

Effect of disinfection with peracetic acid on the microbial community of a seawater aquaculture recirculation system for Pacific white shrimp (*Litopenaeus vannamei*)

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Abstract

When tropical shrimps are kept in recirculating aquaculture systems (RAS), one of the limiting factors is the maintenance of a sufficient water quality, and therefore, often disinfectants like peracetic acid (PAA) are added to the water either as prophylactic or treatment measure. In this study, PAA in concentrations of 0.1 mg/L, 1 mg/L and 10 mg/L was applied continuously for 56 days to small-scale seawater RAS stocked with *Litopenaeus vannamei*. Treatment with 0.1 mg/L did not result in a reduction in the total bacterial amount and therefore was not effective. A concentration of 10 mg/L led to significant changes in the chemical water parameters already after 2 days and was therefore not recommendable. A concentration of 1 mg/L led to increased levels of ammonia and nitrite within 2 days and to a significant increase in the bacterial amount in the water, most probably due to an enhanced growth of heterotrophic bacteria. The microflora showed significant fluctuations, and there were indications that the welfare of the shrimps was affected. Using 1 mg PAA/L for prophylactic use is therefore also not recommendable but might be an alternative option for short-term treatment in cases of disease outbreaks.

KEYWORDS

bacterial community, disinfection, microflora, Pacific white shrimp, peracetic acid

1 | INTRODUCTION

During the past few years, the production of Pacific white shrimp (*Litopenaeus vannamei*) has increased in European countries due to the possibility of keeping these animals, independent from natural sea water, on inland farms in recirculating aquaculture systems (RAS) (Bauer et al., 2018). The primary advantages of keeping shrimps in RAS are a reduced consumption of water, a low environmental impact and the possibility of high stocking densities and therefore high productivity. Challenges that occur when tropical shrimp are kept in northern Europe are maintaining a high water temperature

and an appropriate salinity. Pacific white shrimp are very tolerant against low and moderate salinity levels (Bray, Lawrence, & Leungtrujillo, 1994; Jayasankar et al., 2009) so that RAS can be operated at 10 to 13 ‰ salinity, which lowers the cost for artificial sea salt and reduces the pollution of the wastewater caused by high salinity. In northern European countries, waste heat from biogas plants can be used for heating the water in RAS so that the production of shrimp in these systems is a sustainable option for the local production of high-quality food. Due to the current development, fresh marine shrimp can be offered to customers in areas far away from the sea.

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Nevertheless, 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). By choosing specific pathogen-free post-larvae for stocking these systems, the infection risk for viral pathogens can be reduced. However, due to the high stocking densities and the large amount of organic material in recirculating water from non-utilized feed and faeces, very high numbers of bacteria in water and on all surfaces of the system might occur (Bauer et al., 2018). Additionally, the accumulation of micro-particles in the water leads to an increased growth of bacteria on these solids (Wold et al., 2014). Most bacteria present in RAS have only a low pathogenic potential for shrimp but might cause heavy losses under suboptimal farming conditions. Bacteria in the system attach to every possible surface, including the walls of the tanks, the filters and materials inside the filters and also to the surface of the animals. Bacteria from the surrounding water are colonizing the carapaces and the gills of shrimp. When these bacteria are taken up by shrimp, they may even colonize the intestine. For fish, it is known that a dense population of bacteria from the physiological microflora on mucosal surfaces acts as an important component of the external infection barrier (Balcazar et al., 2006) and the same might be true for the surface of shrimp. Also in shrimp, a diverse and stable microflora might therefore prevent bacterial infections. Nevertheless, within this physiological bacterial microflora, also potentially harmful organisms might occur. Under suboptimal conditions, the colonization of surfaces with potentially pathogenic bacteria can be the starting point of bacterial infections (Abraham, Sharon, & Ofek, 1999). In marine aquaculture facilities, especially high diversity of *Vibrio* spp. can be found. Among these bacteria, there are also potential pathogenic species that might induce disease (Bauer et al., 2018).

In RAS for fish production, different approaches are used to reduce the risk of bacterial infections. One approach relies on removing bacteria from the water to achieve a reduction in bacterial numbers on fish mucosa as well. With this aim in mind, physical and chemical methods are used to reduce the total amount of bacteria in the water. One of the most frequently used chemical substances for reducing possible pathogens in aquaculture systems is peracetic acid (PAA), which is considered as highly efficient against pathogens and environmentally friendly (Kitis, 2004). Due to its low molecular weight, PAA is able to pass through outer membranes of bacteria and react with internal cell components leading to cell damage and release of cellular components (Finnegan et al., 2010). Thus, the substance is able to reduce all bacteria in the system in a very non-specific manner. PAA was used in experimental studies as a prophylactic measure over long-term periods to reduce the total amount of bacteria generally or as an alternative treatment for specific bacterial pathogens, like *Flavobacterium* spp., *Aeromonas salmonicida* or *Yersinia ruckeri* to reduce antibiotic use (Liu, Pedersen, Straus, Kloas, & Meinelt, 2017; Liu, Straus, Pedersen, & Meinelt, 2018; Marchand et al., 2012; Meinelt et al., 2015). Most research on PAA was carried out in freshwater systems, and it is known that higher salinity levels accelerate the degradation of PAA. Obviously, especially due to the effect of ions like Ca^{2+}

or Mg^{2+} , in sea water, the degradation of PAA is faster than in pure NaCl-solutions. Furthermore, it was assumed that a lower Mg^{2+} and Ca^{2+} ratio compared with the Na^+ and K^+ ratio forces a faster degradation (Liu, Steinberg, Straus, Pedersen, & Meinelt, 2014).

In aquaculture, there are two different strategies used to apply PAA products: pulse applications, short-term treatments using high concentrations of PAA, up to several times per day with concentrations of 1–2 mg PAA/L or continuous applications of concentrations below 0.2 mg PAA/L (Liu, Straus, Pedersen, & Meinelt, 2017). In fish aquaculture systems, it could be shown that pulse application inhibits biofilm formation in the tanks, whereas continuous application of PAA promotes biofilm formation (Liu, Straus, et al., 2017). By pulse application therefore, the amount of bacteria in the system could be reduced and stable biofilms were prevented. On the other hand, it is known that stable biofilms are important for the stability of the whole system and that chemical or physical treatments influence not only the amount but also the composition of the bacterial microflora in the system (Blancheton, Attramadal, Michaud, d'Orbcastel, & Vadstein, 2013; Gullian, Espinosa-Faller, Nunez, & Lopez-Barahona, 2012; Wietz, Hall, & Hoj, 2009). This might lead to a selection of bacteria that are more resistant to the used substance or method and therefore might destabilize the physiological microflora. Also, such unstable microflora favours colonization by fast-growing “r-strategist” populations, which comprises most pathogenic bacteria (Attramadal et al., 2014; Skjermo, Salvesen, Oie, Olsen, & Vadstein, 1997; Skjermo & Vadstein, 1999). Another approach to prevent clinically relevant bacterial infections is therefore the stabilization of the physiological microflora by not disturbing the composition of the bacterial flora. This approach aims at a stable and diverse microflora where different bacterial species occupy practically all available ecological niches so that pathogenic bacteria are impeded from asserting themselves within the system. It could be shown that in a stable microbial environment characterized by high amounts of slow-growing K-strategists and low amounts of rapid growing opportunistic pathogenic r-strategists, fish survival rates were increased (Attramadal et al., 2014; Skjermo et al., 1997). It can be assumed that this is transferable to shrimp aquaculture as well.

Most research activities were performed in aquaculture systems for fish. However, no systematic evaluation is available on the effect of PAA in seawater shrimp aquaculture. In the present study, therefore, the effect of different concentrations of PAA on the microflora in RAS for *L. vannamei* was tested. As the microflora should be kept as stable as possible despite the PAA treatment, a continuous application of PAA was performed.

2 | MATERIAL AND METHODS

2.1 | Examinations on toxic levels of PAA for *L. vannamei*

To determine the PAA concentrations for use in the main experiment, four pretests were performed to examine toxic levels of PAA

for *L. vannamei*. In a first experiment, 59-day-old animals ($n = 125$) and, in a second experiment, 21-day-old animals ($n = 155$) were used. Both experiments were performed in 12 plastic aquaria with a water volume of two litres each. In the first experiment, 10 shrimps and, in the second experiment, 12 shrimps were placed in each of the aquaria. In the first experiment, the water was adjusted to a salinity of 10‰ and a temperature of 30°C, and in the second experiment, to a salinity of 30‰ and a temperature of 30°C. Each of the aquaria was aerated and equipped with an identical amount of plastic fibre as holding material for the shrimp. In both experiments, PAA at concentrations of 1 mg/L, 0.1 mg/L or 0.01 mg/L was put in three of the aquaria in order to perform the test in triplicate. In every experiment, three control tanks remained untreated. After adding PAA to the water, the shrimps were kept in the aquaria for a period of 10 hr.

In third and fourth experiments, the same experimental set-ups were used (experiment 3: 67-day-old animals ($n = 125$), salinity 10‰, water temperature 30°C; experiment 4: 31-day-old animals ($n = 155$), salinity 30‰, water temperature 30°C). The PAA concentrations were changed to 1 mg/L, 10 mg/L and 100 mg/L, respectively, and three tanks remained untreated as controls. Observation time was prolonged to 12 hr after applying PAA.

2.2 | Influence of different concentrations of PAA on bacterial growth

In order to test the effectiveness of PAA against bacteria, one isolate each of *Aeromonas hydrophila*, *Pseudomonas fluorescens* and *Vibrio parilis* was used. All isolates were incubated on three Columbia sheep blood agar plates (Oxoid) for 24 hr at 25°C. The bacteria were dissolved homogeneously in veal infusion broth, and the optical density of each suspension was adjusted to 0.2. The bacterial suspensions were aliquoted and mixed with PAA concentrations of 1 mg/L, 0.1 mg/L and 0.01 mg/L, respectively. PAA was diluted in a 0.9% saline solution (NaCl). As negative control, veal infusion broth mixed with the same concentrations of PAA was used. After a 24-hr incubation period at 25°C, a 10-step dilution series with dilution steps of 1:10 each was made with the incubated culture media. Each of these dilutions was spread out in triplicate on Columbia sheep blood agar plates. Bacterial colonies were counted after a 48-hr incubation period at 25°C.

The experiment was repeated with *Vibrio parilis* incubated with PAA concentrations of 4, 40, 400, 4,000 and 40,000 mg/L under the same conditions.

2.3 | Recirculating aquaculture systems

The main experiment was performed in four laboratory-scaled RAS. Each RAS consisted of three keeping tanks with 100 L volume each and one reservoir tank for all technical devices with a volume of 150 L. In the reservoir tank with a pump that ensured a constant water circulation in the entire RAS, filter material, a skimmer and

aerators were placed. All four RAS were maintained with the addition of food for six weeks before shrimp were introduced into the holding tanks. Water temperature was adjusted to 30°C and water salinity to 30‰.

2.4 | Shrimps

The RAS were stocked with post-larvae from *L. vannamei* (PL 12, approx. 12 days old), and 230 shrimp were added to each holding tank. After a four-week acclimatization period, at the start of the experiment, the shrimp were approximately 39 days old with a mean body weight of 0.14 ± 0.18 g and a mean body length of 2.37 ± 1.37 cm. Automatic feeders (Eheim GmbH & C. KG) installed in the individual tanks allowed the shrimp to be fed on a regular basis. Up until day 9, the daily amount of food was 10.5 g per RAS. This was reduced after day 9, and for the rest of the duration of the experiment, only a total amount of 4.5 g food was dispensed daily per RAS.

2.5 | Application of PAA

The PAA product Wofasteril E400 (Kesla Pharma Wolfen GmbH) was used in the experiment. Three of the RAS were treated with different concentrations of PAA and the fourth RAS served as untreated control. According to the results from the pretest, final PAA concentrations of 0.1 mg/L, 1 mg/L and 10 mg/L should be achieved. However, to avoid strong concentration peaks, the corresponding amount of PAA was added continuously to the tanks using peristaltic pumps. The needed amounts of PAA were calculated as three times the single dose of 0.1 mg/L, 1 mg/L and 10 mg/L per 24 hr. Thus, after adding a single dose of the desired amount of PAA to the RAS (the RAS with a final concentration of 0.1 mg/L received 45 mg/450 L; the RAS with a final concentration of 1 mg/L received 450 mg/450 L; and to the RAS with a final concentration of 10 mg/L: 4,500 mg/450 L were added), the doses needed to maintain this level were added continuously with peristaltic pumps to the RAS (RAS with 0.1 L⁻¹: 5.64 mg/450 L/hr; RAS with 1 mg/L: 56.4 mg/450 L/hr; RAS with 10 mg/L: 564 mg/450 L/hr). The concentrations of PAA in the three RAS were controlled by analysing samples from the tanks by the DPD method (Liu et al., 2014). The measured amounts of PAA in those samples were for the RAS treated with 0.1 L⁻¹: 0.16 ± 0.06 mg/L; for the RAS treated with 1 mg/L: 0.25 ± 0.12 mg/L; and for the RAS treated with 10 mg/L: 8.75 ± 2.88 mg/L.

2.6 | Experimental design

During the experiment, samples were taken six days before adding PAA and two, nine, 29 and 56 days after commencing PAA application. Chemical water parameters (pH, NH₄⁺, NO₂⁻, NO₃⁻) were measured daily for the first 2 weeks of the experiment and at each of the

additional sampling time-points. To assess the effect of PAA on the bacterial microflora in the RAS at each sampling time-point, water samples from each tank, swabs from the biofilm of the surface of each tank and swabs from the transition from the carapax to the abdominal segments of three shrimp specimens per tank were taken. Additionally, swabs from the abdominal cavity of three shrimp specimens per tank were taken at the first and the last sampling time-points. For this, the shrimp were killed individually in a 1 L plastic aquarium with iced water at a temperature of $0 \pm 1^\circ\text{C}$. 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 were completely surrounded by iceless water. Shrimp were left in iced water for at least two minutes. The abdominal cavity was then opened with a sterile scalpel and sampled with a swab.

To investigate the total amount of bacteria in the water samples, dilution series with sterilized water of the same salinity were prepared from undiluted samples to a dilution level of 10^{-5} and each dilution was spread on two sheep blood agar plates containing 30‰ 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, and the amount of CFU per ml of tank water was calculated. The amount of morphologically different CFUs 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 30‰ artificial sea salt. After a 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 tanks surfaces, the carapaces and the abdominal cavities of shrimp specimens were plated on blood agar plates containing 30‰ 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 amount of bacterial colonies was assessed semi-quantitatively. On average, subcultures of bacteria were prepared after one day of incubation. Afterwards, from macroscopically different colonies, one colony was picked with a loop and plated and then fractionated on a separate blood agar plate. One day later, the subcultures were checked for purity and were stored at -80°C in 2 ml of veal infusion broth until further analysis for the bacterial species.

2.7 | 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, Hilden, Germany), 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, $63\text{--}58^\circ\text{C}$ 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 with known 16S rRNA sequences using the online databases EzBioCloud (<http://www.ezbiocloud.net>) and Blast (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

2.8 | 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, and 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).

2.9 | 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 on ranks was performed, followed by an all-pairwise multiple comparison procedure. The Tukey test was used for comparing groups with an equal number of data, and 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 for

the data on the microbial community in respect to their relationship between different treatment groups and different sample types.

3 | RESULTS

3.1 | Pretests

3.1.1 | Toxicity of PAA for *L. vannamei*

None of the shrimp died in any of the four test groups treated with PAA concentrations of 0.1 up to 100 mg/L. In addition, no changes in the behaviour of the animals could be recognized. It could be concluded that for a 12-hr period, PAA concentrations of up to 100 mg/L are tolerable for *L. vannamei*.

3.1.2 | Influence of PAA on bacterial growth under laboratory conditions

In the first experiment, similar results were obtained for all three tested bacterial isolates. In the control group without PAA, 10^8 CFU/ml were counted for all three bacterial isolates. No reduction in bacterial growth was observed when PAA at a concentration of 0.1 mg/L was added to the bacterial suspensions. A reduction to 10^7 or 10^4 CFU/ml was seen when the bacterial suspensions were treated with a PAA concentration of 1 mg/L or 10 mg/L.

In the second experiment with *Vibrio parilis*, the highest amount of bacteria could be detected in the untreated control group (1.05×10^9 CfU). Treatment with a PAA concentration of 4 mg/L only led to a very slight reduction in bacterial growth (1.02×10^9 CfU). However, after a treatment with a PAA concentration of 40 mg/L a significant reduction in bacterial growth to 9.55×10^8 CfU was observed. No bacterial growth at all could be detected at all PAA concentrations above 40 mg/L.

3.2 | Main experiment

3.2.1 | Animals

In all four RAS, the shrimp grew and gained weight over the experimental period. The animals treated with a PAA concentration of 0.1 mg/L were statistically significantly smaller at the end of the experiment compared with the animals in the control group and the group treated with a PAA concentration of 1 mg/L. Animals from the group treated with a PAA concentration of 0.1 mg/L were also lighter in weight compared with those from the control group, and also, their antenna length was shorter compared with both other groups (Figure 1). The relation between antenna length and body length at day 56 was statistically significantly smaller for those

animals treated with a PAA concentration of 1 mg/L compared with those animals in the control group.

3.2.2 | Water chemistry

The concentration of the nitrogen compounds, ammonia and nitrite in recirculating water remained relatively stable in the untreated control RAS and in the RAS treated with a PAA concentration of 0.1 mg/L. In the RAS treated with PAA concentrations of 1 mg/L and 10 mg/L, two days after commencing PAA application, a statistically significant ($p = <.05$) increase in the concentration of ammonia and nitrite could be measured. To avoid negative effects on the shrimp, in all four RAS, the water was changed completely at this time-point and the amount of feed given was reduced for the rest of the experiment from a daily amount of 10.5 g per RAS to one of 4.5 g per RAS. At this time-point, the PAA addition was suspended to the RAS treated with a PAA concentration of 10 mg/L and the RAS was removed from the experiment in order to avoid shrimp losses. At day 29 post-start of PAA addition, statistically significantly higher concentrations of ammonia and nitrite were again measured in the RAS treated with 1 mg/L ($p = <.05$) compared to the control RAS and the RAS treated with a PAA concentration of 0.1 mg/L (Figure 2). As expected in a running system without a denitrification unit, the nitrate levels increased during the experimental period in all RAS, whereas this increase was highest in the control group. As similarly described for other seawater RAS as well, the pH value decreased continuously during the course of the experiment and the most highly significant changes were detected in the RAS treated with PAA at a concentration of 1 mg/L (Figure 2).

3.2.3 | Bacterial community

A taxonomic characterization of the bacterial community was performed by cultivating bacterial species. In this analysis, a total of 91 bacterial species were isolated from all examined samples (Table 1). The greatest number of bacterial species was isolated from the carapaces of the shrimp and from water samples ($n = 61$). Almost the same number of bacterial species was found in the samples from the biofilms from the tanks ($n = 56$) while the lowest number of bacterial species was found in the samples from the abdominal cavity of the shrimp ($n = 34$) (Table 1–4). The detected bacterial species belonged to 34 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 distribution of the different phyla was similar in all groups before application of PAA but changed with time after commencing PAA application. 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

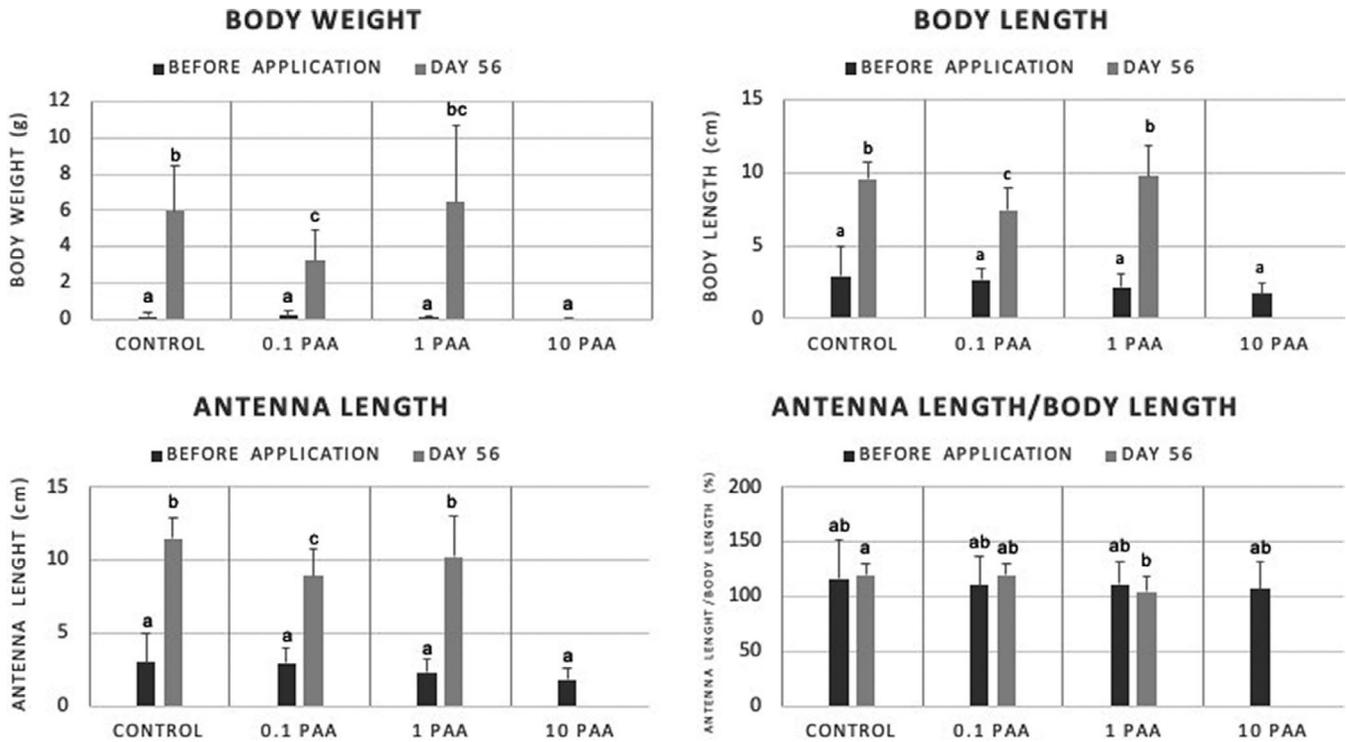


FIGURE 1 Body weight (g), body length (cm), antenna length (cm) and relation between antenna length and body length (%) of *Litopenaeus vannamei* before and 56 days after continuous application of different amounts of peracetic acid (PAA). The statistically significant differences between the treatments are marked by different letters

each treatment group in samples from water, biofilms and carapaces, the closest relationship was seen between the samples from the control group and the samples from the group treated with a PAA concentration of 0.1 mg/L. Compared with this, the microbial composition of the samples from the group treated with a PAA concentration of 1 mg/L differed to a larger extent from the composition in the control group, and samples from the group treated with a PAA concentration of 10 mg/L differed the most to all other samples (Figure 3). For the samples taken from the abdominal cavity, no clear pattern could be recognized. Nevertheless, samples from shrimp from the RAS treated with a PAA concentration of 10 mg/L could not be included in this analysis (Figure 3).

Bacterial community in recirculating water

Total number of bacteria in recirculating water. The bacterial load of recirculating water was assessed in the four RAS by bacterial culturing. Before applying PAA, no differences in the bacterial load in the water were detected (Figure 4). Up until day two after commencing the application, the amount of bacteria in the water increased in the control RAS as well as in the RAS treated with a PAA concentration of 1 mg/L. Due to the large water exchange and the reduced feeding, the bacterial amount in all RAS was significantly reduced in all three remaining RAS up until day 9 after commencing PAA application. At the following sampling points, an increase in bacteria in the water could be detected that was lowest in the control RAS and highest in the RAS treated with a PAA concentration of 1 mg/L (Figure 4).

Composition of the bacterial community in water. The bacterial composition in recirculating water changed in a moderate way in all four

RAS during the experimental period. *Vibrio* sp. was the most abundant genus in all four RAS at almost every sampling day and represented around 40%–60% of all isolated bacteria (Figure 5). After exchanging a large proportion of water and reducing the amount of feed given to the shrimps at day 9 after commencing PAA application, the bacterial composition changed in all three remaining RAS (control and PAA concentrations of 0.1 mg/L and 1 mg/L). Then, higher numbers of *Citrobacter* sp. could be detected. Whereas the composition in the control RAS and in the RAS treated with a PAA concentration of 0.1 mg/L was similar at all sampling days, in the RAS treated with a PAA concentration of 1 mg/L, a reduced number of bacterial species and a very high amount of *Vibrio* sp. (90% of all bacteria identified) were found. In contrast, two days after PAA application in the RAS treated with a PAA concentration of 10 mg/L, a reduced number of *Vibrio* sp. (27% of all detected bacteria) and higher amounts of other bacterial species especially *Thalassospira* spp. and *Bacillus* spp. were found (Figure 5). The *Vibrio* species detected in the water samples changed over time in the control RAS and in the RAS treated with a PAA concentration of 0.1 and 1 mg/L. At the beginning of the experiment, mainly *V. alginolyticus* could be detected, whereas at days 29 and 56 after commencing PAA application, higher amounts of *V. harveyi* were isolated (Table 1).

Bacterial community in biofilms on tank surfaces

Bacterial composition in the biofilms on tank surfaces also changed in a moderate way in all four RAS during the experimental period. Like in the water, *Vibrio* sp. was the most abundant genus (Figure 6). The large water exchange before day nine and the reduced feeding

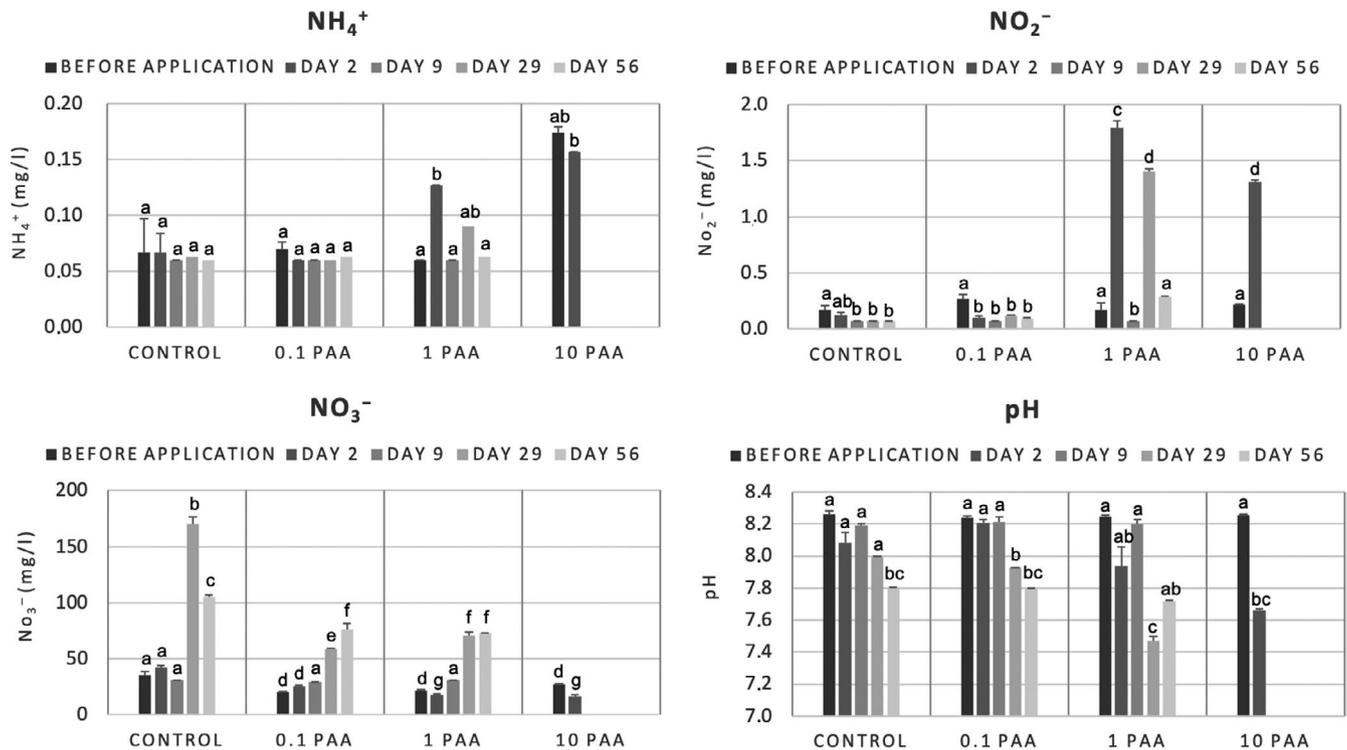


FIGURE 2 Measured amounts of ammonia (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and pH values in four seawater recirculating aquaculture systems stocked with *Litopenaeus vannamei* and treated with different concentrations of peracetic acid (PAA). Shown are measurements before PAA application and 2, 9, 29 and 56 days after continuous application of PAA. The statistically significant differences between the treatments are marked by different letters

of the shrimp changed the composition of the bacterial community in the biofilms in all three remaining RAS to a greater extent. This could be seen in particular at day 9 after commencing PAA application. At this time-point, higher amounts of bacterial species, like *Virgibacillus* spp., were present, which could not be isolated in such large amounts at previous sampling time-points. *Photobacterium* spp. could only be detected in the samples from RAS treated with PAA, especially at days 29 and 56 after application and at higher percentages in the RAS treated with a PAA concentration of 1 mg/L. The largest changes in the composition of the microflora in biofilms were found in the RAS treated with a PAA concentration of 10 mg/L two days after commencing the PAA application. Then, a significant reduction in *Vibrio* sp. from 58% before PAA application to 20% after two days of PAA application and higher percentages of other bacterial species were found (Figure 6). Similar to the composition in the water, the amount of *V. alginolyticus* decreased over time, whereas the amount of *V. harveyi* increased in the control RAS and in the RAS treated with a PAA concentration of 0.1 and 1 mg/L, respectively (Table 2).

Bacterial community on the carapaces of shrimp

The composition of the bacterial community on the surfaces of the carapaces of the shrimp showed the largest differences in all four RAS during the experimental period. *Vibrio* sp. was the most abundant genus also on the carapaces, but to a lesser percentage than in the samples from the water and the biofilms of the tank surfaces. A change in microbial composition was seen again after the large

water exchange and the reduction in food at day 9 after commencing PAA application. Unlike in the community in water and biofilms, the number of different bacterial species did not decrease, but a higher diversity was seen. In the RAS treated with a PAA concentration of 10 mg/L two days after commencing the PAA application, there was not a large change in the bacteria composition as was seen in water and biofilm samples. On the carapaces of the shrimp from the RAS treated with a PAA concentration of 1 mg/L, the number of different bacteria that could be detected was reduced significantly at both later sampling time-points, days 29 and 56 (Figure 7).

Bacterial community in the abdominal cavity of shrimp

In total, the number of different bacterial isolates was much lower in the microflora from the abdominal cavity compared with those from the water or the surfaces of tanks or carapaces. The composition of the microflora in the abdominal cavity from the examined shrimps did not alter greatly between the different treatment groups. Nevertheless, *Photobacterium* spp. could not be detected at the end of the experimental period at 56 days in all three remaining RAS (Figure 8).

3.2.4 | Relationship between the samples from different origins in the four treatment groups

In general, in the three RAS, which were sampled for the entire 56-day experimental period, the microflora in the abdominal cavity

TABLE 1 Mean abundances of bacterial species isolated from tank water from four seawater RAS treated with different concentrations of PAA (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L) before start of the application and 2, 9, 29 and 56 days after start of continuous PAA application

Tank water				Control				
Phylum	Class	Genus	Species	Before PAA	Day 2	Day 9	Day 29	
Actinobacteria	Actinobacteria	<i>Arthobacter</i>	<i>A. ardleyensis</i>	0	0	0	0	
		<i>Brevibacterium</i>	<i>B. casei</i>	0	0	0	0	
		<i>Microbacterium</i>	<i>M. saccharophilum</i>	0	0	0	0	
		<i>Pseudarthrobacter</i>	<i>Pseudoarthrobacter</i> spp.	0	0	0	0	
Bacteroidetes	Flavobacteriia	<i>Tenacibaculum</i>	<i>T. discolor</i>	0	0	11	0	
			<i>T. mesophilum</i>	11	0	0	0	
			<i>Tenacibaculum</i> spp.	0	0	0	0	
Firmicutes	Bacilli	<i>Bacillus</i>	<i>B. altitudinis</i>	0	0	0	0	
			<i>B. aquimaris</i>	44	0	0	0	
			<i>B. arsenicus</i>	0	0	0	0	
			<i>B. cereus</i>	22	0	11	11	
			<i>B. circulans</i>	0	0	0	0	
			<i>B. megaterium</i>	0	0	0	0	
			<i>B. mojavensis</i>	0	0	0	0	
			<i>B. mycoides</i>	0	0	0	0	
			<i>B. vietnamensis</i>	0	0	0	0	
			<i>Bacillus</i> spp.	0	11	0	0	
			<i>Halobacillus</i>	<i>H. trueperi</i>	0	0	0	0
			<i>Paenibacillus</i>	<i>Paenibacillus</i> spp.	0	0	11	0
			<i>Planococcus</i>	<i>P. dechangensis</i>	0	0	0	11
		<i>Psychrobacillus</i>	<i>P. psychrodurans</i>	0	0	0	0	
		<i>Staphylococcus</i>	<i>S. epidermidis</i>	0	0	0	0	
			<i>S. haemolyticus</i>	0	22	0	0	
			<i>S. warneri</i>	0	0	0	0	
			<i>S. xylosus</i>	22	0	0	22	
			<i>Staphylococcus</i> spp.	0	22	11	22	
			<i>Virgibacillus</i>	<i>V. halodenitrificans</i>	0	0	11	0
Proteobacteria	α-Proteobacteria		<i>Nitratireductor</i>	<i>N. kimnyeongensis</i>	0	0	0	0
		<i>Paracoccus</i>	<i>P. hibiscisoli</i>	0	0	0	0	
		<i>Thalassospira</i>	<i>T. indica</i>	0	0	0	0	
			<i>T. xiamenensis</i>	0	0	0	0	
		β-Proteobacteria	<i>Hydrogenophaga</i>	<i>H. palleronii</i>	0	0	11	0
	γ-Proteobacteria	<i>Alcanivorax</i>	<i>A. profundus</i>	0	100	0	0	
			<i>A. venustensis</i>	0	0	0	0	
		<i>Alteromonas</i>	<i>A. abrolhosensis</i>	0	0	0	0	
			<i>A. mediterranea</i>	0	0	0	0	
		<i>Bowmanella</i>	<i>B. denitrificans</i>	0	0	0	0	
		<i>Citrobacter</i>	<i>Citrobacter</i> spp.	0	0	22	0	
		<i>Halomonas</i>	<i>H. aquamarina</i>	0	0	0	0	
		<i>Idiomarina</i>	<i>I. fontislapidosi</i>	0	0	0	0	
			<i>I. loihiensis</i>	0	0	0	11	

Day 56	0.1 PAA					1 PAA					10 PAA	
	Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2
0	0	0	0	0	11	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	11	0	0	0	0
0	0	0	0	0	0	0	0	0	0	11	0	0
0	0	0	0	0	0	11	0	0	0	0	0	0
11	0	0	0	0	0	0	0	67	0	0	0	0
0	44	0	0	0	0	22	0	0	0	0	44	0
11	0	0	0	0	22	0	0	0	0	33	0	0
0	0	0	0	0	0	0	0	0	0	0	0	11
0	22	22	0	11	0	0	11	0	0	0	0	0
0	0	0	0	11	0	0	0	0	0	0	0	0
11	11	0	0	0	0	11	11	11	0	0	22	11
0	0	33	0	0	0	0	22	0	0	0	0	0
0	0	11	0	0	0	0	0	0	0	0	0	11
0	0	0	11	0	0	0	0	0	0	0	0	0
0	0	0	11	0	0	0	0	0	0	0	0	0
0	0	0	0	11	0	0	0	0	0	0	0	0
0	0	0	0	0	44	0	11	0	0	11	0	11
0	0	0	0	0	0	0	0	11	0	0	0	0
0	0	0	0	0	0	0	0	11	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	22	0
67	33	0	0	22	44	0	0	0	0	67	0	0
0	0	22	0	0	0	0	0	0	0	0	0	44
0	0	0	0	0	0	0	0	0	11	0	0	0
0	11	11	0	33	0	22	0	0	0	0	0	0
0	0	11	56	33	11	0	67	56	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	11	0	0	0	0	0	0	0	0	0
0	0	33	0	0	0	0	33	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	44
0	0	0	0	0	0	0	0	0	0	0	0	33
0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	100	0	0	0	0	67	0	0	0	0	11
0	0	0	0	0	33	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	22	0	0
0	0	0	0	0	0	0	22	0	0	0	0	0
0	0	11	0	0	0	0	11	0	0	0	0	0
0	0	0	44	0	0	0	0	22	0	0	0	0
0	0	0	0	0	0	0	0	11	0	0	0	0
0	0	0	22	0	0	0	0	0	0	0	0	0
0	0	0	0	22	0	0	0	0	0	0	0	0

(Continues)

TABLE 1 Continued

Tank water				Control			
Phylum	Class	Genus	Species	Before PAA	Day 2	Day 9	Day 29
		<i>Marinobacter</i>	<i>M. hydrocarbonoclasticus</i>	22	44	11	78
			<i>M. xestospongiae</i>	0	0	0	0
		<i>Photobacterium</i>	<i>P. damsela</i>	67	0	0	0
			<i>P. jeanii</i>	0	0	0	0
		<i>Pseudoalteromonas</i>	<i>P. lipolytica</i>	0	0	0	0
			<i>P. ruthenica</i>	0	0	0	0
			<i>P. shioyasakiensis</i>	0	0	0	0
		<i>Spongiibacter</i>	<i>S. marinus</i>	0	0	0	67
		<i>Vibrio</i>	<i>V. alginolyticus</i>	100	22	89	67
			<i>V. brasiliensis</i>	0	0	0	0
			<i>V. fortis</i>	22	0	0	0
			<i>V. harveyi</i>	0	56	0	89
			<i>V. hepatarius</i>	44	0	0	0
			<i>V. mytili</i>	33	0	0	0
			<i>V. parahaemolyticus</i>	0	11	0	0
			<i>V. pectenicida</i>	0	0	0	0
			<i>V. pelagius</i>	0	0	0	11
			<i>V. rotiferianus</i>	0	100	0	0
			<i>V. xuii</i>	0	56	0	0
			<i>Vibrio</i> spp.	0	33	0	33

Abbreviations: PAA, peracetic acid; RAS, recirculating aquaculture systems.

differed to the greatest extent to that isolated from the water, biofilms on tank surfaces or shrimp carapaces (Figure 9). In the control group, the composition of the bacterial microflora of the water was closely related to the microflora of the biofilms of tank surfaces and the biofilms of the carapaces of shrimp. The composition of the bacteria from the abdominal cavity was more different from those in the water and the closest related to those from the carapaces. After treating the RAS with a PAA concentration of 0.1 mg/L, the largest differences in the bacterial composition were seen between the abdominal cavity and the carapaces of shrimp, whereas the closest relationship was seen between water samples and samples from the biofilms in the tanks. After treatment with a PAA concentration of 1 mg/L, especially the composition of the bacterial community in the water and on the carapaces of shrimp was very similar, and most differences were seen between the microflora of the abdominal cavity of shrimp and the biofilms of tank surfaces. As there was only one sampling day for the RAS treated with a PAA concentration of 10 mg/L after application, no correlations in the microflora of the samples from different origins could be detected for this RAS.

3.2.5 | 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 PAA application in all four RAS and the mean of the Shannon–Wiener indices ranged between 1.65 and 1.89. In water, the tank biofilm and the biofilm on the carapaces, the bacterial diversity remained high during the experiment in the control group and in the group treated with a PAA concentration of 0.1 mg/L. In the latter group, the microbial diversity even increased in the biofilms of tank surfaces compared with the control RAS, reaching a Shannon–Wiener index of 2.33 at the end of the experiment. Larger changes in bacterial diversity were found in the RAS treated with a PAA concentration of 1 mg/L. In this RAS, the diversity in samples from water, biofilms from tank and carapaces was very low especially at day 29 after commencing the application of PAA and decreased to a Shannon–Wiener index of 1. No differences were seen in the bacterial diversity from samples from the abdominal cavities from shrimps of all RAS (Figure 10).

4 | DISCUSSION

In the present study, the effects of PAA at different concentrations on the bacterial microflora were analysed in seawater RAS for Pacific white shrimp, *Litopenaeus vannamei*. Therefore, the bacterial composition in the water, on the biofilms of tank surfaces and on the carapaces of shrimp and in the abdominal cavity was investigated.

Day 56	0.1 PAA					1 PAA					10 PAA	
	Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2
22	33	89	11	33	0	11	22	11	11	11	0	0
0	0	0	0	0	33	0	0	0	0	0	0	0
0	67	0	0	0	0	56	0	0	0	0	100	0
0	0	0	0	44	0	0	0	0	0	0	0	0
0	0	0	0	0	33	0	0	0	0	11	0	0
0	0	0	0	0	0	0	22	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	22	0	0
0	0	0	0	33	0	0	0	0	0	0	0	0
0	78	33	89	33	22	100	44	89	100	0	33	0
0	0	11	0	0	0	0	0	0	0	0	0	0
0	100	0	56	0	0	22	0	0	0	0	56	0
67	33	0	0	89	56	0	33	0	89	78	67	0
0	33	0	0	0	0	33	0	0	0	0	67	0
0	0	0	0	0	0	0	0	22	0	0	0	0
0	56	0	11	0	11	33	0	22	0	0	67	0
44	0	0	0	0	44	0	0	0	0	100	0	0
11	0	11	0	0	0	0	0	0	11	0	0	0
0	0	44	22	0	0	0	78	56	0	0	0	0
0	0	11	0	0	0	0	44	0	0	0	0	0
11	0	11	11	33	22	44	11	0	0	11	89	67

Additionally, the performance of the shrimps and the chemical water quality were analysed.

PAA is widely used in aquaculture systems for fish production either prophylactically as a general water disinfectant with the aim of keeping the animals in the systems healthy (Liu, Pedersen, et al., 2017) or, in the case of a disease outbreak due to bacterial or parasitic infections, to reduce these pathogens. As there are many regulations regarding the use of therapeutic agents for treating animals for human consumption and especially considering a reduction in the use of antibiotics, alternative strategies to maintain the health of aquatic animals in recirculation aquaculture systems are becoming increasingly important. Studies on the application of PAA documented that the substance is environmentally friendly because it degrades to biodegradable residues (Kitis, 2004). PAA can reduce the growth of fish-pathogenic bacteria *in vitro* at a dosage of 1 mg/L (Marchand et al., 2012; Meinelt et al., 2015), and it is effective against the parasite *Ichthyophthirius multifiliis* (Meinelt et al., 2009; Meinelt, Richert, Stuber, & Braunig, 2007; Meinelt, Staaks, Staaks, Stueber, & Braunig, 2007). The reducing effect of PAA on the growth of different bacterial species observed *in vitro* could be confirmed in the present study as well because PAA concentrations of 1 mg/L or higher led to a significant reduction in bacterial growth. Most studies on PAA were performed in freshwater aquaculture systems stocked with different fish species.

Nonetheless, PAA could also be an interesting substance for RAS for the cultivation of crustaceans like shrimp in order to maintain optimal water quality or to reduce the abundance of pathogens. For example, it could be shown that adding 10 mg PAA/L can effectively suppress the pathogenicity of the oomycete, *Aphanomyces astaci*, the pathogenic agent in crayfish plague (Jussila, Makkonen, & Kokko, 2011). For fish, PAA seems to be welfare-friendly, as a true habituation of fish to the substance associated with a decrease in the cortisol response after repeated exposure to PAA has been seen (Gesto et al., 2018; Liu, Pedersen, et al., 2017). The toxicity of PAA varied between different fish species, and the mean 24-hr LC₅₀ values ranged from 2.8 to 9.3 mg/L PAA (Straus, Meinelt, Liu, & Pedersen, 2018). Additionally, it could be shown that hydrogen peroxide (H₂O₂), one of the components present in commercially available PAA mixtures, has an additive effect on the toxicity, the mixtures being more toxic at a higher H₂O₂: PAA ratio (Liu, Straus, Pedersen, & Meinelt, 2015). For the planktonic crustacean, *Daphnia magna*, the commercial formulation Wofasteril E 400 showed the lowest toxicity because of a low H₂O₂: PAA ratio compared with other tested formulations (Liu et al., 2015). Wofasteril E 400 was therefore chosen for application in the present study. There are very few data available on the toxicity of PAA on crustaceans in general and even fewer on shrimp. In the signal crayfish (*Pacifastacus leniusculus*), histological alterations especially in the gills were recognized

TABLE 2 Mean abundances of bacterial species isolated from the biofilms of tank surfaces from four seawater RAS treated with different concentrations of PAA (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L) before start of the application and 2, 9, 29 and 56 days after start of continuous PAA application

Biofilm of tank surfaces				Control					
Phylum	Class	Genus	Species	Before PAA	Day 2	Day 9	Day 29	Day 56	
Actinobacteria	Actinobacteria	<i>Brevibacterium</i>	<i>B. casei</i>	0	0	11	0	0	
		<i>Microbacterium</i>	<i>M. saccharophilum</i>	0	0	0	0	0	
Bacteroidetes	Flavobacteriia	<i>Tenacibaculum</i>	<i>T. discolor</i>	0	0	11	0	0	
Firmicutes	Bacilli	<i>Bacillus</i>	<i>B. aquimaris</i>	11	0	0	0	0	
			<i>B. arsenicus</i>	0	0	0	0	0	
			<i>B. cereus</i>	56	89	67	33	0	
			<i>B. circulans</i>	0	0	0	0	0	
			<i>B. licheniformis</i>	0	0	11	0	0	
			<i>B. mojavensis</i>	0	0	0	0	0	
			<i>B. nakamurai</i>	0	0	0	0	0	
			<i>B. velezensis</i>	0	0	0	0	0	
			<i>Bacillus</i> spp.	0	0	11	0	0	
			<i>Facklamia</i>	<i>F. hominis</i>	0	0	0	0	56
			<i>Lysinibacillus</i>	<i>L. xylanilyticus</i>	0	0	0	0	0
			<i>Oceanobacillus</i>	<i>O. oncorhynchi</i>	0	0	11	0	0
			<i>Paenibacillus</i>	<i>Paenibacillus</i> spp.	0	0	11	0	0
			<i>Staphylococcus</i>	<i>S. epidermidis</i>	0	0	0	0	22
				<i>S. warneri</i>	0	0	0	0	0
				<i>S. xylosus</i>	22	0	0	11	0
				<i>Staphylococcus</i> spp.	0	0	0	11	0
			<i>Virgibacillus</i>	<i>V. halodenitrificans</i>	0	0	33	0	0
				<i>V. salarius</i>	0	0	22	33	0
Proteobacteria	α -Proteobacteria	<i>Nitratireductor</i>		<i>N. kimnyeongensis</i>	0	0	0	0	0
		β -Proteobacteria		<i>Hydrogenophaga</i>	<i>H. palleronii</i>	0	0	11	0
			γ -Proteobacteria	<i>Alcanivorax</i>	<i>A. profundus</i>	0	100	0	0
<i>A. venustensis</i>	0	0		0	22	0			
<i>Alteromonas</i>	<i>A. abrolhosensis</i>	0		0	0	0	11		
<i>Bowmanella</i>	<i>B. denitrificans</i>	0		0	0	0	0		
<i>Citrobacter</i>	<i>Citrobacter</i> spp.	0		0	33	0	0		
<i>Halomonas</i>	<i>H. aquamarina</i>	0		0	0	0	0		
<i>Marinobacter</i>	<i>M. hydrocarbonoclasticus</i>	0		11	0	56	33		
	<i>M. xestospongiae</i>	0		0	0	0	0		
<i>Photobacterium</i>	<i>P. damsela</i>	44		0	0	0	0		
	<i>P. jeanii</i>	0		0	0	44	0		
	<i>Pseudoalteromonas</i>	<i>P. lipolytica</i>	0	0	0	0	0		
<i>P. ruthenica</i>		0	0	0	0	0			
<i>P. shioyasakiensis</i>		0	0	0	0	0			

0.1 PAA					1 PAA					10 PAA	
Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	44	0	0	0	0	0	0	0
0	0	0	0	0	0	0	11	0	0	0	0
0	11	0	0	0	11	0	0	0	0	0	11
0	0	0	0	0	0	0	0	0	0	11	0
56	89	56	56	0	56	56	67	0	0	33	22
0	11	0	0	0	0	11	0	0	0	0	33
0	0	0	0	0	0	0	0	0	0	0	0
0	0	22	0	0	0	0	56	0	11	0	0
0	0	0	0	0	0	0	33	0	0	0	0
0	0	33	0	0	0	0	0	0	0	0	0
0	0	0	0	11	11	0	0	0	0	0	0
0	0	0	0	33	0	0	0	0	11	0	0
0	0	0	0	0	0	0	0	0	0	0	78
0	0	0	0	0	0	0	0	0	0	0	0
0	0	22	0	0	0	0	0	0	0	0	0
0	0	0	0	56	0	0	0	0	0	0	0
0	0	0	11	0	0	0	0	0	0	0	0
11	33	11	22	22	22	0	33	0	0	11	22
0	0	0	33	0	0	0	0	0	0	0	22
0	0	22	0	0	0	0	11	0	0	0	0
0	0	0	22	0	0	0	33	0	0	0	0
0	0	0	0	0	0	0	33	0	0	0	0
0	0	56	0	0	0	0	11	0	0	0	0
0	89	0	0	0	0	100	0	0	0	0	44
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	44	0	0	0	0	44	0	0
0	44	0	0	0	0	67	0	0	0	0	33
0	0	56	0	0	0	0	44	0	0	0	0
0	0	33	0	0	11	0	11	0	0	0	0
0	78	0	78	22	0	0	11	33	0	0	78
0	0	0	0	67	0	0	0	0	56	0	0
100	0	0	0	0	0	0	0	0	0	100	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	33	0	0	0	0	0	0	22
0	0	0	0	0	0	11	0	0	0	0	11
0	0	0	22	11	0	0	0	56	56	0	0

(Continues)

TABLE 2 Continued

Biofilm of tank surfaces				Control				
Phylum	Class	Genus	Species	Before PAA	Day 2	Day 9	Day 29	Day 56
		<i>Pseudomonas</i>	<i>P. mendocina</i>	0	0	0	0	0
			<i>P. oryzihabitans</i>	0	0	0	0	0
			<i>P. songnenensis</i>	0	0	0	0	0
			<i>P. stutzeri</i>	0	0	11	0	0
			<i>Pseudomonas</i> spp.	0	0	0	0	0
		<i>Spongiibacter</i>	<i>S. marinus</i>	0	0	0	11	0
		<i>Vibrio</i>	<i>V. alginolyticus</i>	100	100	100	89	0
			<i>V. brasiliensis</i>	0	0	0	0	0
			<i>V. fortis</i>	11	0	22	0	0
			<i>V. harveyi</i>	67	0	0	100	100
			<i>V. hepatarius</i>	0	0	0	0	0
			<i>V. mytili</i>	22	0	33	0	11
			<i>V. parahaemolyticus</i>	22	33	0	0	33
			<i>V. pectenicida</i>	0	0	0	0	0
			<i>V. pelagius</i>	0	0	0	11	0
			<i>V. rotiferianus</i>	0	100	11	0	0
			<i>V. xuii</i>	0	11	44	11	0
			<i>Vibrio</i> spp.	44	78	0	0	0

Abbreviations: PAA, peracetic acid; RAS, recirculating aquaculture systems.

TABLE 3 Mean abundances of bacterial species isolated from the biofilms of shrimp carapaces from four seawater RAS treated with different concentrations of PAA (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L) before start of the application and 2, 9, 29 and 56 days after start of continuous PAA application

Biofilm on carapaces				Control				
Phylum	Class	Genus	Species	Before PAA	Day 2	Day 9	Day 29	
Actinobacteria	Actinobacteria	<i>Arthobacter</i>	<i>Arthobacter</i> spp.	11	0	22	0	
			<i>Brevibacterium</i>	<i>B. casei</i>	0	11	11	0
			<i>Corynebacterium</i>	<i>C. glyciniphilum</i>	11	0	0	0
			<i>Microbacterium</i>	<i>M. saccharophilum</i>	0	0	0	0
			<i>Micrococcus</i>	<i>M. luteus</i>	0	0	0	0
			<i>Streptomyces</i>	<i>S. violaceus</i>	0	0	0	0
Bacteroidetes	Flavobacteriia	<i>Tenacibaculum</i>	<i>T. discolor</i>	0	0	33	0	
			<i>T. mesophilum</i>	11	0	0	0	
			<i>Tenacibaculum</i> spp.	0	0	0	0	
Firmicutes	Bacilli	<i>Bacillus</i>	<i>B. altitudinis</i>	0	0	0	0	
			<i>B. aquimaris</i>	22	0	0	11	
			<i>B. arsenicus</i>	0	0	0	0	
			<i>B. cereus</i>	11	0	11	11	
			<i>B. circulans</i>	0	67	0	0	
			<i>B. licheniformis</i>	0	0	0	0	
			<i>B. megaterium</i>	0	0	0	0	
			<i>B. mojavensis</i>	11	0	0	0	

0.1 PAA					1 PAA					10 PAA	
Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2
0	0	0	0	0	22	0	0	0	0	0	0
0	11	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	11	0	0	0	0	33
0	0	0	0	11	0	11	0	0	0	0	67
0	33	0	0	0	0	0	0	0	0	0	0
0		0	33	0	0	0	0	0	0	0	0
100	67	100	100	44	33	100	100	100	0	56	11
0	22	0	0	0	0	0	0	0	0	0	0
100	0	44	0	0	0	0	11	0	0	67	0
0	0	0	100	33	0	0	0	100	100	11	0
0	0	67	0	0	0	0	0	0	0	0	0
0	0	22	0	0	0	0	44	0	0	22	0
100	67	11	0	67	67	0	11	0	22	11	0
0	0	0	0	100	0	0	0	0	22	0	0
0	78	0	22	0	0	22	0	0	11	0	22
0	44	56	0	0	22	89	44	0	0	0	33
0	78	33	33	0	0	33	44	0	44	0	11
22	67	0	44	33	11	56	33	0	33	44	44

Day 56	0.1 PAA					1 PAA					10 PAA	
	Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2
0	22	0	0	0	0	0	0	0	0	0	11	0
0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	11	0	0	0	0	22	0	0
0	0	22	0	0	0	0	0	0	0	0	11	0
0	33	0	0	0	0	11	0	0	0	0	0	0
22	0	0	11	0	0	0	0	33	0	44	0	0
0	44	0	0	0	0	0	0	0	0	0	33	0
0	0	0	0	0	22	0	0	0	0	11	0	0
0	0	0	0	0	0	0	0	0	0	0	0	11
0	22	11	0	11	0	11	0	0	0	0	11	0
0	0	0	0	0	0	22	0	0	0	0	0	0
22	33	22	22	11	0	0	0	22	0	0	11	0
0	0	11	0	0	0	0	22	0	0	0	0	33
0	0	0	0	0	0	0	0	0	0	0	11	0
0	0	0	11	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0

(Continues)

TABLE 3 Continued

Biofilm on carapaces				Control			
Phylum	Class	Genus	Species	Before PAA	Day 2	Day 9	Day 29
			<i>B. subtilis</i>	0	0	0	0
			<i>B. vietnamensis</i>	0	0	0	0
			<i>Bacillus</i> spp.	11	0	0	0
		<i>Halobacillus</i>	<i>H. trueperi</i>	0	0	11	0
		<i>Lysinibacillus</i>	<i>L. xylanilyticus</i>	0	0	0	0
		<i>Staphylococcus</i>	<i>S. epidermidis</i>	0	0	0	0
			<i>S. petrasii</i>	0	0	0	0
			<i>S. warneri</i>	0	0	0	44
			<i>S. xylosus</i>	0	0	56	0
			<i>Staphylococcus</i> spp.	0	89	0	0
		<i>Streptococcus</i>	<i>Streptococcus</i> spp.	0	0	0	0
		<i>Virgibacillus</i>	<i>V. halodenitrificans</i>	0	0	100	0
			<i>V. salarius</i>	0	0	0	0
Proteobacteria	α -Proteobacteria	<i>Nitratireductor</i>	<i>N. kimnyeongensis</i>	0	0	11	0
		<i>Paracoccus</i>	<i>P. hibiscisoli</i>	0	0	0	0
	β -Proteobacteria	<i>Hydrogenophaga</i>	<i>H. palleronii</i>	0	0	22	0
	γ -Proteobacteria	<i>Aestuariibacter</i>	<i>A. aggregatus</i>	0	0	0	0
		<i>Alcanivorax</i>	<i>A. profundus</i>	0	100	0	0
		<i>Alteromonas</i>	<i>A. abrolhosensis</i>	0	0	0	0
			<i>A. mediterranea</i>	0	0	0	0
		<i>Bowmanella</i>	<i>B. denitrificans</i>	0	22	0	0
		<i>Citrobacter</i>	<i>Citrobacter</i> spp.	0	0	22	0
		<i>Halomonas</i>	<i>H. aquamarina</i>	0	0	0	0
		<i>Marinobacter</i>	<i>M. hydrocarbonoclasticus</i>	22	22	11	89
			<i>M. xestospongiae</i>	0	0	0	11
		<i>Photobacterium</i>	<i>P. damsela</i>	89	0	0	0
			<i>P. jeanii</i>	0	0	0	0
		<i>Pseudoalteromonas</i>	<i>P. lipolytica</i>	0	0	0	0
			<i>P. ruthenica</i>	0	0	0	0
			<i>P. shioyasakiensis</i>	0	0	11	0
		<i>Pseudomonas</i>	<i>P. gallaeciensis</i>	56	0	0	0
		<i>Spongiibacter</i>	<i>S. marinus</i>	0	0	11	56
		<i>Vibrio</i>	<i>V. alginolyticus</i>	67	89	78	11
			<i>V. brasiliensis</i>	0	0	0	0
			<i>V. fortis</i>	44	0	0	0
			<i>V. harveyi</i>	0	0	0	33
			<i>V. hepatarius</i>	67	0	0	0
			<i>V. mytili</i>	22	0	0	0
			<i>V. parahaemolyticus</i>	44	0	0	0
			<i>V. pectenicida</i>	0	0	0	0
			<i>V. pelagius</i>	0	0	0	0
			<i>V. rotiferianus</i>	0	56	11	0
			<i>V. xuii</i>	0	44	11	22
			<i>Vibrio</i> spp.	11	44	0	11

Abbreviations: PAA, peracetic acid; RAS, recirculating aquaculture systems.

Day 56	0.1 PAA					1 PAA					10 PAA	
	Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2	Day 9	Day 29	Day 56	Before PAA	Day 2
100	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	11	11	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	11
0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	11
67	33	0	0	11	0	0	0	0	11	67	67	0
0	0	11	0	0	0	0	22	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0
0	44	56	11	11	0	11	0	11	0	0	0	11
0	0	0	11	0	22	0	100	0	0	11	0	0
0	0	22	0	0	67	0	0	22	0	0	0	0
0	0	0	33	11	0	0	0	33	0	0	0	0
0	0	0	0	11	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	11	0	0	0	0
0	0	0	0	0	0	0	11	0	0	0	0	0
0	0	0	100	0	0	0	0	44	0	0	0	0
0	0	0	0	0	0	0	0	56	0	0	0	0
0	100	0	0	0	0	0	78	0	0	0	0	56
22	0	0	22	0	11	0	0	0	0	0	0	0
0	0	0	0	0	0	0	33	0	0	0	0	0
0	0	56	0	0	0	0	33	0	0	0	0	22
0	0	0	11	0	0	0	0	33	0	0	0	0
0	0	0	11	0	0	22	0	11	0	0	0	0
11	11	100	0	100	11	0	56	11	11	11	0	11
0	0	0	0	22	22	0	0	11	0	0	0	0
0	89	0	0	0	0	67	0	0	0	0	100	0
0	0	0	0	11	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	11	0	0	0	0	0
0	0	0	44	22	33	0	0	11	11	22	0	0
0	11	0	0	0	0	33	0	0	0	0	67	0
0	0	0	22	11	0	0	0	33	11	0	0	0
0	89	0	89	33	11	89	89	89	89	0	44	67
0	0	11	0	0	0	0	0	0	0	0	0	0
0	33	0	0	0	0	56	0	0	0	0	67	0
44	0	0	0	44	67	33	0	0	67	56	0	0
0	56	0	0	0	0	0	0	0	0	0	33	0
0	0	0	0	0	0	11	0	33	0	0	0	0
0	11	0	0	0	0	0	11	0	0	0	44	0
56	0	0	0	0	89	0	0	0	0	100	0	0
11	0	0	0	0	11	0	0	0	0	33	0	22
0	11	0	11	0	0	0	89	11	0	0	0	0
0	0	11	0	0	0	0	22	0	0	22	0	22
0	0	11	22	22	11	0	0	0	0	22	0	33

TABLE 4 Mean abundances of bacterial species isolated from the abdominal cavities of shrimp from four seawater RAS treated with different concentrations of PAA (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L) before start of the application and 56 days after start of continuous PAA application

Abdominal cavity	Phylum	Class	Genus	Species	Control		0.1 PAA		1 PAA		10 PAA	
					Before PAA	Day 56						
Actinobacteria	Actinobacteria	Arthobacter	Arthobacter	<i>A. creatinolyticus</i>	11	0	0	0	0	0	0	0
				<i>Arthobacter</i> spp.	0	0	33	0	11	0	0	
				<i>B. casei</i>	0	0	0	11	0	0	0	
				<i>M. saccharophilum</i>	0	0	0	22	0	33	0	
				<i>Streptomyces</i>	0	11	33	0	22	0	0	
				<i>T. discolor</i>	0	11	0	0	0	44	56	
				<i>T. mesophilum</i>	0	0	44	0	0	0	0	
				<i>Tenacibaculum</i> spp.	0	0	0	44	0	0	0	
				<i>B. aquimaris</i>	0	0	44	0	0	0	22	
				<i>B. cereus</i>	0	0	11	0	22	0	11	
Bacterioidetes	Flavobacteriia	Tenacibaculum	Tenacibaculum	<i>B. subtilis</i>	0	11	0	44	0	44	0	
				<i>P. citreus</i>	0	0	0	11	0	11	0	
				<i>S. epidermidis</i>	33	33	44	44	22	67	56	
				<i>S. hominis</i>	0	67	0	33	0	44	0	
				<i>Staphylococcus</i> spp.	0	0	0	0	11	11	0	
				<i>N. kimyeongensis</i>	0	0	11	0	0	0	0	
				<i>M. hydrocarbonoclasticus</i>	33	44	11	11	22	11	0	
				<i>M. xestospongiae</i>	0	0	0	33	0	11	0	
				<i>P. damsela</i>	67	0	100	0	67	0	89	
				<i>P. lipolytica</i>	0	0	0	11	0	0	0	
Firmicutes	Bacilli	Bacillus	Bacillus	<i>P. ruthenica</i>	0	0	0	0	11	0	0	
				<i>P. shioyasakiensis</i>	0	11	0	0	0	33	0	
				<i>P. gallaeciensis</i>	22	0	0	0	0	0	33	
				<i>Planococcus</i>	0	0	0	0	0	0	0	
				<i>Staphylococcus</i>	0	0	0	0	0	0	0	
				<i>Staphylococcus</i>	0	0	0	0	11	11	0	
				<i>Nitritireductor</i>	0	0	11	0	0	0	0	
				<i>Marinobacter</i>	33	44	11	11	22	11	0	
				<i>Photobacterium</i>	0	0	0	33	0	11	0	
				<i>Pseudoalteromonas</i>	67	0	100	0	67	0	89	
Proteobacteria	α -Proteobacteria	Nitritireductor	Nitritireductor	<i>P. lipolytica</i>	0	0	0	11	0	0	0	
				<i>P. ruthenica</i>	0	0	0	0	11	0	0	
				<i>P. shioyasakiensis</i>	0	11	0	0	0	33	0	
				<i>P. gallaeciensis</i>	22	0	0	0	0	0	33	
				<i>Nitritireductor</i>	0	0	11	0	0	0	0	
				<i>Marinobacter</i>	33	44	11	11	22	11	0	
				<i>Photobacterium</i>	0	0	0	33	0	11	0	
				<i>Pseudoalteromonas</i>	67	0	100	0	67	0	89	
				<i>P. lipolytica</i>	0	0	0	11	0	0	0	
				<i>P. ruthenica</i>	0	0	0	0	11	0	0	

(Continues)

TABLE 4 (Continued)

Abdominal cavity	Class	Genus	Species	Control		0.1 PAA		1 PAA		10 PAA	
				Before PAA	Day 56						
		<i>Vibrio</i>	<i>V. alginolyticus</i>	89	0	89	0	100	0	67	0
			<i>V. fortis</i>	44	0	44	0	22	0	89	0
			<i>V. harveyi</i>	0	56	11	67	0	33	0	0
			<i>V. hepatarius</i>	22	0	78	0	11	0	11	0
			<i>V. mytili</i>	0	0	33	0	0	0	11	0
			<i>V. parahaemolyticus</i>	22	0	44	11	11	0	56	0
			<i>V. pectenicida</i>	0	44	0	100	0	100	0	0
			<i>V. pelagius</i>	0	0	0	11	0	0	0	0
			<i>V. rotiferianus</i>	0	0	0	0	0	0	22	0
			<i>V. xuii</i>	0	0	0	22	0	0	0	0
			<i>Vibrio</i> spp.	22	11	0	0	11	11	0	0

Abbreviations: PAA, peracetic acid; RAS, recirculating aquaculture systems.

after seven-day treatment with 2 mg PAA/L. These alterations were even more pronounced when the crayfish were treated with 10 mg PAA/L for an equal duration (Chupani, Zuskova, Stara, Velisek, & Kouba, 2016). However, none of the crayfish died, and after a recovery period of seven days, gill morphology returned to normal levels (Chupani et al., 2016). This is in accordance with the results of the preliminary experiment of the present study where all shrimp survived the exposure to PAA concentrations even up to 100 mg PAA/L. No losses were seen also in the main experiment; however, the RAS treated with a PAA concentration of 10 mg/L had to be removed from the experiment already two days after commencing PAA application because of the severe changes in chemical water parameters which subsequently would have affected the health of the shrimps. In the remaining RAS treated with two different PAA concentrations, alterations in shrimp performance were detected compared with the shrimp from the untreated control RAS. The different performance of the shrimp in both PAA treated RAS might indicate a slightly negative effect of a continuous PAA application on shrimp welfare during the 56-day observation period, even at very low concentrations. Nevertheless, it was not examined whether the alterations improved again after the PAA supplementation had been stopped. Hence, more research on shrimp welfare after disinfection treatment methods with PAA is needed.

In this study, PAA was applied to seawater RAS with a salinity of 30‰. It is known that the degradation of PAA is related to the salinity of water and that higher salinities lead to a faster degradation (Liu et al., 2014). The effect of a PAA prophylaxis or treatments in seawater RAS therefore might be reduced compared with freshwater RAS, and the maintenance of an effective concentration might be a challenge (Liu et al., 2014). For seawater RAS, multiple applications or additional PAA dosages are thus recommended to maintain effective concentrations (Liu et al., 2014). To avoid potential harm to the biofilter in RAS during PAA application, it is nevertheless suggested to apply PAA only at reduced flow rates (Liu, Pedersen, et al., 2017). Taking this into account, in the present study, PAA was applied to the RAS at normal flow rates, not as pulse application but continuously. Thus, stable PAA concentrations in the RAS should be maintained, and especially, short-term high PAA concentrations in the biofilter should be avoided. The addition of PAA at the higher dosage of 10 mg/L in the present study interfered with the removal of nitrogenous compounds, which suggests that the bacteria of the biofilter were affected. Because this effect was not anticipated, the biofilter was not sampled and this assumption could not be confirmed. For freshwater RAS, it could be shown that a continuous application did not lead to PAA accumulation and it was assumed that a fast degradation of the substance was attenuated by microbial adaptation (Liu, Straus, et al., 2017). An enhanced biofilm formation was seen visually, and it was suggested that acetic acid and acetate as active ingredients of PAA acted as an easy degradable dissolved organic substance, which could promote the growth of especially heterotrophic bacteria. Nevertheless, an examination of the composition of the microflora was not performed (Liu, Straus, et al., 2017). Our former studies on the microbial community in freshwater RAS showed that

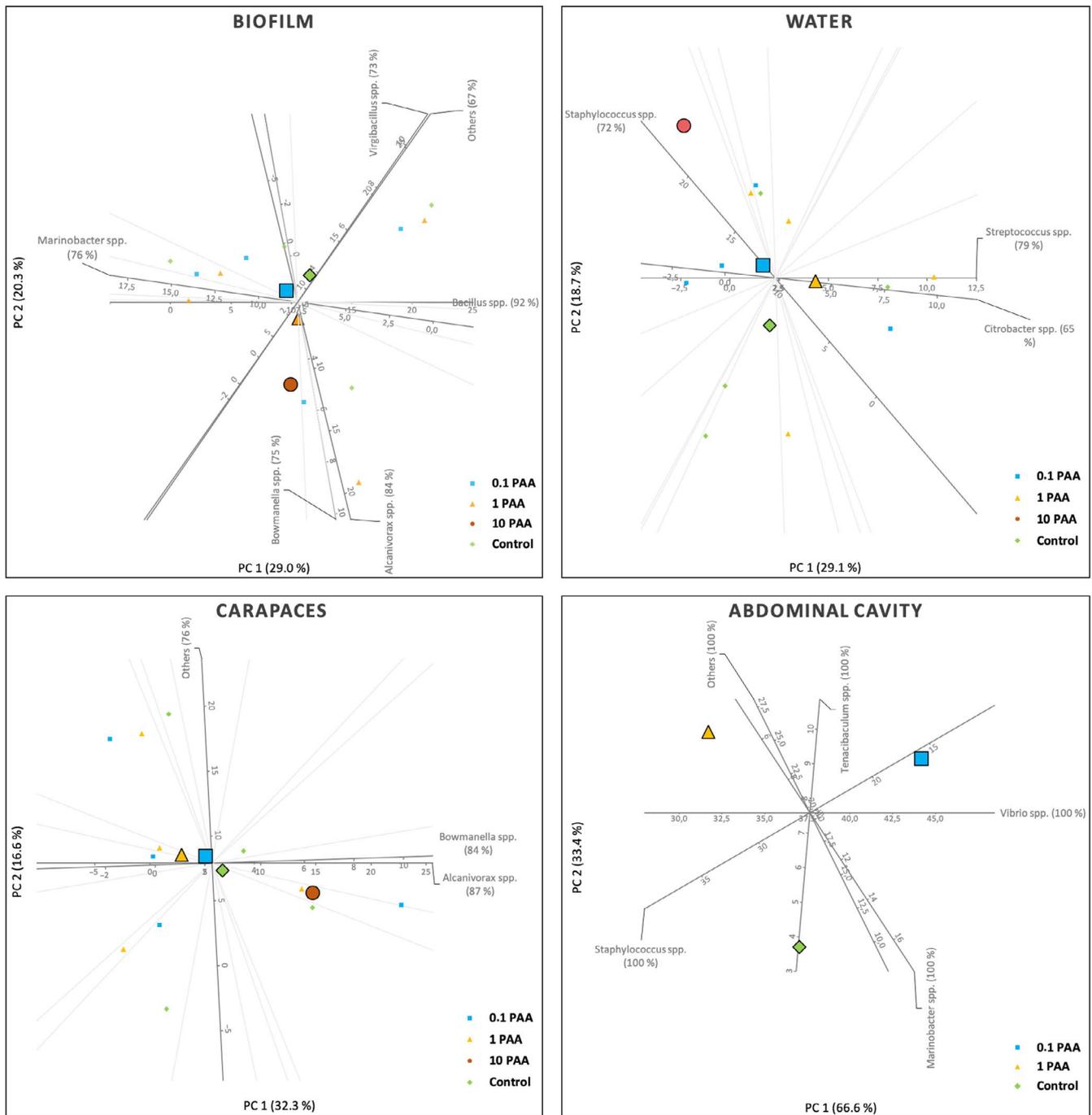


FIGURE 3 Principal component analysis (PCA) of the composition of the bacterial microflora from the biofilms of tank surfaces, the water, the biofilms from shrimp carapaces and the abdominal cavity from shrimp from four seawater recirculating aquaculture systems (RAS) treated with different concentrations of peracetic acid (PAA) (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L) before adding PAA, and 2, 9, 29 and 56 days after commencing continuous PAA application. The data from the different sampling time-points 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]

a stable microflora seems to be very important in order to avoid the rapid growth of potentially fish-pathogenic bacteria (Jung-Schroers, Adamek, Boley, Korshun, & Steinhagen, 2019; Jung-Schroers et al., 2016, 2018). The maintenance of stable chemical and microbiological water conditions in the RAS was therefore another reason for applying the PAA continuously. Regarding the chemical water

parameters, in freshwater RAS, it could be seen that the pH value increased when PAA was continuously applied (Liu, Straus, et al., 2017). In seawater RAS, the pH value decreases regularly over time, and in the present study, decreased pH values could be measured in all four RAS during the observation period. The most stable conditions regarding pH levels in water were therefore maintained in the control

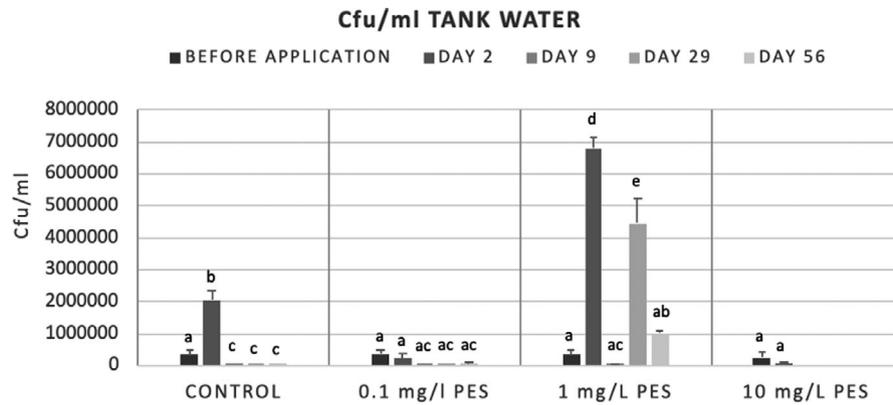


FIGURE 4 Colony-forming units (Cfu) in the water of four seawater recirculating aquaculture systems stocked with *Litopenaeus vannamei* treated with different concentrations of peracetic acid (PAA) (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L) before the start of the application and 2, 9, 29 and 56 days after commencing continuous PAA application. Shown are the total numbers of Cfus. The statistically significant differences between the treatments are marked by different letters

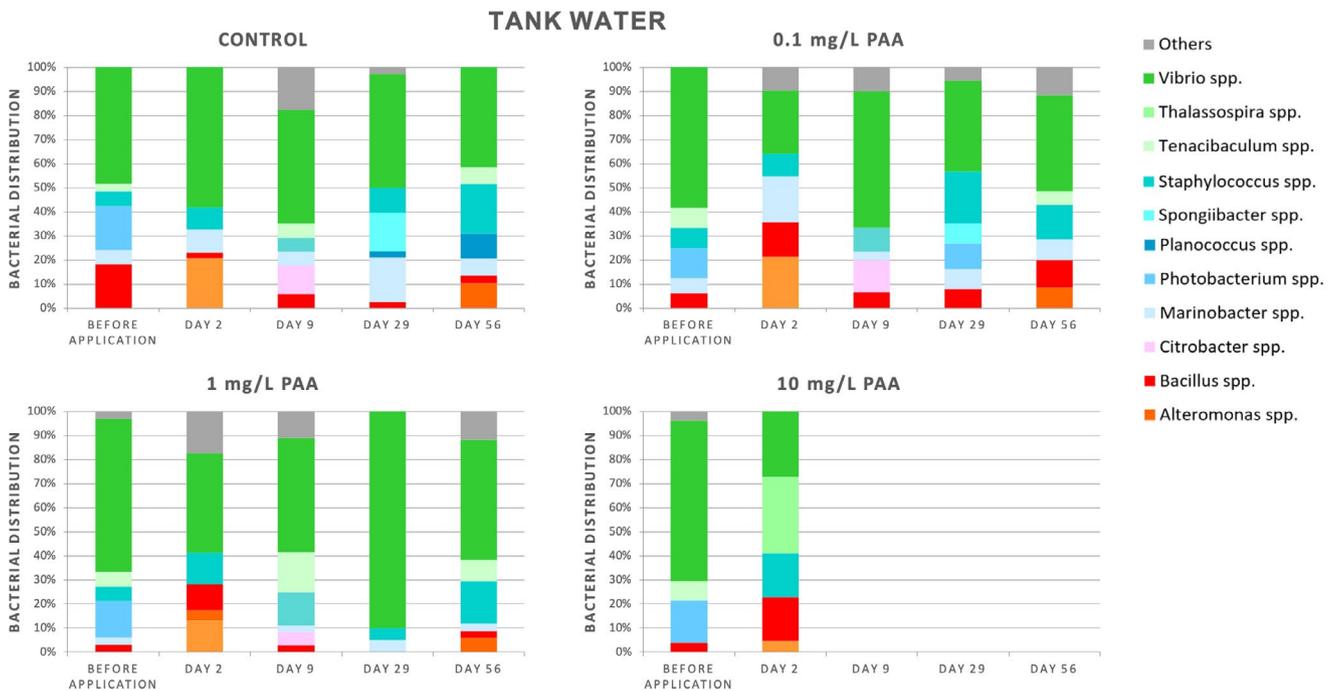


FIGURE 5 Composition of the bacterial microflora in the recirculating water of four seawater recirculating aquaculture systems stocked with *Litopenaeus vannamei* and treated with different concentrations of peracetic acid (PAA) (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L). Columns represent the composition of the bacterial microflora before the start of the application and 2, 9, 29 and 56 days after starting a continuous application of PAA in the above-mentioned concentrations. Shown are the bacterial species that could be detected at least at one sampling time-point 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]

RAS and in the RAS treated with the lowest PAA concentration. It can be assumed that adding PAA at a concentration of 1 mg/L influences the pH value. Other chemical water parameters were also influenced by PAA treatments, in particular at concentrations of 1 mg/L upwards. The increase in ammonia and nitrite concentrations in recirculating water as measured shortly after commencing continuous PAA applications in the present study can be interpreted as a sign of the disruption of the biological filtration and indicates a reduction in nitrifying bacteria. This is in contrast to previous findings in freshwater

RAS treated continuously with PAA in which nitrite and nitrate levels were not higher than in a control RAS (Liu, Straus, et al., 2017).

In the present study, a reduction in the abundance of bacteria in water, as was expected by applying PAA at higher concentrations to the RAS, was not achieved, neither with 1 mg/L nor with 10 mg/L. The total number of bacteria in the water even increased significantly, especially two days after applying 1 mg/L. Additionally, a shift in the bacterial community seemed to be provoked by applying PAA at concentrations of 1 or 10 mg/L to the RAS. Nevertheless, the

BIOFILM OF TANK SURFACES

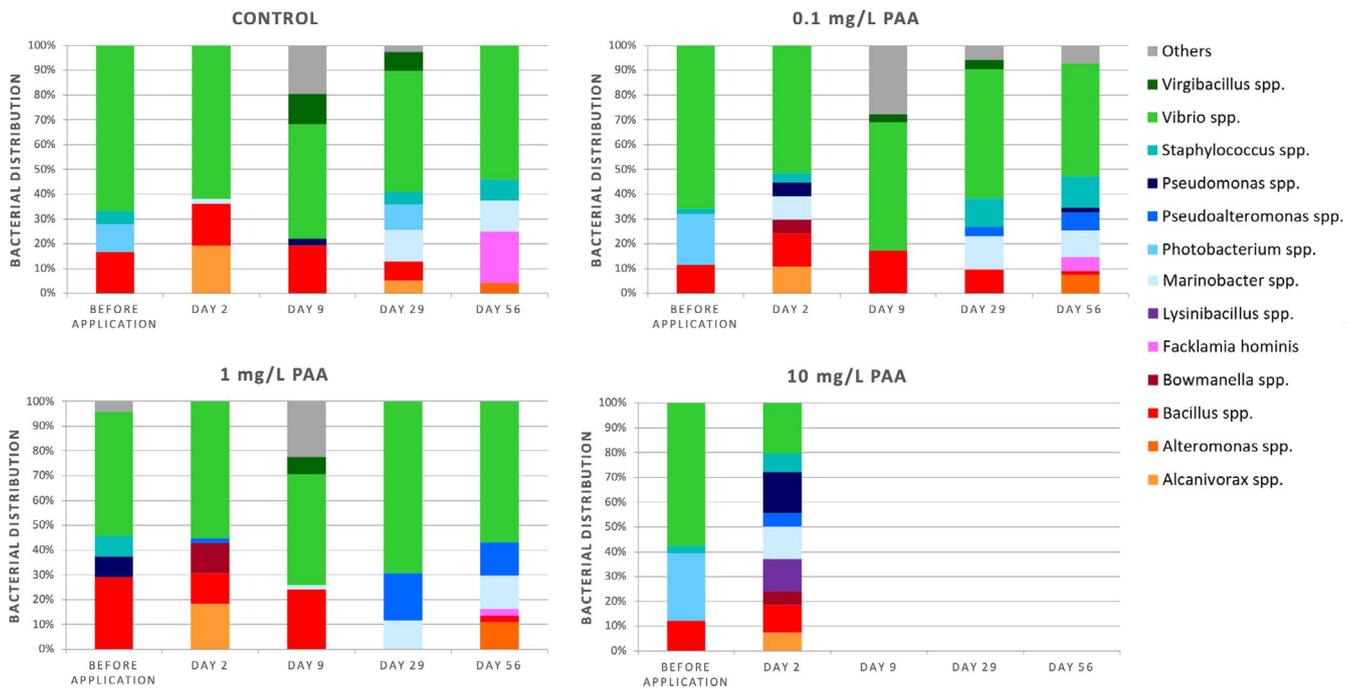


FIGURE 6 Composition of the bacterial microflora in biofilms on tank surfaces in four seawater recirculating aquaculture systems stocked with *Litopenaeus vannamei* and treated with different concentrations of peracetic acid (PAA) (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L). Columns show the composition of the bacterial microflora before the start of the application and 2, 9, 29 and 56 days after starting a continuous PAA application. Shown are the bacterial species that could be detected at least at one sampling time-point 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]

CARAPACES

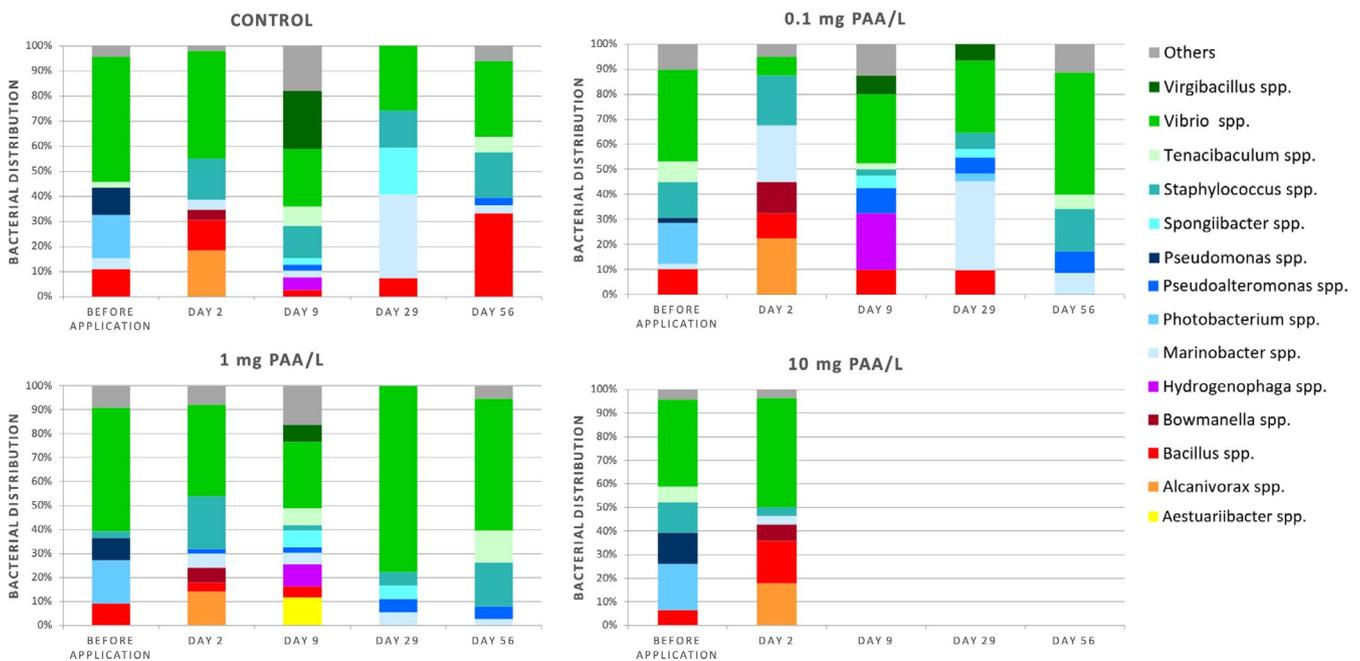


FIGURE 7 Composition of the bacterial microflora on the carapaces of *Litopenaeus vannamei* kept in four seawater recirculating aquaculture systems and treated with different concentrations of peracetic acid (PAA) (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L). Columns depict the composition of the bacterial microflora before the start of the application and 2, 9, 29 and 56 days after commencing a continuous PAA application. Shown are the bacterial species that could be detected at least at one sampling time-point 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]

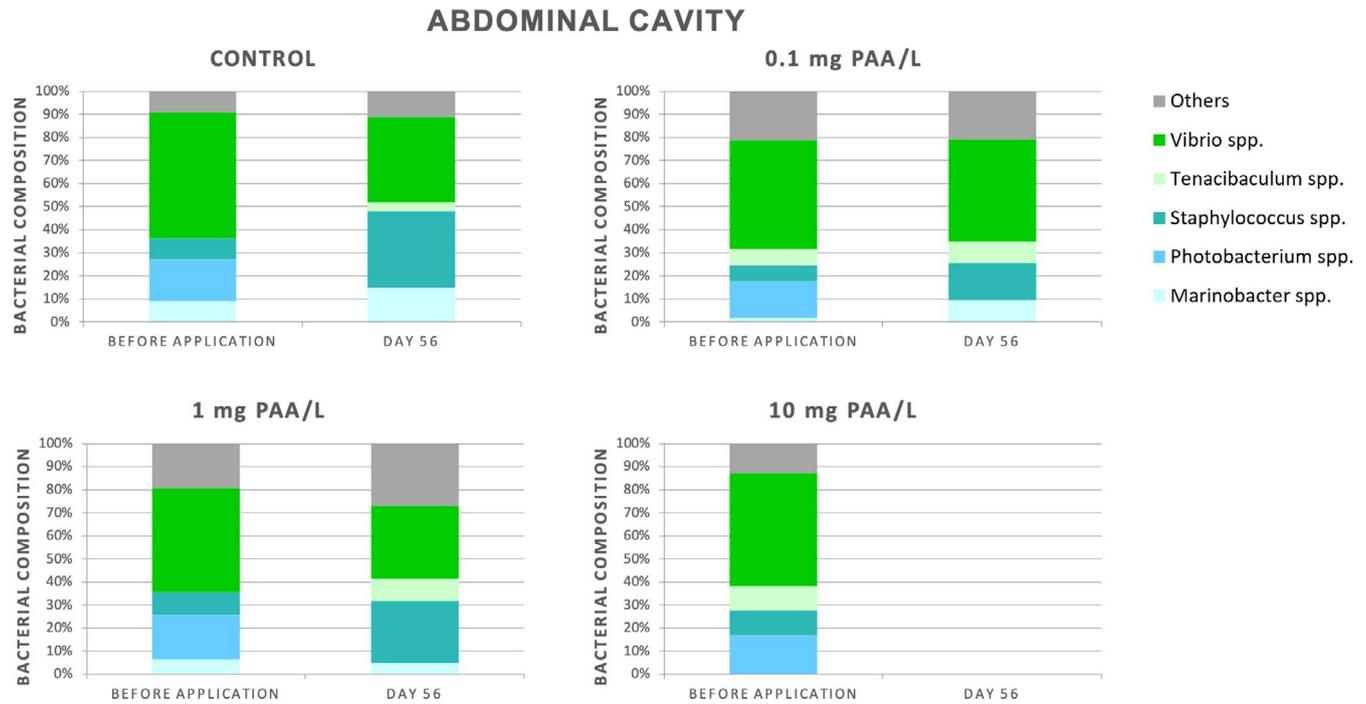


FIGURE 8 Composition of the bacterial microflora in swabs taken from the abdominal cavity of *Litopenaeus vannamei* kept in four seawater recirculating aquaculture systems and treated with different concentrations of peracetic acid (PAA) (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L). The analysis was performed before and 56 days after commencing a continuous PAA application. Shown are the bacterial species that could be detected at least at one sampling time-point 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]

bacterial community was analysed by cultural techniques followed by molecular biological identification. By culturing, it is known that not all bacterial species might be detected as not all are growing on agar plates. This has to be considered when interpreting the data. As mentioned previously, in freshwater RAS, an increased biofilm formation was described after continuous application of PAA (Liu, Straus, et al., 2017). In the present study, the thickness of biofilms was not measured but an enhanced biofilm formation was visually not observed in the PAA treated RAS. The higher amounts of bacteria in tank water nevertheless indicated an enhanced reproduction of bacteria that were probably able to use PAA degradation products as a nutritional source. It could be seen that the composition of the bacteria within the RAS changed in relation to the amount of PAA applied to the tanks and that the composition of the biofilms at tank surfaces and carapaces of shrimp changed differently. Especially in the RAS treated with a PAA concentration of 10 mg/L, a significant reduction in the amount of *Vibrio* sp. to 20% was recognized already two days after commencing the application. In contrast to this, the mean amount of *Vibrio* sp. was around 40%–60% of all detected bacteria and in all kinds of samples. This mean value is in accordance with findings in other seawater RAS and also in the oceans where *Vibrio* sp. represent the prevailing organism in the physiological microflora. The amount of *Vibrio* sp. within the physiological microflora in marine and brackish habitats can reach up to 40% (Bauer et al., 2018; Jeyasanta, Lilly, & Patterson, 2017; Urakawa & Rivera, 2006). A significant reduction in the abundance of these

organisms in a seawater RAS running at 30‰ therefore can be regarded as a sign of destabilization of the system. By adding PAA at a concentration of 1 mg/L to the RAS, also changes in the bacterial composition occurred that also indicated a destabilization of the microflora in the RAS. Such unsteady environments which offer unexploited nutrient resources can favour fast-growing bacteria with an r-selection strategy. To avoid such unstable conditions in the water, the importance of microbial matured water in RAS was underlined by different authors (Attramadal et al., 2014; Skjermo et al., 1997; Skjermo & Vadstein, 1999). Stable water conditions most likely favour the colonization of recirculating water and biofilms by bacteria with a K-selection strategy. K-selection mainly occurs in communities that are close to the carrying capacity (CC) of a system, where the CC is the number of bacteria that can be sustained in the system over a long period of time (Attramadal et al., 2016). According to this, the selection pressure of bacteria is used to prevent the proliferation of opportunistic bacteria with an r-selection strategy by filling all possible niches in the system with non-opportunistic bacteria. In this respect, matured water colonized by these non-opportunistic bacteria with a K-selection strategy would protect fish from bacterial diseases caused by opportunistic fast replicating bacteria with r-selection strategy (Skjermo et al., 1997).

It can be assumed that these findings are relevant for RAS stocked with shrimp as well. In the present study, the microbial community in water and biofilms of tanks surfaces and carapaces of the shrimp remained largely unchanged in the control RAS and

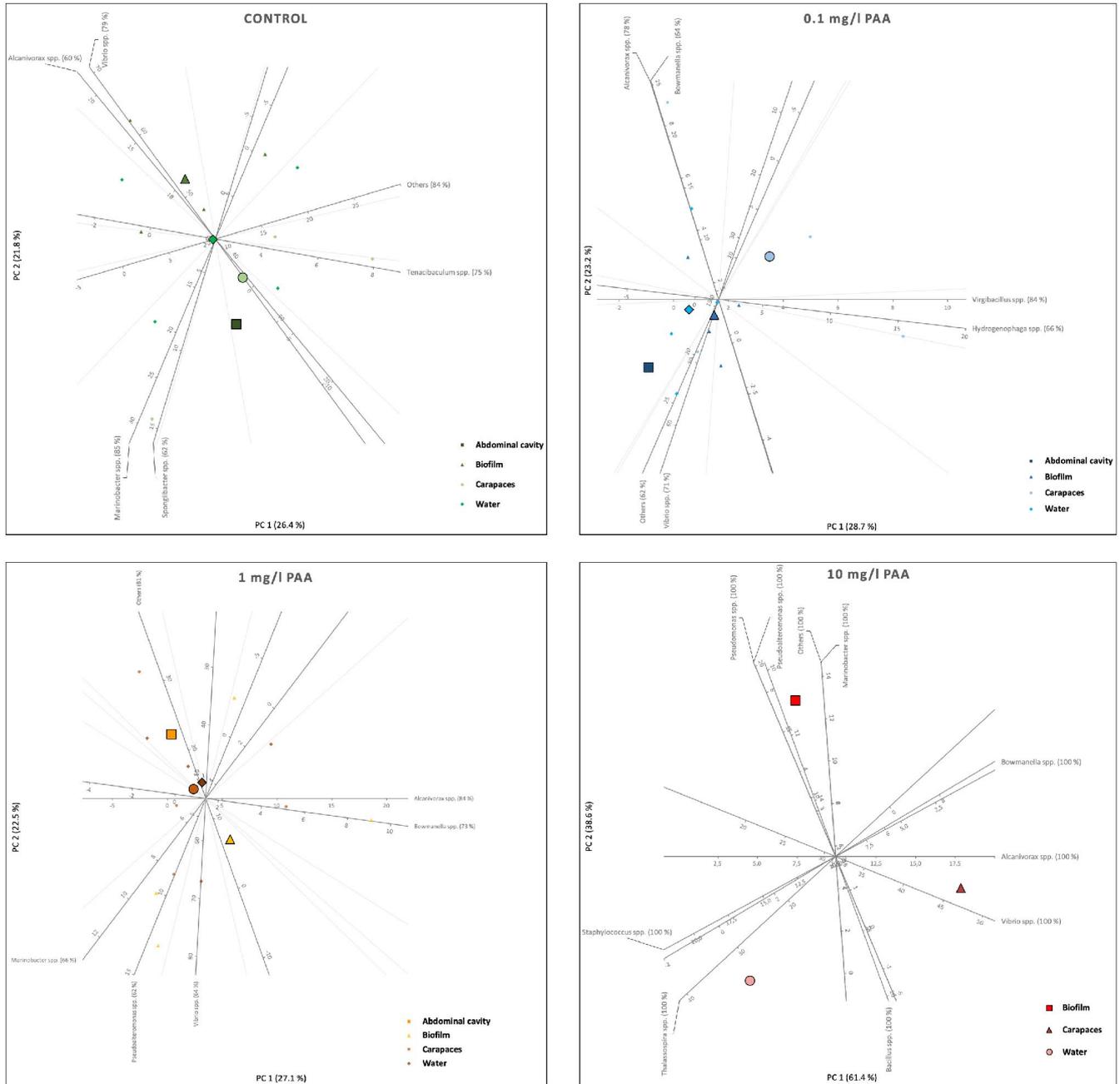


FIGURE 9 Principal component analysis (PCA) of the composition of the bacterial microflora in water, biofilms on tank surfaces, shrimp carapaces and in the abdominal cavity from shrimp from four seawater recirculating aquaculture systems (RAS) treated with different concentrations of peracetic acid (PAA) (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L). Samples were collected before the start of the application and 2, 9, 29 and 56 days after commencing a continuous PAA application. In the RAS treated with a PAA concentration of 10 mg/L, samples were collected only two days after commencing continuous PAA application. The data from the different sampling time-points are indicated with small symbols, and the mean values of the samples from the different sample locations are indicated with large, bordered symbols [Colour figure can be viewed at wileyonlinelibrary.com]

in the RAS treated with a PAA concentration of 0.1 mg/L. Thus, it can be assumed that the microflora was not influenced largely by very small amounts of PAA. Especially, the bacterial composition of the biofilm of the tank surfaces in the RAS treated with a PAA concentration of 0.1 mg/L remained very similar to biofilms in the untreated control group. Biofilms can act as reservoirs for potentially pathogenic bacteria (King et al., 2004), and therefore, stable biofilms formed by harmless bacterial species are believed to reduce the risk

of infections caused by opportunistic pathogens. Nevertheless, effective treatment or water disinfection in case of a disease outbreak would not be achieved by adding PAA in a small amount of 0.1 mg/L to the RAS, and therefore, this application has no practical value. On the other hand, we could clearly see an effect of PAA concentrations of 1 mg/L water or higher on the microflora and the occurring destabilization is also not helpful when applying the substance for prophylactic reasons. The conditions, which are anticipated to be improved

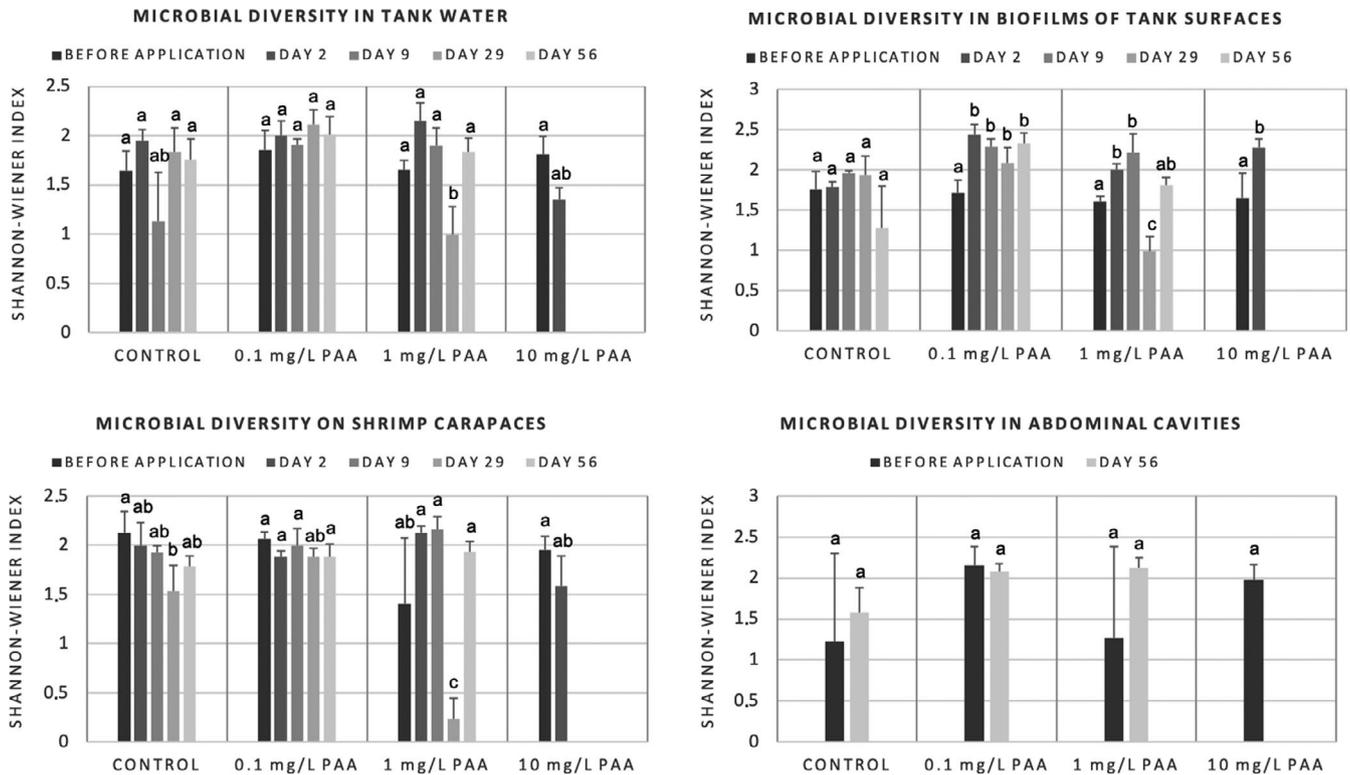


FIGURE 10 Shannon–Wiener indices of diversity describing the composition of the bacterial microflora from four seawater recirculating aquaculture systems (RAS) treated with different concentrations of peracetic acid (PAA) (0 mg/L = control; 0.1 mg/L; 1 mg/L; 10 mg/L). Depicted are index values from the recirculating water, the biofilms of tank surfaces and from shrimp carapaces, and from the abdominal cavity of shrimp before the start of the application and 2, 9, 29 and 56 days after commencing continuous PAA application. In the RAS treated with a PAA concentration of 10 mg/L, samples were collected only 2 days after commencing continuous PAA application and the abdominal cavity of shrimps was sampled before the application of PAA. The statistically significant differences between the treatments are marked by different letters

by adding PAA, might even be impaired by the application, and the risk of bacterial infections might increase because of an enhanced growth of heterotrophic bacteria that might act as facultative pathogens. In all four RAS, there was a closer relationship between the microflora in samples taken from the water and the biofilms on tank surfaces and carapaces that are exposed directly to the water, than between the microflora from the abdominal cavities. A systemic effect of the PAA application could be excluded, as the composition of the bacteria isolated from the abdominal cavities of the shrimp remained very similar in all RAS.

Nevertheless, in the current study, differences in the microbial community were not only seen due to PAA application but also due to a large water exchange and reduced amount of food at day nine after commencing PAA application. This clearly indicates that management measures are of great importance for maintaining optimal keeping conditions in aquaculture systems and that these measures are more suitable for prophylaxis treatment of disease outbreaks than water disinfection.

In conclusion, using PAA in seawater aquaculture systems affected the microflora in the water and on biofilms depending on the used concentration. The highest continuously applied PAA concentration tested, at 10 mg/L, led to significant changes in chemical water parameters, in particular a drop in the pH value and to high

levels of ammonia and nitrite, already after two days of application. A continuous application of PAA at a concentration of 1 mg/L, which is often used in aquaculture systems for fish, also led to increased levels of ammonia and nitrite within two days of application and to a significant increase in the amount of bacteria in water. In the preliminary experiment, it could be demonstrated that PAA at a concentration of 1 mg/L was able to significantly reduce bacterial growth of different species *in vitro*. Therefore, it can be assumed that the bacterial number in the water increased in the aquaculture systems because heterotrophic bacteria were able to use degradation products of PAA as a nutritional source. There were indications that the welfare of the shrimps was affected because the relation between body length and antennae length was significantly smaller than in those shrimps in the control RAS. In the RAS treated with a PAA concentration of 0.1 mg/L, the chemical and microbiological parameters were comparable to those of the untreated control RAS or even more stable during the experimental period. In this RAS, a reduction in the total amount of bacteria was achieved by decreasing the amount of food given and by exchanging the water, but obviously not by the effect of PAA. Applying PAA continuously at concentrations of 0.1 mg/L therefore seems not to be effective in seawater aquaculture systems for shrimp, neither for prophylactic use nor as an alternative treatment for specific pathogens. Continuous application

of 10 mg/L also is not recommendable for use in seawater shrimp aquaculture because this had a strong impact on chemical water parameters. Applying 1 mg/L PAA in seawater shrimp aquaculture might be an alternative in cases of disease outbreaks due to bacterial or parasitic infections for short periods of time. Nonetheless, further research on the specific effect in a running seawater system is needed. A continuous prophylactic use of PAA in this concentration is not recommendable as no bacterial reduction was achieved and the chemical and microbiological parameters fluctuated strongly due to the application, leading to a destabilization of the system. Whether repeated pulse applications of PAA are more effective in seawater shrimp aquaculture either for prophylactic or as an alternative treatment has to be investigated further.

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CONFLICT OF INTERESTS

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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