria in recirculating water

Before starting the UV irradiation, no differences in the bacterial load in the water from the six RAS were detected (Figure 4). Up to day 42 after start of the UV treatment, the number of bacteria increased significantly in the RAS treated with 7 W UV light, whereas in the control RAS the bacterial amount stayed similar as before the start of the experiment (Figure 4). Also, in the RAS treated with 9 W UV light the bacterial load increased over time and was significantly higher at day 42 compared to day 7 after start of the experiment (Figure 4).

Composition of the bacterial community

The bacterial composition in recirculating water, biofilms of tank surfaces and biofilms on shrimp carapaces changed in all differently treated RAS during the experimental period. After start of the experiment, at day seven, *Vibrio* sp. was the most abundant genus in all RAS at every sampling day and in all types of samples and represented around 40%–80% of all detected bacteria (Figure 5). The amount of *Vibrio* spp. decreased until day 42 in all sample types taken from the control RAS whereas its amount increased significantly in water sample and samples from the biofilms of tank surfaces from the RAS treated with 9 W UV light. The *Vibrio* species detected in the water samples and in the biofilm samples from tank surfaces and shrimp carapaces changed over time in all six RAS. At the beginning of the experiment, mainly *V. alginolyticus* could be detected in the tank water and in the biofilms of the tanks, whereas at later time points higher amounts of *V. parahaemolyticus* and of bacteria from a not clearly assignable *Vibrio* species were isolated (Tables 1–3). On day 42, *V. parahaemolyticus* showed the highest mean abundance in the tank biofilms of the RAS treated with 9 W UV light, and in all six examined holding tanks, this species was found in high amounts. The amount of *Pseudomonas* spp. increased until the end of the experiment in the control RAS and in the 7 W UV-treated RAS in all types of samples. This increase was not measured in high amounts in the water samples taken from the 9 W UV-treated RAS. Before start of the experiment bacteria like *Lactobacillus* spp., *Bowmanella denitrificans* and *Staphylococcus* spp. could be detected in high amounts in water and tank biofilm samples, but these species were not found any more in any of the RAS in high numbers at later time points. On shrimp carapaces, especially the amount of *Pseudomonas* spp. increased till day 42 in all RAS. Samples from the abdominal cavity of shrimp carapaces were taken before start of the experiment, when all animals were kept in a holding tank together and at the last sampling time point at day 42. In all differently treated shrimp, the composition of the microflora in the abdominal cavity changed significantly during the experimental time and most bacterial species that could be detected before the start of the experiment could not be detected anymore on day 42. Especially, bacteria from the phyla Actinobacteria, Bacteroidetes and Firmicutes could not be detected anymore on day 42 and almost only Proteobacteria were found. The amount of *Vibrio* spp. and *Pseudomonas* spp. increased but the number of different bacterial species in total decreased in all differently treated RAS in a similar way (Table 4).

TABLE 1 Mean abundances of bacterial species isolated from tank water from six RAS after treatment with 0 W (control), 7 W and 9 W UV light before and 7, 28 and 42 days after start of continuous UV irradiation

				Control				7 W UV light				9 W UV light			
Phylum	Class	Genus	Species	Before start	Day 7	Day 28	Day 42	Before start	Day 7	Day 28	Day 42	Before start	Day 7	Day 28	Day 42
Actinobacteria	Actinobacteria	*Micrococcus*	*M. luteus*	7	0	0	0	0	0	0	0	0	0	0	
		Microbacterium	*M. lacus*	17	0	0	0	0	17	0	0	0	0	0	0
		Nocardia	*N. salmonicida*	0	0	0	0	0	8	0	0	0	0	0	0
Bacteriodetes	Flavobacteriia	*Tenacibaculum*	*T. discolor*	0	0	33	0	0	0	25	8	0	0	58	0
Firmicutes	Bacilli	*Bacillus*	*B. altitudinis*	7	0	0	0	0	0	0	0	20	0	0	0
			B. cereus	0	8	33	0	0	25	0	0	0	0	8	8
			B. megaterium	0	17	0	8	0	0	0	8	0	0	0	0
			B. pumilus	0	8	0	0	0	0	0	0	0	0	0	0
			B. vietnamensis	0	25	0	0	0	17	0	0	0	0	0	0
			Bacillus spp.	0	0	0	8	0	0	0	0	0	0	0	0
		Lactobacillus	*Lactobacillus* spp.	80	0	0	0	**100**	0	0	0	**100**	0	0	0
		Sediminihabitans	*S. luteus*	0	0	0	0	0	0	0	0	7	0	0	0
		Sporosarcina	*S. contaminans*	0	0	17	0	0	0	0	0	0	0	0	0
		Staphylococcus	*S. arlettae*	0	0	8	0	0	0	0	0	0	0	0	0
			S. epidermidis	7	0	0	0	20	25	0	0	20	17	0	0
			S. haemolyticus	27	0	0	0	47	0	0	0	47	0	0	0
			S. pasteuri	0	0	0	0	0	0	0	0	7	0	0	0
			S. xylosus	0	0	0	8	0	0	0	8	0	0	0	33
			Staphylococcus spp.	0	17	0	0	0	0	0	0	0	0	0	0
Proteobacteria	α-Proteobacteria	*Aestuariispira*	*A. insulae*	80	0	0	0	80	0	0	0	80	0	0	0
	γ-Proteobacteria	*Acinetobacter*	*A. jejuni*	7	0	0	0	20	0	0	0	0	0	0	0
		Bowmanella	*B. denitrificans*	80	0	0	0	**100**	0	0	0	**100**	0	17	0
			Bowmanella spp.	0	17	0	0	0	0	0	0	0	0	0	0
		Marinobacter	*M. xestospongiae*	0	0	0	0	0	0	0	0	0	8	0	0
		Morganella	*Morganella* spp.	0	17	0	0	0	0	0	0	0	0	0	0
		Pseudoalteromonas	*P. ulvae*	0	0	0	50	0	0	0	50	0	0	0	0
			Pseudoalteromonas spp.	60	0	67	0	**93**	0	42	0	**100**	0	25	0

(Continues)

TABLE 1 (Continued)

Phylum	Class	Genus	Species	Control				7 W UV light				9 W UV light			
				Before start	Day 7	Day 28	Day 42	Before start	Day 7	Day 28	Day 42	Before start	Day 7	Day 28	Day 42
		Pseudomonas	*P. gallaeciensis*	0	0	0	0	0	0	8	0	0	0	8	0
			P. hussainii	0	0	25	50	0	0	0	50	0	25	17	25
			P. mendocina	7	0	0	0	20	0	0	0	0	17	0	0
			Pseudomonas spp.	27	8	0	58	27	67	0	42	27	58	0	33
		Shewanella	*S. putrefaciens*	0	0	50	0	0	0	42	8	20	0	42	0
		Vibrio	*V. alginolyticus*	73	**92**	8	0	80	**100**	25	17	80	**100**	33	25
			V. fortis	0	0	17	0	0	0	58	0	0	0	33	0
			V. harveyi	0	42	0	0	0	50	0	0	0	25	0	0
			V. mytili	27	0	0	0	27	0	0	8	27	0	0	0
			V. parahaemolyticus	27	0	**92**	58	27	0	67	50	33	0	75	58
			V. pelagius	60	42	0	17	**93**	17		25	53	25	0	67
			Vibrio spp.	13	25	**100**	58	0	25	**100**	42		33	**100**	**92**

Abbreviation: RAS, recirculating aquaculture systems.

Diversity of the bacterial community

The diversity of bacterial isolates from water, biofilms of tank surfaces and shrimp carapaces and from the abdominal cavity of shrimp was similar before the start of the UV irradiation in all RAS and the means of the Shannon–Wiener indices ranged between 2.12 and 2.77. In all types of samples from the control RAS as well as from all UV-treated RAS, the bacterial diversity decreased during the experiment. This decrease was significant in all RAS and at all sampling time points for samples from the carapaces and from the abdominal cavity of shrimp and no differences between both UV-treated RAS and the control RAS were detected. In the tank water, the decrease of the microbial diversity was statistically significant only in the RAS treated with either 7 W or 9 W UV light and on days 7 and 42. The microbial diversity in samples from the biofilms of tank surfaces decreased significantly only in the RAS treated with 7 W UV light on day 7 (Figure 6).

3.2 | Ozonation

3.2.1 | Animals

Similar like in the UV irradiation experiment, also in the ozone experiment in all RAS the shrimp showed no clinical signs of a disease. The animals grew and gained weight over the experimental period, but the body length of shrimp specimens from ozone-treated RAS was significantly shorter at the end of the experiment compared to shrimp specimen from the control RAS (Figure 7).

3.2.2 | Water chemistry

The pH values stayed constant at pH 7.8 ± 0.1 in the control and the ozone-treated RAS. The concentrations of ammonia and nitrite increased significantly during the experimental period in the control RAS, whereas in the ozone-treated RAS no statistically significant change in the amounts of these nitrogen compounds was detected (Figure 8). Especially, the nitrite concentrations stayed below 0.1 mg/L in the ozone-treated RAS. Nitrate concentrations decreased over time in both RAS, but only in the ozone-treated RAS this decrease was statistically significant until day 31 (Figure 8). The dissolved and the total organic carbon (DOC and TOC) were measured at days 2 and 31 after start of the experiment. At day 2, already a lower amount of TOC was measured in ozone-treated RAS, though no statistically significant difference to the control RAS was seen and a significant decrease in the amount of TOC was seen only in the control RAS at day 31. In all RAS, the amount of DOC stayed constant and a significant reduction in the amount of filterable substances was found on day 31 (Figure 8).

3.2.3 | Bacterial community

In total, 88 bacterial species were isolated from all examined samples. Most different species were isolated from the carapaces of the

TABLE 2 Mean abundances of bacterial species isolated from the biofilms of tank surfaces from six RAS after treatment with 0 W (control), 7 W and 9 W UV light before and 7, 28 and 42 days after start of continuous UV irradiation

				Control				7 W UV light				9 W UV light			
Phylum	Class	Genus	Species	Before start	Day 7	Day 28	Day 42	Before start	Day 7	Day 28	Day 42	Before start	Day 7	Day 28	Day 42
Actinobacteria	Actinobacteria	*Arthrobacter*	*Arthrobacter* spp.	0	0	0	0	0	17	0	0	0	0	0	0
Bacteriodetes	Flavobacteriia	*Tenacibaculum*	*T. discolor*	0	0	0	0	0	0	8	0	0	0	0	0
Firmicutes	Bacilli	*Bacillus*	*B. cereus*	0	0	17	8	0	0	0	0	0	0	8	0
			B. mycoides	27	0	0	0	27	0	0	0	27	0	0	0
			B. vietnamensis	0	33	0	0	0	33	0	0	0	0	0	0
		Filibacter	*F. tadaridae*	0	0	0	0	7	0	0	0	0	0	0	0
		Lactobacillus	*Lactobacillus* spp.	80	0	0	0	80	0	0	0	80	0	0	0
		Staphylococcus	*S. epidermidis*	0	0	0	0	0	0	0	0	0	42	0	0
Proteobacteria	γ-Proteobacteria	*Aeromonas*	*A. media*	7	0	0	0	0	0	0	0	0	0	0	0
		Bowmanella	*B. denitrificans*	80	0	0	0	80	0	17	0	80	0	17	0
		Pseudoalteromonas	*P. shioyasakiensis*	0	0	0	42	0	0	0	0	0	0	0	0
			Pseudoalteromonas spp.	80	0	67	0	80	0	0	0	80	0	0	0
		Pseudomonas	*P. hussainii*	0	0	**100**	75	0	0	33	58	0	0	8	**83**
			P. mendocina	60	25	0	0	80	0	17	0	80	0	8	0
			Pseudomonas spp.	0	33	0	**83**	0	8	8	25	0	17	0	0
		Shewanella	*S. putrefaciens*	0	17	0	33	0	17	33	75	0	17	25	8
		Vibrio	*V. alginolyticus*	**100**	**83**	33	42	**100**	**83**	25	42	**100**	**92**	50	75
			V. brasiliensis	27	0	0	0	27	0	0	0	27	0	0	0
			V. fortis	0	0	50	0	0	0	67	0	0	0	8	0
			V. harveyi	7	50	0	0	0	0	0	0	0	42	0	0
			V. mytili	0	0	0	0	0	0	0	8	0	0	0	0
			V. parahaemolyticus	80	0	67	33	80	17	**83**	58	80	0	**92**	**100**
			V. pelagius	80	**83**	0	8	80	17	0	33	80	33	0	0
			Vibrio spp.	0	50	**100**	50	0	25	**100**	25	0	0	**100**	**100**

Abbreviation: RAS, recirculating aquaculture systems.

TABLE 3 Mean abundances of bacterial species isolated from the biofilms of shrimp carapaces from six RAS after treatment with 0 W (control), 7 W and 9 W UV light before and 7, 28 and 42 days after start of continuous UV irradiation

				Control				7 W UV light				9 W UV light			
Phylum	Class	Genus	Species	Before start	Day 7	Day 28	Day 42	Before start	Day 7	Day 28	Day 42	Before start	Day 7	Day 28	Day 42
Actinobacteria	Actinobacteria	*Arthrobacter*	*Arthrobacter* spp.	19	17	0	0	19	0	0	0	19	17	0	0
		Microbacterium	*M. lacus*	0	0	0	0	0	25	0	0	0	0	0	0
		Mycobacterium	*M. syngnathidarum*	5	0	0	0	5	0	0	0	5	0	0	0
		Pseudarthrobacter	*Pseudarthrobacter* spp.	0	0	0	0	0	0	0	0	0	25	0	0
Bacteriodetes	Flavobacteriia	*Chryseobacterium*	*C. aquaticum*	5	0	0	0	5	0	0	0	5	0	0	0
			Chryseobacterium spp.	5	0	0	0	5	0	0	0	5	0	0	0
		Tenacibaculum	*T. discolor*	0	0	8	0	0	0	58	0	0	0	50	0
Firmicutes	Bacilli	*Bacillus*	*B. cereus*	0	25	8	0	0	8	0	0	0	0	8	8
			B. vietnamensis	0	50	0	0	0	58	0	0	0	0	0	0
		Exiguobacterium	*E. aurantiacum*	33	0	0	0	33	0	0	0	33	0	0	0
			E. profundum	5	0	0	0	5	0	0	0	5	0	0	0
		Lactobacillus	*Lactobacillus* spp.	**86**	0	0	0	**86**	0	0	0	**86**	0	0	0
		Staphylococcus	*S. arlettae*	0	0	8	0	0	0	0	0	0	0	0	0
			S. epidermidis	0	0	0	0	0	17	0	0	0	17	0	0
			S. haemolyticus	33	0	0	0	33	0	0	0	33	0	0	0
			S. warneri	5	0	0	0	5	0	0	0	5	0	0	0
			S. xylosus	0	0	0	25	0	0	0	33	0	0	0	42
			Staphylococcus spp.	0	0	0	0	0	0	0	0	0	17	0	0
Proteobacteria	α-Proteobacteria	*Paracoccus*	*P. marcusii*	8	0	0	0	8	0	0	0	8	0	0	0
	γ-Proteobacteria	*Acinetobacter*	*A. lwoffii*	5	0	0	0	5	0	0	0	5	0	0	0
		Alteromonas	*A. abrolhosensis*	0	0	0	0	0	0	0	0	0	0	8	0
		Bowmanella	*B. denitrificans*	**100**	**92**	33	0	**100**	58	0	0	**100**	75	0	0
		Marinobacter	*M. hydrocarbonoclasticus*	0	17	0	0	0	0	0	0	0	17	0	0
		Morganella	*Morganella* spp.	0	0	0	0	0	17	0	0	0	0	0	0
		Pseudoalteromonas	*P. ulvae*	0	0	0	58	0	0	0	0	0	0	0	0
			Pseudoalteromonas spp.	14	0	8	0	14	0	33	0	14	0	0	0
		Pseudomonas	*P. hussainii*	0	0	17	50	0	0	8	**100**	0	0	0	**83**
			P. mendocina	5	33	0	0	5	25	8	0	5	0	25	0
			P. pachastrellae	0	0	0	0	0	25	0	0	0	0	0	0

(Continues)

TABLE 3 (Continued)

Phylum	Class	Genus	Species	Control			7 W UV light			9 W UV light		
				Before start	Day 7	Day 28	Day 42	Before start	Day 7	Day 28	Day 42	Before start	Day 7	Day 28	Day 42
			P. stutzeri	5	0	0	0	5	0	0	0	5	0	0	0
			Pseudomonas spp.	0	17	25	67	0	0	0	17	0	17	0	42
		Shewanella	*S. putrefaciens*	0	42	58	0	0	33	67	0	0	25	50	17
		Stenotrophomonas	*Stenotrophomonas* spp.	62	0	0	0	62	0	0	0	62	0	0	0
		Vibrio	*V. alginolyticus*	0	**92**	33	58	0	**92**	58	67	0	**92**	42	75
			V. fortis	0	0	42	0	0	0	67	0	0	0	50	0
			V. harveyi	0	**83**	0	0	0	50	0	0	0	42	0	0
			V. mytili	57	0	0	0	57	0	0	0	57	0	0	0
			V. orientalis	0	0	0	0	0	17	0	0	0	17	0	0
			V. parahaemolyticus	14	0	**100**	67	14	0	58	33	14	17	58	67
			V. pelagius	19	8	0	8	19	33	0	0	19	8	0	0
			Vibrio spp.	0	25	**83**	50	0	17	**83**	42	0	25	**100**	**83**

Abbreviation: RAS, recirculating aquaculture systems.

shrimp ($n = 67$). In the tank water and from the biofilms of tank, each 47 different bacterial species were found, and from samples of the abdominal cavities of shrimp, the lowest number of different species, in total 29 species, were detected (Tables 5–8). The detected bacterial species belonged to 44 bacterial genera and most of the isolated bacteria belonged to the phylum Proteobacteria. Like in the UV experiment, to a lesser extent, bacteria from the phyla Firmicutes, Actinobacteria and Bacteroidetes were isolated (Tables 5–8). The distribution of the different phyla was similar in all RAS before application of ozone and changed with time after commencing ozone application.

The PCA analysis showed that in samples of different origin (tank water, biofilms in the tanks, carapaces of the shrimp and abdominal cavity of the shrimp) differences in the composition of the microflora occurred in general over time. When evaluating the mean values for the control RAS and the ozone-treated RAS especially in samples from the tank biofilms, the carapaces and the abdominal cavities, only slight differences occurred depending on the different treatments. The composition of the microflora in the tank water was very similar in both RAS (Figure 9). In general, the microflora in the abdominal cavity differed to the greatest extent to those of all other types of samples. However, the composition of the bacterial microflora in the water was closely related to the microflora of the biofilms of tank surfaces and the biofilms of the carapaces of shrimp.

Total number of bacteria in recirculating water

Up to day nine after commencing ozone application, the number of bacteria in the water increased especially in the control RAS but also in the ozone treated RAS. Nevertheless, this increase was not statistically significant as large differences were seen in the individual holding tanks of the RAS. Until the end of the experiment at day 31 almost the same numbers of bacteria like at the start of the experiment were measured in all RAS (Figure 10).

Composition of the bacterial community

The bacterial composition in recirculating water, biofilms of tank surfaces and biofilms on shrimp carapaces changed in all RAS during the experimental period despite their ozone treatment. Before start of the experiment, *Vibrio* spp. represented 27% of all bacterial species in the water and in biofilms of tank surfaces. On the shrimp carapaces, only around 7.5%, and in the abdominal cavities of shrimp, only around 2.8% of bacterial isolates belonged to the genus Vibrio. After start of the experiment, the amount of *Vibrio* sp. increased in all RAS and in all sample types, and from day two, *Vibrio* spp. was the most abundant genus (Figure 11). The *Vibrio* species detected changed over time in all RAS, and especially, the amount of *V. parahaemolyticus* increased. This was comparable in the control and in the ozone-treated RAS. Additionally, in samples of biofilms of tank surfaces and of shrimp carapaces of the ozone-treated RAS, the amount of *V. harveyi* increased as well (Table 5-8). Before start of the experiment, high amounts of *Staphylococcus* spp. could be detected in water samples and in samples of shrimp carapaces and abdominal cavities.

TABLE 4 Mean abundances of bacterial species isolated from the abdominal cavities from shrimp kept in six RAS treated with 0 W (control), 7 W and 9 W UV light before and 42 days after start of continuous UV irradiation

				Control		7 W UV light		9 W UV light	
Phylum	Class	Genus	Species	Before start	Day 42	Before start	Day 42	Before start	Day 42
Actinobacteria	Actinobacteria	*Arthrobacter*	*A. citreus*	5	0	5	0	5	0
			Arthrobacter spp.	5	0	5	17	5	0
		Clavibacter	*C. michiganensis*	5	0	5	0	5	0
		Micrococcus	*M. luteus*	0	0	0	8	0	0
		Mycolicibacterium	*M. fortuitum*	5	0	5	0	5	0
Bacteriodetes	Flavobacteriia	*Chryseobacterium*	*Chryseobacterium* spp.	29	0	29	0	29	0
Firmicutes	Bacilli	*Bacillus*	*B. altitudinis*	29	0	29	0	29	0
			B. megaterium	0	8	0	0	0	0
		Exiguobacterium	*E. aurantiacum*	14	0	14	0	14	0
		Lactobacillus	*Lactobacillus* spp.	57	0	57	0	57	0
		Paenibacillus	*P. peoriae*	19	0	19	0	19	0
		Staphylococcus	*S. epidermidis*	19	0	19	0	19	0
			S. haemolyticus	14	0	14	0	14	0
			S. xylosus	0	25	0	42	0	50
Proteobacteria	α-Proteobacteria	*Aestuariispira*	*A. insulae*	14	0	14	0	14	0
	γ-Proteobacteria	*Acinetobacter*	*A. jejuni*	14	0	14	0	14	0
		Bowmanella	*B. denitrificans*	**86**	0	**86**	0	**86**	0
		Pseudoalteromonas	*P. ulvae*	0	75	0	25	0	0
		Pseudomonas	*P. hussainii*	0	75	0	**92**	0	**92**
			P. mendocina	29	0	29	0	29	0
			Pseudomonas spp.	0	42	0	50	0	58
		Shewanella	*S. putrefaciens*	0	0	0	8	0	0
		Vibrio	*V. alginolyticus*	0	50	0	50	0	33
			V. brasiliensis	48	0	48	0	48	0
			V. parahaemolyticus	33	75	33	17	33	**83**
			V. pelagius	0	0	0	25	0	0
			Vibrio spp.	0	58	0	50	0	**92**

Abbreviation: RAS, recirculating aquaculture systems.

These species occurred in lower numbers at the following sampling time points. In general, the changes in the microbial composition were similar in the control and the ozone-treated RAS and almost no differences occurred due to ozonation of the water.

Diversity of the bacterial community

The diversity of bacterial isolates from water, biofilms of tank surfaces and shrimp carapaces and from the abdominal cavity of shrimp was similar before the start of ozone application in all RAS, and the mean of the Shannon–Wiener indices ranged between 1.68 and 1.86. In water, tank biofilms and biofilms on the carapaces, the bacterial diversity remained on a similar level during the experimental period in the control and the ozone-treated RAS. Only in samples of the abdominal cavities, a significantly higher diversity was measured at day 31 in all differently treated RAS (Figure 12).

4 | DISCUSSION

In the present study, the effects of UV irradiation and ozone application in a brackish water RAS for Pacific White shrimp, *L. vannamei*, were analysed. Identical RAS with several holding tanks were used for both experiments and the examined shrimp specimens originated from the same breeding farm. Although samples were taken at slightly different time points after the start of each experiment and the ozone experiment was performed for an 11 days shorter period, the data from the experiments are comparable in general. In both experiments, the bacterial compositions in the water, on the biofilms of tank surfaces and on shrimp carapaces and in the abdominal cavities of shrimp were investigated. Additionally, the performance of the shrimp and the chemical water quality in the RAS were analysed. As for each treatment, two independent RAS were used, and as from each RAS, two tanks were sampled, and inter- and intra-RAS

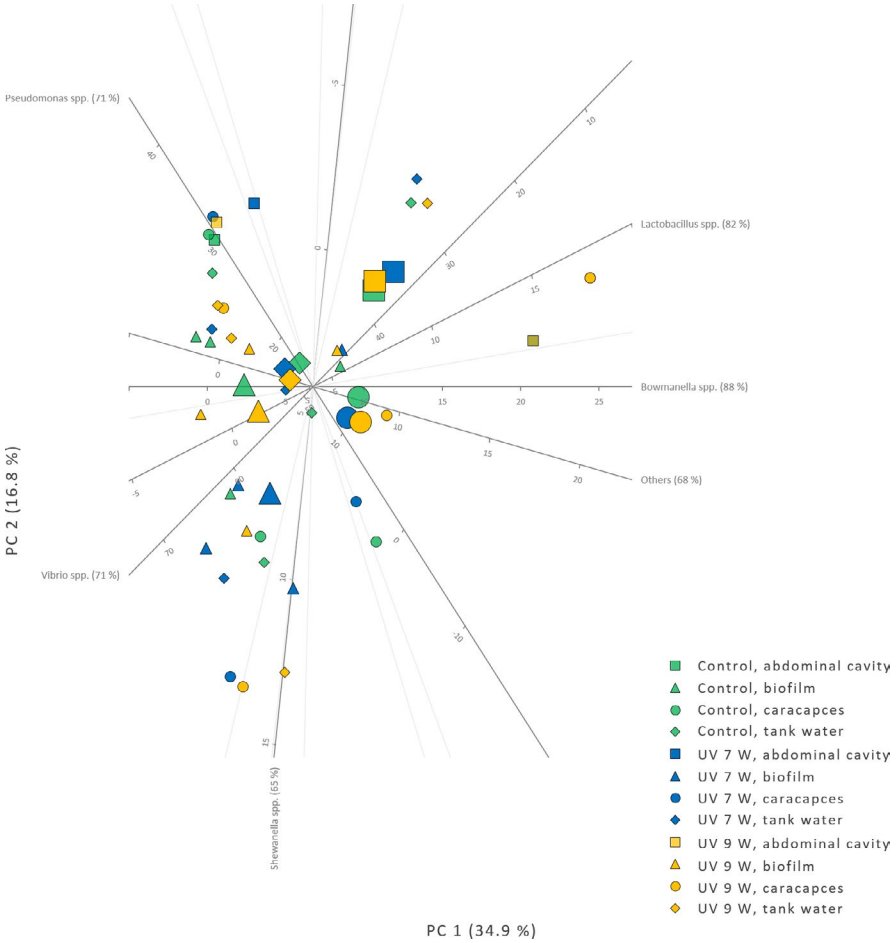

FIGURE 3 Principal component analysis (PCA) of the composition of the bacterial microflora from the biofilms of tank surfaces, the water, the biofilms from shrimp carapaces and the abdominal cavity from shrimp from six recirculating aquaculture systems (RAS) treated with 0 W (control), 7 W and 9 W UV light before and 7, 28 and 42 days after start of a continuous UV irradiation. Samples from the abdominal cavities are only shown before and 42 days after start of UV irradiation. The data from the different sampling timepoints are indicated with small symbols, and the mean values of the samples from the differently treated RAS are indicated in large, bordered symbols [Colour figure can be viewed at wileyonlinelibrary.com]

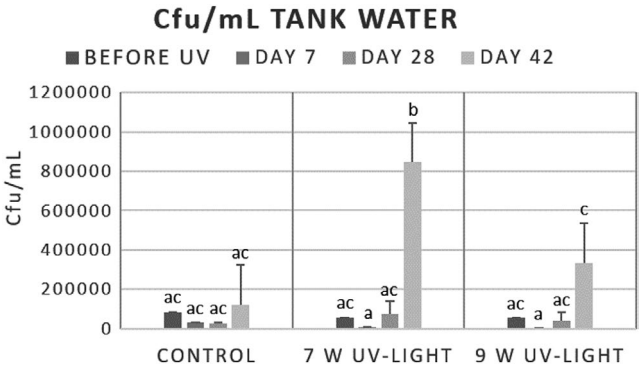

FIGURE 4 Colony-forming units (Cfu) in the water of six RAS stocked with *Litopenaeus vannamei* treated with 0 W (control), 7 W and 9 W UV light before and 7, 28 and 42 days after start of a continuous UV irradiation. Shown are mean values and standard deviations of Cfu from two RAS with two holding tanks each per treatment. The statistically significant differences between the treatments are marked by different letters

variabilities were examined. The two samples taken from the same RAS are therefore not completely independent from each other, and in the statistical analysis, the overall variance might be underestimated, while the statistical significance might be overestimated. The interpretation of the statistically significant findings was therefore made carefully, especially when only slight differences were seen.

Ultraviolet irradiation as well as ozone application are widely used in aquaculture systems for finfish production mainly prophylactically for a general water disinfection with the aim of reducing the number of heterotrophic bacteria in the system and thus preventing the animals from disease outbreaks due to infections with facultative pathogens. Both methods are known for many years for their use in finfish aquaculture and even in shellfish facilities, studies on the effectiveness of UV irradiation were performed. It could be shown already more than 40 years ago that, when a pathogenic bacterium is present, the potential for a disease outbreak in the animals was much greater in non-UV-treated water than in UV-treated water (Brown & Russo, 1979). In general, the advantages of the use of UV irradiation over ozone application are lower costs and an easier maintenance of the reactors. Additionally, UV irradiation generates toxic residuals to a much lesser extent than ozone application. Main disadvantages of UV irradiation are the reduced efficiency in turbid water (Summerfelt, 2003), the robustness of several pathogens against UV irradiation (Huyben et al., 2018; Lazarova et al., 1999; Liltved & Cripps, 1999) and the formation of photoproducts like ozone that can be harmful for animals (Björnsson, 2004).

Ozone application on the other hand is more costly and very complex. However, in RAS the costs can be lowered when other gases like oxygen are applied to a specific tank separated from the holding tanks as only an ozone reactor has to be installed

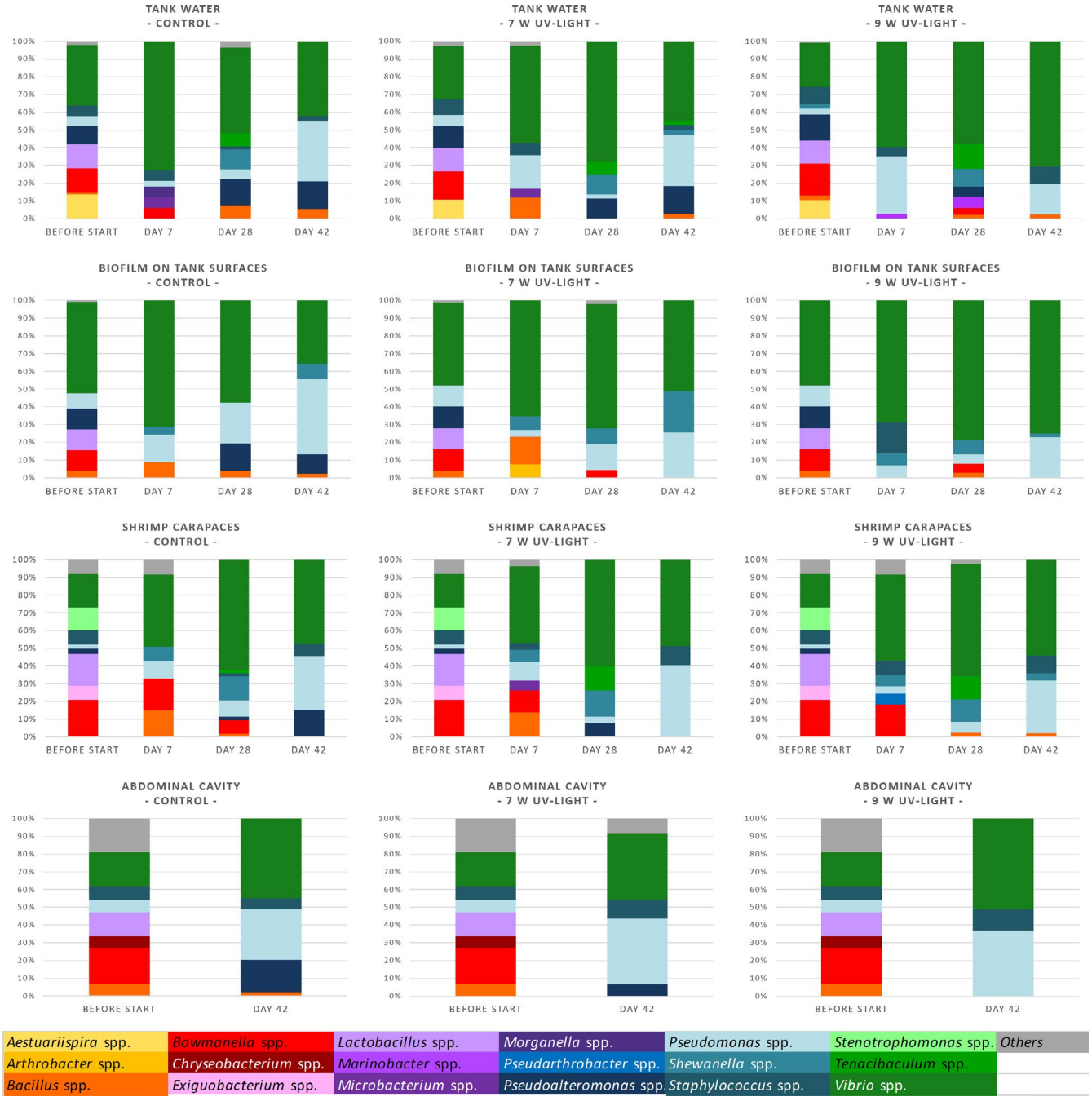

FIGURE 5 Composition of the bacterial microflora in six RAS stocked with *Litopenaeus vannamei* and treated with 0 W (control), 7 W and 9 W UV light. Columns represent the composition of the microflora in tank water, biofilms of tank surfaces and shrimp carapaces before and 7, 28 and 42 days after start of a continuous UV irradiation. The composition of bacteria in the abdominal cavities of shrimp is shown before and 42 days after start of UV irradiation. Shown are the bacterial species that could be detected at least at one sampling timepoint in one of the samples with an abundance of 10% or more. All other bacterial species are summarized under the heading "others." [Colour figure can be viewed at wileyonlinelibrary.com]

additionally and because the water in RAS is reused the amount of needed ozone is reduced too (Summerfelt, Sharrer, Tsukuda, & Gearheart, 2009). It is usually recommended that free ozone, that might be toxic for the animals kept in the RAS, should be degraded before the ozone treated water is introduced into the holding tanks, but during the last years studies have been performed on direct application of ozone in the water of the animal holding tanks for finfish and shellfish (Powell & Scolding, 2016). It could be shown that direct application of ozone can even improve productivity and welfare of farmed animals and it also appears to correlate with reduced infection and disease and an improved water quality (Powell & Scolding, 2016). Nevertheless, also deleterious effects of direct ozonation are documented including behavioural abnormalities, changes in physiology, tissue damage and mortality

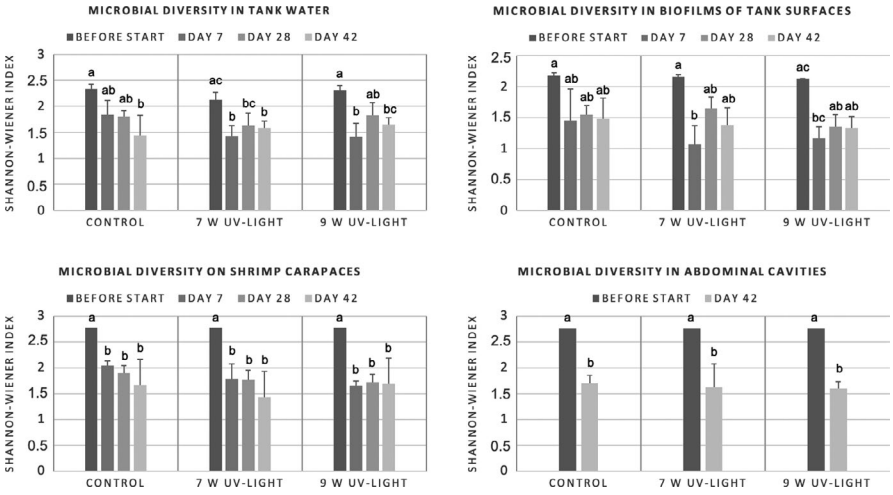

FIGURE 6 Shannon–Wiener indices of diversity describing the composition of the bacterial microflora from six RAS treated with 0 W (control), 7 W and 9 W UV light. Depicted are index values from the recirculating water, the biofilms of tank surfaces and from shrimp carapaces 7, 28 and 42 days after start of a continuous UV irradiation. From the abdominal cavity of shrimp, data before and 42 days after start of UV irradiation are shown. The statistically significant differences between the treatments are marked by different letters. RAS, recirculating aquaculture systems

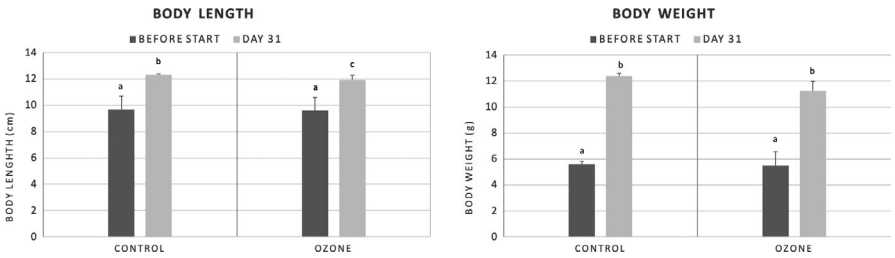

FIGURE 7 Body length (cm) and body weight (g) of *Litopenaeus vannamei* before and 31 days after treatment of the holding water with 0 mg/hr ozone (control) and 5–50 mg/hr ozone (redox potential adjusted to 350 mV). The figure is showing the mean values of two RAS with each two holding tanks for every treatment. The statistically significant differences between the treatments are marked by different letters. RAS, recirculating aquaculture systems

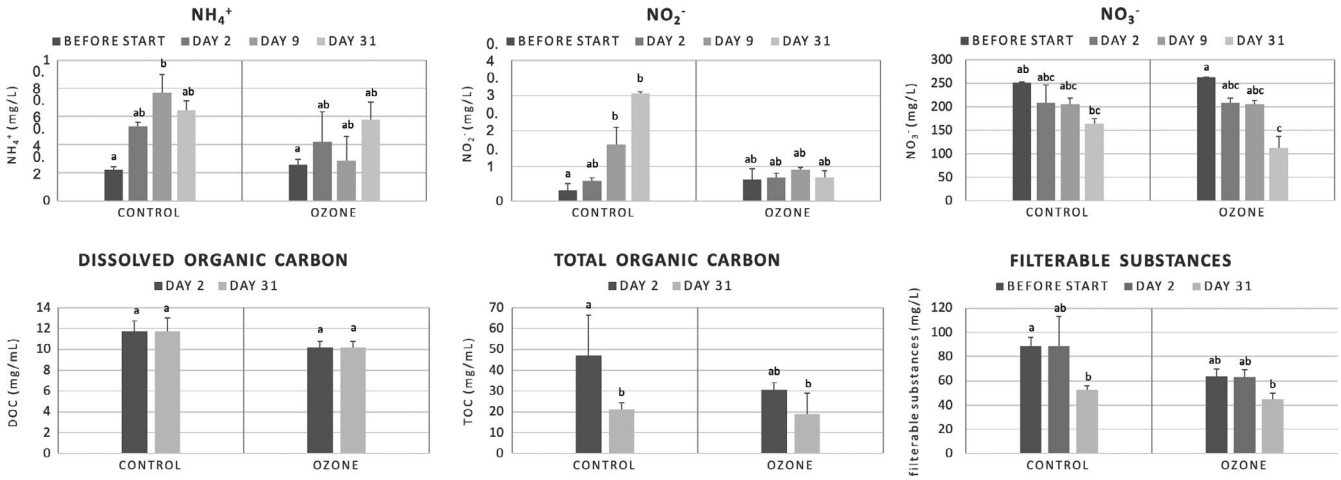

FIGURE 8 Concentration of ammonia (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), dissolved organic carbon (DOC), total organic carbon (TOC) and filterable substances in four RAS stocked with *Litopenaeus vannamei* and treated with 0 mg/hr ozone (control) and 5–50 mg/hr ozone (redox potential adjusted to 350 mV). Shown are mean values and standard deviations of measurements of NH_4^+, NO_2^- and NO_3^- before and 2, 9 and 31 days after start of ozone application and of DOC, TOC and filterable substances on days 2 and 31 after start of ozone application. The statistically significant differences between the treatments are marked by different letters

and further studies especially on the safe dose of ozone for animals in aquaculture are necessary (Powell & Scolding, 2016).

When comparing the positive effects of UV irradiation and ozone application, especially ozone has additional advantages and its application was shown to be beneficial for different finfish species. In a low-exchange water recirculating system for rainbow trout (*Oncorhynchus mykiss*), water ozonation improved fish performance without significantly impacting fish health and welfare (Good, Davidson, Welsh, Snekvik, & Summerfelt, 2011). Also, for "Srikandi" tilapia (*Oreochromis aureus* × *niloticus*) it could be shown that the fastest growth rates were achieved when the water for fish holding tanks was ozonated for 10–15 min before

TABLE 5 Mean abundances of bacterial species isolated from tank water from four RAS after treatment with 0 mg ozone/hr (control) and 5–50 mg ozone/hr (redox potential adjusted to 350 mV) before and 2, 9 and 31 days after start of ozone application

				Control				Ozone			
Phylum	Class	Genus	Species	Before start	Day 2	Day 9	Day 31	Before start	Day 2	Day 9	Day 31
Actinobacteria	Actinobacteria	*Actinomyces*	*Actinomyces* spp.	0	0	17	0	0	0	0	0
			A. oxydans	0	0	0	25	0	0	0	0
			Arthrobacter spp.	17	25	8	42	58	8	33	17
		Corynebacterium	*C. glyciniphilum*	0	0	0	0	0	8	0	0
		Curtobacterium	*C. herbarum*	0	0	0	25	0	0	0	33
			G. terrae	0	25	0	0	0	17	0	0
		Mycolicibacterium	*M. porcinum*	0	0	17	0	0	0	8	0
		Streptomyces	*Streptomyces* spp.	0	0	0	0	0	0	0	33
Bacteriodetes	Flavobacteriia	*Tenacibaculum*	*T. discolor*	0	0	8	0	0	0	17	0
			T. mesophilum	0	**83**	0	0	0	75	0	0
Firmicutes	Bacilli	*Bacillus*	*B. cereus*	0	0	8	0	0	0	0	0
			B. megaterium	0	0	0	42	0	0	0	8
			B. mycoides	0	0	0	0	8	0	0	0
			B. psychrosaccharolyticus	0	0	0	0	8	0	0	0
			Bacillus spp.	0	0	17	0	0	0	17	0
		Exiguobacterium	*Exiguobacterium* spp.	0	0	0	8	0	0	0	0
		Lactobacillus	*Lactobacillus* spp.	0	0	0	17	0	0	0	42
		Sporosarcina	*S. globispora*	8	0	0	0	0	0	0	0
		Staphylococcus	*S. capitis*	0	0	0	17	0	0	0	42
			S. epidermidis	**83**	0	0	8	42	0	0	8
			S. saprophyticus	0	0	8	0	0	17	0	0
			S. xylosus	0	8	8	0	8	8	25	0
Proteobacteria	γ-Proteobacteria	*Acinetobacter*	*A. calcoaceticus*	0	17	0	0	0	8	0	0
			A. lwoffii	0	0	0	0	0	0	8	0
		Aestuariibacter	*A. aggregatus*	8	0	0	0	0	0	0	0
		Alteromonas	*A. abrolhosensis*	0	0	0	0	8	0	0	0
		Bowmanella	*B. denitrificans*	8	0	0	42	8	0	33	33
		Marinobacter	*M. hydrocarbonoclasticus*	42	0	0	0	25	0	0	0
		Motilimonas	*M. eburnea*	0	8	8	0	0	0	25	8
		Pantoea	*P. agglomerans*	0	0	0	67	0	0	0	**83**
		Pseudoalteromonas	*P. piscicida*	0	0	**92**	0	0	0	67	0
		Pseudomonas	*P. abyssi*	0	0	17	0	0	0	8	0
			P. hussainii	0	50	33	0	0	75	33	0
			P. mangrovi	17	0	42	0	17	8	50	0
			P. mendocina	67	0	0	0	**83**	0	0	0
			P. pachastrellae	17	0	0	0	0	0	0	0
			P. stutzeri	0	0	0	0	0	8	**83**	0
			Pseudomonas spp.	0	8	75	8	8	17	0	0
		Stenotrophomonas	*Stenotrophomonas* spp.	0	17	0	0	0	0	0	0
		Vibrio	*V. alginolyticus*	0	58	33	0	0	17	8	0
			V. campbelli	0	17	0	0	0	25	0	0
			V. harveyi	**83**	58	75	58	50	67	67	67

(Continues)

TABLE 5 (Continued)

Phylum	Class	Genus	Species	Control				Ozone			
				Before start	Day 2	Day 9	Day 31	Before start	Day 2	Day 9	Day 31
			V. mytili	0	0	17	0	0	0	0	0
			V. orientalis	0	0	0	8	0	0	0	0
			V. parahaemolyticus	0	25	50	**83**	0	0	42	58
			V. rotiferianus	0	25	0	8	0	50	0	0
			Vibrio spp.	17	**83**	58	67	25	67	**83**	58

Abbreviation: RAS, recirculating aquaculture systems.

TABLE 6 Mean abundances of bacterial species isolated from biofilms of tank surfaces from four RAS after treatment with 0 mg ozone/hr (control) and 5–50 mg ozone/hr (redox potential adjusted to 350 mV) before and 2, 9 and 31 days after start of ozone application

Phylum	Class	Genus	Species	Control				Ozone			
				Before start	Day 2	Day 9	Day 31	Before start	Day 2	Day 9	Day 31
Actinobacteria	Actinobacteria	*Arthrobacter*	*A. aurescens*	0	0	8	0	0	17	0	0
			Arthrobacter spp.	8	42	0	75	0	0	0	33
		Cellulosimicrobium	*C. cellulans*	0	0	0	0	0	0	0	8
		Gordonia	*G. rubripertincta*	0	0	0	0	0	0	8	0
			G. terrae	0	25	8	0	0	33	42	25
		Microbacterium	*M. saccharophilum*	0	0	8	0	0	0	0	0
		Mycolicibacterium	*M. porcinum*	0	0	25	0	0	0	50	0
		Streptomyces	*Streptomyces* spp.	0	0	0	0	0	0	0	8
Bacteriodetes	Flavobacteriia	*Tenacibaculum*	*T. discolor*	0	0	8	0	17	0	0	0
			T. mesophilum	0	0	0	0	0	33	0	0
Firmicutes	Bacilli	*Bacillus*	*B. arsenicus*	0	0	0	0	0	8	0	0
			B. cereus	8	8	0	0	8	0	8	0
			B. horikoshii	0	0	0	0	0	8	0	0
			B. mycoides	0	0	0	0	8	0	0	0
			B. vietnamensis	8	0	0	0	8	0	0	0
			Bacillus spp.	0	0	8	0	0	8	0	17
		Lactobacillus	*Lactobacillus* spp.	0	0	0	25	0	0	0	17
		Psychrobacillus	*P. psychrodurans*	0	0	0	0	0	17	0	0
		Staphylococcus	*S. capitis*	0	0	0	0	0	0	0	33
			S. epidermidis	17	0	0	75	50	0	0	17
			S. haemolyticus	0	0	0	0	0	8	0	0
			Staphylococcus spp.	8	0	0	8	8	0	0	8
		Streptococcus	*S. salivarius*	0	0	0	0	0	25	0	0
Proteobacteria	α-Proteobacteria	*Epibacterium*	*E. mobile*	0	0	0	0	0	0	8	0
	β-Proteobacteria	*Ralstonia*	*Ralstonia* spp.	0	0	0	8	0	0	0	25
	γ-Proteobacteria	*Acinetobacter*	*A. calcoaceticus*	0	17	0	0	0	75	0	0
		Bowmanella	*B. denitrificans*	25	8	17	50	8	17	8	42
		Marinobacter	*M. hydrocarbonoclasticus*	50	0	50	0	42	0	8	0
		Motilimonas	*M. eburnea*	0	8	8	17	0	0	0	0
		Pseudoalteromonas	*P. piscicida*	0	0	58	0	0	25	67	0
			P. shioyasakiensis	58	0	8	0	33	0	0	0

(Continues)

TABLE 6 (Continued)

Phylum	Class	Genus	Species	Control				Ozone			
				Before start	Day 2	Day 9	Day 31	Before start	Day 2	Day 9	Day 31
		Pseudomonas	*P. abyssi*	0	0	0	0	0	0	8	0
			P. hussainii	0	17	50	8	8	50	25	0
			P. mangrovi	0	17	25	0	8	42	25	0
			P. mendocina	75	8	0	0	58	0	0	0
			P. pachastrellae	0	0	0	0	8	0	0	0
			Pseudomonas spp.	0	75	42	8	8	33	50	8
		Shewanella	*S. putrefaciens*	8	0	0	0	0	0	0	0
		Vibrio	*V. alginolyticus*	25	**83**	50	8	0	67	42	8
			V. campbelli	0	25	0	0	0	0	0	0
			V. harveyi	**92**	25	50	**83**	**100**	17	**83**	**92**
			V. mytili	0	0	0	0	0	0	25	0
			V. parahaemolyticus	0	67	**100**	**100**	0	**83**	**92**	**100**
			V. rotiferianus	8	42	0	17	0	42	0	17
			V. xuii	0	0	0	8	0	0	0	8
			Vibrio spp.	0	8	25	0	8	58	42	42

Abbreviation: RAS, recirculating aquaculture systems.

TABLE 7 Mean abundances of bacterial species isolated from biofilms of shrimp carapaces from four RAS after treatment with 0 mg ozone/hr (control) and 5–50 mg ozone/hr (redox potential adjusted to 350 mV) before and 2, 9 and 31 days after start of ozone application

Phylum	Class	Genus	Species	Control				Ozone			
				Before start	Day 2	Day 9	Day 31	Before start	Day 2	Day 9	Day 31
Actinobacteria	Actinobacteria	*Actinomyces*	*Actinomyces* spp.	0	0	0	0	0	0	0	8
			Arthrobacter spp.	17	17	25	33	8	17	25	25
		Curtobacterium	*C. herbarum*	0	0	0	50	0	0	0	58
		Frigoribacterium	*F. faeni*	0	0	0	0	0	0	8	0
		Gordonia	*G. rubripertincta*	0	0	0	8	0	0	0	33
			G. terrae	0	17	0	0	0	0	0	0
		Mycobacterium	*M. chelonae*	0	0	0	0	0	0	0	8
		Mycolicibacterium	*M. peregrinum*	0	0	0	8	0	0	0	8
			M. porcinum	0	0	33	0	8	0	25	0
		Nocardia	*N. cyriacigeorgica*	0	0	0	0	0	0	8	0
		Oerskovia	*O. turbata*	0	0	0	0	8	0	0	0
		Sanguibacter	*S. keddieii*	0	0	17	0	0	0	0	0
Bacteriodetes	Flavobacteriia	*Tenacibaculum*	*T. discolor*	0	8	42	0	0	8	33	0
			T. mesophilum	0	50	0	0	0	17	0	0
	Sphingobacteriia	*Pedobacter*	*P. jejuensis*	0	0	0	0	0	0	0	8
Firmicutes	Bacilli	*Bacillus*	*B. arsenicus*	0	8	0	25	0	0	0	0
			B. cereus	0	0	0	0	0	0	0	17
			B. loiseleuriae	0	0	0	0	8	0	0	0
			B. mycoides	0	0	0	0	25	0	0	0
			B. psychrosaccharolyticus	0	0	0	0	25	0	0	0
			B. pumilus	0	0	0	0	0	0	8	0
			B. thuringiensis	0	0	0	0	8	0	0	0

(Continues)

TABLE 7 (Continued)

Phylum	Class	Genus	Species	Control				Ozone			
				Before start	Day 2	Day 9	Day 31	Before start	Day 2	Day 9	Day 31
			B. toyonensis	0	0	0	0	0	0	8	0
			Bacillus spp.	0	0	0	0	0	8	50	0
		Brevibacillus	*B. porteri*	0	0	0	0	0	0	8	0
		Lactobacillus	*Lactobacillus* spp.	0	0	0	17	0	0	0	25
		Paenibacillus	*P. amylolyticus*	0	0	0	8	0	0	0	0
		Staphylococcus	*S. aureus*	0	0	0	0	0	0	17	0
			S. capitis	0	0	0	42	0	0	0	58
			S. epidermidis	75	0	0	17	**92**	0	0	17
			S. haemolyticus	0	0	0	0	0	0	0	8
			S. saprophyticus	0	0	0	0	8	0	0	0
			S. warneri	0	0	0	25	0	0	0	0
			S. xylosus	0	0	0	0	0	0	8	0
			Staphylococcus spp.	8	0	0	8	17	0	0	0
		Streptococcus	*S. salivarius*	0	8	0	0	0	0	0	0
Proteobacteria	α-Proteobacteria	*Epibacterium*	*E. mobile*	0	0	8	8	0	0	0	0
		Ruegeria	*R. atlantica*	8	0	0	0	0	0	0	0
		Sulfitobacter	*S. faviae*	0	0	0	0	0	25	0	0
		Thalassospira	*T. xiamenensis*	0	0	0	0	8	0	0	0
	β-Proteobacteria	*Ralstonia*	*Ralstonia spp.*	0	0	0	0	0	0	0	25
	γ-Proteobacteria	*Acinetobacter*	*A. calcoaceticus*	0	17	0	0	0	0	0	0
		Alteromonas	*A. abrolhosensis*	0	0	0	0	8	0	8	8
		Bowmanella	*B. denitrificans*	33	17	17	58	17	42	17	58
		Marinobacter	*M. hydrocarbonoclasticus*	58	25	8	0	50	0	0	0
		Marinomonas	*M. ostreistagni*	0	0	8	0	0	0	0	0
		Motilimonas	*M. eburnea*	0	25	0	0	0	0	0	0
		Pantoea	*P. agglomerans*	0	0	0	17	0	17	0	0
		Pseudoalteromonas	*P. piscicida*	0	0	**83**	25	0	0	25	0
			P. shioyasakiensis	33	0	0	0	33	0	0	0
		Pseudomonas	*P. abyssi*	0	0	33	0	0	0	17	0
			P. hussainii	0	33	8	0	0	67	25	17
			P. mangrovi	17	8	17	0	0	42	33	0
			P. mendocina	**83**	8	0	0	33	0	0	0
			P. pachastrellae	8	0	0	0	8	0	0	0
			Pseudomonas spp.	8	8	42	0	0	17	33	0
		Shewanella	*S. putrefaciens*	8	0	0	0	0	0	0	0
		Stenotrophomonas	*Stenotrophomonas* spp.	0	25	0	0	0	67	0	0
		Vibrio	*V. alginolyticus*	0	33	8	0	0	0	0	0
			V. campbelli	0	8	0	0	0	0	0	0
			V. harveyi	33	58	25	75	17	**92**	42	**100**
			V. hepatarius	0	0	17	0	0	0	0	0
			V. mytili	0	0	0	0	0	0	8	0
			V. orientalis	0	0	0	8	0	0	0	0
			V. parahaemolyticus	0	50	33	42	0	33	33	33
			V. rotiferianus	0	33	0	17	0	25	0	0
			Vibrio spp.	0	50	58	42	0	42	58	50

Abbreviation: RAS, recirculating aquaculture systems.

TABLE 8 Mean abundances of bacterial species isolated from the abdominal cavities from shrimp kept in four RAS after treatment with 0 mg ozone/hr (control) and 5–50 mg ozone/hr (redox potential adjusted to 350 mV) before and 31 days after start of ozone application

				Control		Ozone	
Phylum	Class	Genus	Species	Before start	Day 31	Before start	Day 31
Actinobacteria	Actinobacteria	*Actinomyces*	*Actinomyces* spp.	0	0	0	17
			A. humicola	0	8	0	0
			Arthrobacter spp.	8	50	8	42
		Curtobacterium	*C. herbarum*	0	33	0	17
		Frigoribacterium	*F. faeni*	0	25	0	0
		Microbacterium	*M. liquefaciens*	0	8	0	8
		Oerskovia	*O. turbata*	0	0	8	0
Firmicutes	Bacilli	*Bacillus*	*B. cereus*	0	17	0	25
			B. mycoides	8	0	33	0
			Bacillus spp.	0	0	0	8
		Exiguobacterium	*E. sibiricum*	0	8	0	0
		Lactobacillus	*Lactobacillus* spp.	0	33	0	33
		Staphylococcus	*S. capitis*	0	33	0	42
			S. epidermidis	**92**	25	**92**	42
		Streptococcus	*Streptococcus* spp.	8	0	0	0
Proteobacteria	β-Proteobacteria	*Ralstonia*	*Ralstonia* spp.	0	0	0	33
	γ-Proteobacteria	*Bowmanella*	*B. denitrificans*	50	58	33	58
		Halomonas	*H. meridiana*	0	8	0	0
		Marinobacter	*M. hydrocarbonoclasticus*	33	0	17	0
		Motilimonas	*M. eburnea*	17	0	0	0
		Pantoea	*P. agglomerans*	0	0	0	25
		Pseudoalteromonas	*P. shioyasakiensis*	42	0	25	0
		Pseudomonas	*P. mangrovi*	8	0	8	0
			P. mendocina	25	0	50	0
			Pseudomonas spp.	8	0	0	0
		Vibrio	*V. harveyi*	8	50	8	50
			V. parahaemolyticus	0	33	0	42
			V. rotiferianus	8	8	0	0
			Vibrio spp.	0	25	0	67

Abbreviation: RAS, recirculating aquaculture systems.

adding to the tanks probably due to the increased amount of dissolved oxygen in the water (Putro, Adityarini, & Chiang, 2018). In European lobster, ozone application provided significantly higher survival rates in larviculture compared to UV irradiation, although the authors stated that a direct comparison between both methods might not be possible (Middlemiss et al., 2015). In the present study, only slight differences in the performance of the shrimp were seen between both methods. In the UV irradiation experiment, the shrimp from the RAS treated with UV irradiation at a power of 9 W were gaining slightly less body weight compared to the shrimp from both other treatment groups. Shrimp from the UV 9-treated RAS nevertheless were significantly heavier at the start of the experiment compared to shrimp from the other treatment groups, and therefore, this result has to be interpreted carefully. In the ozone experiment, the shrimp were slightly shorter ($p = .015$) compared to those of the control group after 31 days. As both experiments were running only for a short period of time, these observations nevertheless might not be valid.

Differences in the impact of water treatment measures on chemical and microbiological water parameters were seen in the present study between disinfection by UV irradiation and ozone application. Regarding the chemical water quality, UV irradiation led to a reduced amount of ammonia in the water of RAS only when UV reactors with a power of 9 W were used and no reducing effect was seen for the concentrations of nitrite and nitrate. Ozone application on the other hand prevented a nitrite increase and led to a faster decrease of nitrate compared to control RAS. This benefit of ozone is widely known and makes ozone even more effective in RAS than UV irradiation (Honn &

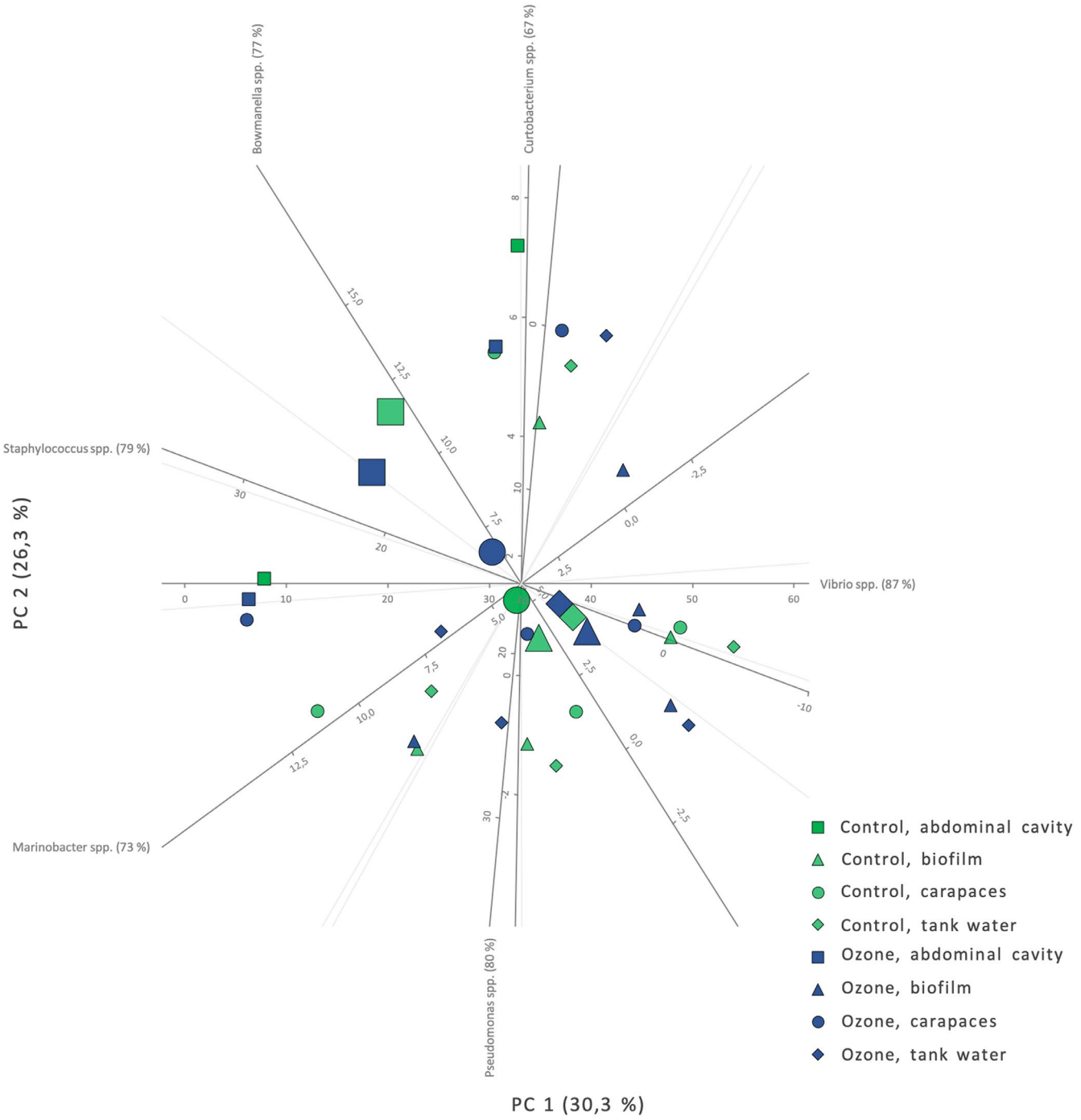

FIGURE 9 Principal component analysis (PCA) of the composition of the bacterial microflora from the biofilms of tank surfaces, the water, the biofilms from shrimp carapaces and the abdominal cavity from shrimp from six recirculating aquaculture systems (RAS) treated with 0 mg/hr ozone (control) and 5–50 mg/hr ozone (redox potential adjusted to 350 mV) before and 2, 9 and 31 days after start of ozone application. Samples from the abdominal cavities are only shown before and 31 days after start of UV irradiation. The data from the different sampling timepoints are indicated with small symbols and the mean values of the samples from the differently treated RAS are indicated in large, bordered symbols [Colour figure can be viewed at wileyonlinelibrary.com]

Chavin, 1976). In sea water from the Mediterranean Sea with a salinity of around 33‰, UV light at 254 nm decreased the amount of total organic carbon (TOC) by 4.2% (Penru et al., 2013). In the present study, even a decrease by 33.46% in the amount of TOC was seen when the RAS water was treated with a 9 W UV reactor, and additionally, the amount of DOC was reduced significantly, too. After ozone application already on day two, a lower amount of TOC was seen compared with the control RAS, but as no samples were analysed before start of the experiment, it can be assumed but cannot be confirmed that the ozone treatment was causing this decrease.

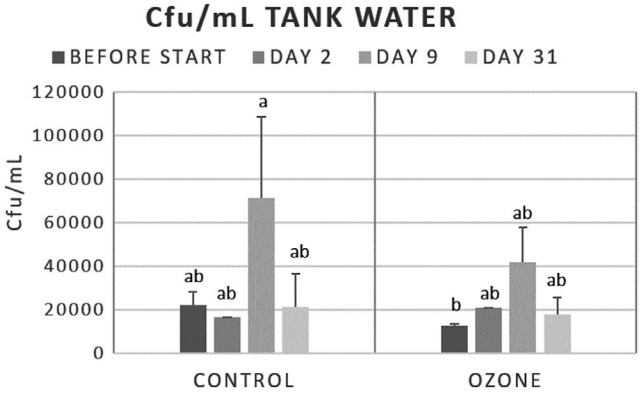

FIGURE 10 Abundance of colony-forming units (Cfu) in the water of six recirculating aquaculture systems (RAS) stocked with *Litopenaeus vannamei* treated with 0 mg/hr ozone (control) and 5–50 mg/hr ozone (redox potential adjusted to 350 mV) before and 2, 9 and 31 days after start of ozone application. Shown are mean values and standard deviations of Cfu from two RAS with two holding tanks each per treatment. The statistically significant differences between the treatments are marked by different letters

The main focus in the present study was laid on the influence of the two disinfection methods on the bacterial community composition in the water, on biofilms of tanks and shrimp carapaces and on the bacteria present in the abdominal cavity of the shrimp. The bacterial community was analysed by cultural techniques followed by molecular biological identification. It is known that not all bacterial species present in the samples might be detected by cultivation methods because not all bacteria are growing on agar plates. This has to be considered when interpreting the data. However, commonly the main bacterial species in aquaculture systems are belonging to the γ-Proteobacteria and are culturable and a representable overview might be possible by the used methods (Jung-Schroers, Adamek, Boley, Korshun, & Steinhagen, 2019).

This analysis indicated that the total bacterial amount in the water could not be decreased significantly over time neither by UV irradiation nor by ozone application. While after application of ozone the bacterial amount in the water stayed on a similar level during the complete experimental duration, in the RAS treated with 7 W UV light the total bacterial amount even increased till the end of the experiment. Since practical conditions were to be tested in the present study, UV irradiation with a power recommended by the manufacturer for the given water volume was tested. By using higher powers of UV irradiation, probably a greater effect on the total amount of bacteria could have been achieved. Therefore, under the present conditions bacterial growth might have had overcompensated the elimination of bacteria by UV irradiation. A conclusion on the efficiency of UV irradiation against bacteria in general is therefore not possible. However, undoubtedly UV irradiation still might have a selective impact on the bacterial community due to different UV sensitivity and doubling times of suspended bacteria. In both RAS treated with 7 W UV light, also the composition of the microflora in biofilms changed to a great extent and differed from those of the control RAS and the 9 W UV-treated RAS. Comparable differences were not detected after the ozone treatment. UV irradiation has a direct impact only on planktonic stages of bacteria suspended in the water. An effect on bacteria organized in biofilms therefore can only be indirect by changing the amount or the composition of planktonic bacteria. Bacterial species show different sensitivity to UV irradiation and by reducing specific species in the water, the amount of these organisms might also be reduced in the biofilms. For other specific species, an increased power of the UV irradiation might be necessary for reduction in the water (Mamane, Colorni, Bar, Ori, & Mozes, 2010). Therefore, in the present study a shift of the population towards specific species that could replicate in high amounts despite the treatment with 7 W UV light might be possible. A resistance of specific bacterial species to UV irradiation could be shown for example in freshwater systems for *Flavobacterium psychrophilum* that requires a fourfold higher UV doses for a 5-log reduction than the typically recommended dose of 30 mJ/cm^2 (Huyben et al., 2018; Sharrer et al., 2005). Additionally, not only the dose of UV irradiation but also the turnover rate relative to the generation time of a specific bacterial species contributed to the efficiency of UV irradiation against a specific organism. There also seem to be different protection kinetics and mechanisms for heterotrophic bacteria for UV irradiation and ozone application. Biotic particles slightly protected bacteria better during UV treatment, but on the other hand, biotic particles in higher amounts reduced the ozone efficacy more than the UV efficiency. Disinfection by UV irradiation seemed not to be more effected by particles than ozone disinfection. There were large differences is inactivation of free-living bacteria versus particle associated bacteria also in ozone disinfection experiments, whereas less differences in the inactivation of free-living versus particle-bound bacteria were seen in 10 UV disinfection experiment (Hess-Erga, Attramadal, & Vadstein, 2008). Other authors still suggest that UV irradiation is limited when bacteria are particle shielded (Huyben et al., 2018). Other water parameters are also influencing the effectiveness of water disinfection methods. Ozone for example showed the best results in reducing the number of *Vibrio* spp. in water for shrimp at a pH of 6.7, whereas at higher or lower pH levels the effect was reduced (Wulansarie, Rengga, & Rustamadji, 2018). In the present study, the pH value was 7.8 and it can be assumed that this was above the optimal range for disinfection of *Vibrio* spp. In a study with European lobster, it could be shown that UV irradiation and ozone application were highly effective at controlling levels of *Vibrio* spp. in the water (Middlemiss et al., 2015). This is in contrast to the present study, where a reduction of *Vibrio* spp. could not be achieved by UV irradiation or ozone application. In general, the composition of the bacterial genera stayed very stable and comparable to the control RAS in the ozone experiment and changed to a larger extent in the UV irradiation experiment. After ozone application, nevertheless a shift in the detected *Vibrio* species was seen. The amount of *V. alginolyticus*, a potential pathogenic bacterium for shrimp that was detected in high amounts before the start of both experiments, decreased significantly over time after treatment with ozone and was not detected anymore on the carapaces of shrimp at the end of the experiment. The amount of *V. parahaemolyticus*, that also has a pathogenic potential for shrimp, increased meanwhile. In the UV irradiation experiment, the amount of *V. alginolyticus* decreased to a far lower extent and was still highly abundant on day 42 in the biofilms

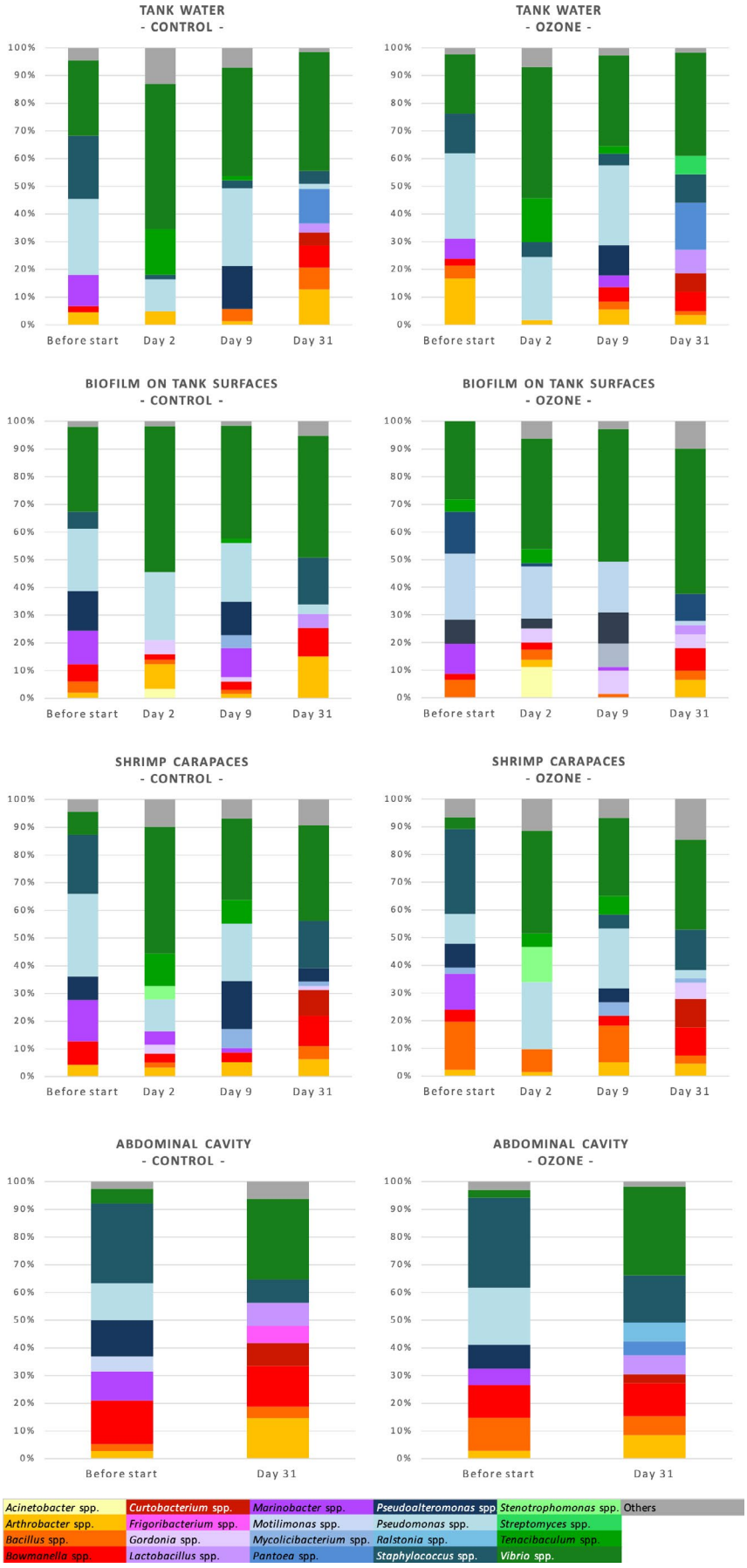

FIGURE 11 Composition of the bacterial microflora in four recirculating aquaculture systems stocked with *Litopenaeus vannamei* and treated with 0 mg/hr ozone (control) and 5–50 mg/hr ozone (redox potential adjusted to 350 mV). Columns represent the composition of the microflora in tank water, biofilms of tank surfaces and shrimp carapaces before and 2, 9 and 31 days after start of ozone application. The composition of bacteria in the abdominal cavities of shrimp is shown before and 31 days after start of ozone application. Shown are the bacterial species that could be detected at least at one sampling timepoint in one of the samples with an abundance of 10% or more. All other bacterial species are summarized under the heading "others." [Colour figure can be viewed at wileyonlinelibrary.com]

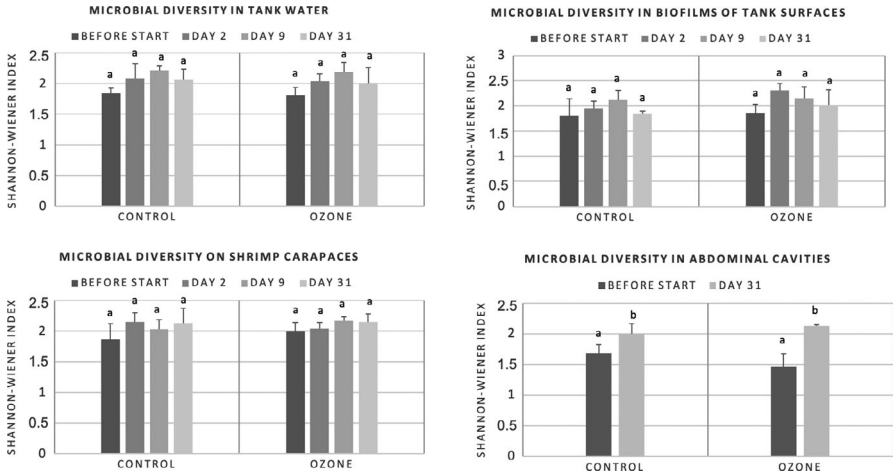

FIGURE 12 Shannon–Wiener indices of diversity describing the composition of the bacterial microflora from four RAS treated with 0 mg/hr ozone (control) and 5–50 mg/hr ozone (redox potential adjusted to 350 mV). Depicted are index values from the recirculating water, the biofilms of tank surfaces and from shrimp carapaces before and 2, 9 and 31 days after start of ozone application. From the abdominal cavity of shrimp, data before and 31 days after start of ozone application are shown. The statistically significant differences between the treatments are marked by different letters

of tank surfaces and on the carapaces of shrimp especially in the RAS treated with 9 W UV light. In this RAS, the amount of *V. parahaemolyticus* increased simultaneously. It is known that *V. alginolyticus* is forming biofilms that are able to survive UV treatments (Snoussi et al., 2009). The results of the present study suggest that *V. alginolyticus* could be eliminated from the RAS by ozone but not by high dosage of UV irradiation. The reason for this different effect on *V. alginolyticus* was probably that a certain amount of these bacteria was organized in biofilms and UV irradiation could only reduce bacteria in the circulating water. This underlines the statements from previous studies, which emphasize that care must be used when determining the effective ozone or UV dose to achieve disinfection because specific pathogens may require much higher dosed for inactivation (Summerfelt, 2003).

It was shown that ozone influenced the dynamic of biofilter communities and stabilizes the bacterial biofilm community and in ozone treated water the amount of γ-Proteobacteria dominated, whereas in non-ozone-treated water the amount of α-Proteobacteria was much higher (Wietz, Hall, & Hoj, 2009). A slightly higher percentual amount of γ-Proteobacteria in the biofilms could also be measured in the present study after ozone treatment, whereas the amount of α-Proteobacteria did not change. In general, the bacterial composition in the RAS changed to a greater extend after UV irradiation but stayed relatively stable and comparable to those of the untreated control RAS after ozone application.

There are also negative effects reported after ozone application or UV irradiation. In the blackhead seabream (*Acanthopagrus schlegelii*), ozone treatment with concentrations of 20 and 40 g ozone/kg feed day^{-1} led to histological changes of gill and liver tissues that were more prominent in fish treated with the higher dose and it was suggested that the ozone dose should not exceed 20 g ozone/kg feed day^{-1} (Kim, Kim, & Park, 2018). In the current study, the application of ozone showed advantages over disinfection by UV irradiation as the chemical water quality could be improved and the microflora in the whole systems stayed more stable. Our former studies on the microbial community in freshwater RAS showed that a stable microflora seems to be very important in order to avoid the rapid growth of potentially fish-pathogenic bacteria (Jung-Schroers et al., 2016, 2018, 2019). Especially, biofilms can act as reservoirs for potentially pathogenic bacteria (King et al., 2004), and therefore, stable biofilms formed by harmless bacterial species, like it was found in the ozone experiment, are believed to reduce the risk of infections caused by opportunistic pathogens. It is known that differences in the microbial community in RAS cannot only be seen due to disinfection but also due to water exchange and reduced feed (Jung-Schroers et al., 2019; Teitge, Peppler, Steinhagen, & Jung-Schroers, 2020). General management strategies in RAS are therefore often at least as important as disinfection measures and as long as a system is well protected against invasion of pathogens from outside sources it is doubtful, whether disinfection within the system itself is useful (Blancheton, Attramadal, Michaud, d'Orbcastel, et al., 2013). These general measures are often even more suitable for prophylaxis treatment of disease outbreaks than water disinfection. The combination of ozone application followed by UV irradiation was shown to be even more effective compared to the use of only one method alone in reducing bacteria counts in freshwater aquaculture systems, and it was suggested that an effective disinfection of water for RAS was possible in this way (Sharrer & Summerfelt, 2007). In the present study, a combined effect of UV irradiation and ozone application was not tested but further studies in brackish water RAS for shrimp should analyse these combined effects.

In conclusion, it could be shown that the use of ozone in brackish water RAS for Pacific white shrimp has beneficial effects on chemical parameters and stabilizing effects in the microbial water quality. UV irradiation in contrast was less effective in optimizing the chemical water quality and led to more significant changes in the microbial composition. This might have an influence on the stability of the physiological microflora over time. Because of these results and as

no negative effects in the animals were seen after ozone application, its use in shrimp RAS seems to be recommendable.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interests.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article. Additional data are available from the corresponding author upon reasonable request.

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