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# UV treatment in RAS influences the rearing water microbiota and reduces the survival of European lobster larvae (*Homarus gammarus*)

Kari J.K. Attramadal [a,b,*], John Vegard Øien [b], Elisabeth Kristensen [b], Jan Ove Evjemo [c], Elin Kjørsvik [a], Olav Vadstein [b], Ingrid Bakke [b]

[a] Department of Biology, NTNU Norwegian University of Science and Technology, Trondheim, Norway
[b] Department of Biotechnology and Food Science, NTNU Norwegian University of Science and Technology, Trondheim, Norway
[c] SINTEF Ocean, Trondheim, Norway

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ABSTRACT

The rearing environment is important for a stable production of good quality lobster juveniles. By providing an environment excluding pathogens and dominated by mutualistic bacteria, the probability of developing healthy host-microbe relationships and produce healthy juveniles is increased. Disinfection of water and sudden increase in the supply of organic matter in culture tanks are processes that open for uncontrolled microbial regrowth in the rearing water. This increase the variability in the development of the microbiota between replicate rearing tanks and promotes selection for potentially harmful opportunistic bacteria. In two start feeding experiments with European lobster (*Homarus gammarus*) we compared the bacterial environment in three types of rearing systems: a recirculating aquaculture system (RAS) with UV treatment directly in front of the rearing raceways, a RAS without disinfection, and a conventional flow through system (FTS). The RAS with no disinfection was hypothesised to stabilise the microbiota of the rearing water, select against opportunistic bacteria, and reduce variability in production outcome between replicate tanks compared to the other systems. As predicted, the three different systems developed significantly different compositions of the microbiota in the rearing water and the larvae. On average, the survival of larvae in RAS without disinfection increased with 43 and 275 %, in the first experiment, and 64 and 18 % in the second experiment, compared to RAS with UV and FTS, respectively. Also, the RAS without disinfection showed less variability in the survival of larvae between replicate tanks and batches compared to the other treatments. The results are promising for controlling the microbiota of the rearing water to improve, increase and stabilise the production of marine larvae by competent use of water treatment and selection regimes. Based on the presented and previous work, RAS is recommended over FTS, and in RAS it is recommended to avoid point-disinfection of the recirculating water, to provide a stable and beneficial microbial environment in the cultivation of marine larvae.

## 1. Introduction

The European lobster (*Homarus gammarus*) is an exclusive and highly priced seafood. Sea ranching, restocking and intensive land-based farming of lobster to market size all require land-based production of Stage IV juveniles. Like most marine species in culture, lobster show a critical period during the first weeks following hatching. High mortality of larvae and low reproducibility between replicate tanks are common in hatcheries during this period, also for lobster. These problems often attribute to infections from opportunistic bacteria that proliferate under aquaculture conditions and cause disease in weakened hosts (Vadstein et al., 2004, 2018a). In a study of the water microbiota in a hatcery for the ornate rock lobster (*Panulirus ornatus*) Payne et al. (2006) demonstrated a dynamic microbial community with distinct changes in the community structure and a major increase in bacterial load the first week after stocking.

The initial colonisation and the subsequent host-microbe relationships that develops depends on which bacteria the larvae encounters, timing, competition, and selection in the environment, and in the host (De Schryver and Vadstein, 2014). According to the ecological theory of r/K-selection (MacArthur and Wilson, 1967), selective pressures drive succession in one of two generalized directions: Selection for

* Corresponding author.
*E-mail address:* Kari.Attramadal@ntnu.no (K.J.K. Attramadal).

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opportunists (r-selection) occurs in unpredictable environments with empty niches and high resource supply per bacterium, which favours fast-growing species. Selection for specialists (K-selection), on the other hand, occurs in stable, predictable environments where the community is close to the carrying capacity (CC) of the system, resource supply is low and the ability to compete successfully for limited resources is favoured. The CC is the maximum number of bacteria that can be sustained in the system over time, and for heterotrophic bacteria in aquaculture systems, the CC is typically determined by the supply of organic matter. The different characteristics of the two selection regimes implies that K-selected microbial communities are more robust to perturbations and more resilient to invasion than r-selected communities.

Water treatment, system design and management can be used to control the selection pressure working on the microbial community to increase the fraction of harmless and beneficial bacteria at the expense of opportunists in aquaculture systems (Vadstein et al., 2018b). A stable supply of organic matter enables a constant, strong competition in the system, which reduce free niches and favours the K-strategists. Likewise, securing a low loss rate of bacteria from the system by reducing water exchange and disinfection, maintain the population close to the CC and a strong competition for the available organic matter. Recirculating aquaculture systems (RAS) without disinfection in the water treatment loop have been suggested as a strategy for microbial control based on three features that promote K-selection: 1. the long retention time of water in the system, 2. the large surface area available for bacterial growth and competition in the biofilter, and 3. the stable microbial CC throughout the system (Attramadal et al., 2012a, b, 2014). In contrast, in flow through systems (FTS) the change in CC from clean intake water to significantly higher substrate levels in the rearing tanks create r-selection and promotes opportunistic proliferation in tanks with low water exchange rates (Fig. 1). Even at a stable CC, disinfection create subsequent r-selection (Hess-Erga et al., 2010) because the number of bacteria competing for the substrate is significantly reduced (Fig. 1).

To the best of our knowledge, this is the first study to test the principles described above in the rearing of lobster larvae. It is also the first study to compare a RAS with no disinfection of the recirculating water to a separate replicate RAS with disinfection directly before the rearing units. Both these system designs are common ways to operate RAS (Blancheton et al., 2013). RAS has been successfully used for commercial cultivation of European lobster (Drengstig and Bergheim, 2013). Other studies have investigated UV-treatment of recirculated water in RAS for other species. Attramadal et al. (2012b) compared the effects of UV-treatment before the biofilter in a RAS to a RAS with moderate ozonation and a FTS for Atlantic cod. Dahle et al. (2020) studied the eff ; ects of various types of treatment of the water returning to replicate tanks in a single RAS for lumpfish, including microfiltration, UV-irradiation and ozonation.

In a FTS the supply of organic matter varies significantly through the system, with a relatively low supply in the intake water followed by a high supply in the rearing tanks. In addition, the loss rate of bacteria from FTS is high due to the high exchange rate from the system. For these two reasons, FTS typically show r-selection of the bacteria of the rearing water. On the other hand, we hypothesise that RAS without disinfection of the recirculating water lead to a constant high competition among the bacteria, due to the stable supply of organic matter throughout the system and the low loss rate of bacteria from it. However, with efficient disinfection of the recirculating water, the loss rate of bacteria from the RAS is increased, and the competition for the available food is thus significantly reduced. RAS without disinfection is therefore predicted to give a more stable, mature microbial community and relatively lower regrowth of bacteria in rearing tanks, than both FTS and RAS with disinfection of the recirculated water. In this study we tested the hypothesis that larvae reared in these three different types of systems will be exposed to different microbial environments and therefore the larvae will develop distinct microbiotas (Attramadal et al., 2016; Ooi et al., 2017; Vestrum et al., 2018). Further, we hypothesized that the expected high microbial regrowth in the rearing water of FTS and RAS with disinfection, would lead to a stochastic recolonization of replicate tanks. This again, could be expected to result in more variable outcomes in microbial composition and possibly also larval performance, between replicate rearing tanks. Further, we predicted that the high bacterial regrowth in the rearing water of FTS and RAS with disinfection would be associated with strong r-selection, and therefore result in a higher relative abundance of detrimental r-strategists. In comparison, limited regrowth of bacteria and continuous K-selection was predicted in the rearing tanks of RAS without disinfection, which was predicted to result in dominance by K-strategists and less variable microbial environments between replicates. Based on the hypothesis that an environment dominated by K-strategists is more beneficial for the larvae, it was predicted that the RAS without disinfection would support a more stable and higher survival of larvae than both FTS and RAS with disinfection.

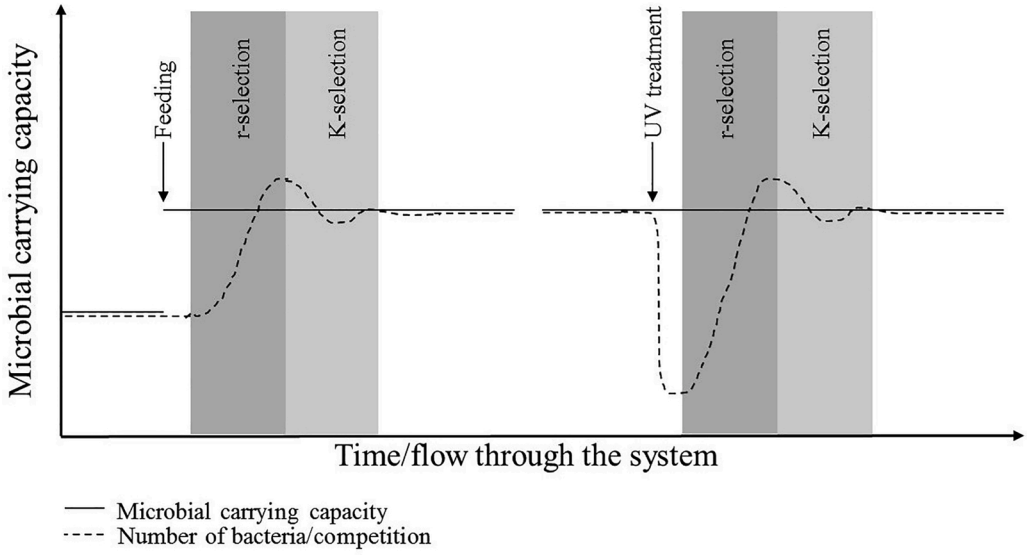


**Fig. 1.** Two aquaculture operations that promote r-selection of heterotrophic bacteria, and increase the probability of detrimental microbial conditions in the rearing tanks. Addition of feed represents an abrupt increase of the microbial carrying capacity in the rearing tanks compared to the intake water, which results in favourable conditions for fast-growing bacteria. Disinfection of intake water is used as a barrier against introduction of pathogens to the system. However, disinfection also decreases bacterial numbers and reduce competition for bioavailable organic matter, promoting growth of opportunistic bacteria.

## 2. Materials and methods

### 2.1. Water treatment systems and rearing regime

Three systems for water treatment (Fig. 2) were compared in first feeding experiments with European lobster larvae (*Homarus gammarus*): one flow through system (FTS), and two different recirculating systems (RAS): one with UV treatment (RAS-UV) and the other with no disinfection (RAS-ND). Two independent experiments were conducted with the same systems, in 2013 and 2014, respectively. Intake water to all three systems was pumped from Trondheimsfjorden (70 m depth), sand filtered, heated, vacuum aerated and UV-filtered (BersonInLine 450, Berson UV-techniek, The Netherlands, in the 2014 experiment only). For the FTS, intake water was continuously added to the rearing tanks, and no water was reused. For the two RAS, intake water was continuously added in the first of two biofilters at a rate of 10 % new water of system volume day$^{-1}$. Each system included two replicate raceways (75 L, 0.25 × 2 m, 15 cm water depth). Inlet was in one end of each raceway, and the outlet was in the other end. Water exchange rate was 0.16 L min$^{-1}$ equal to 3× raceway volume day$^{-1}$. In each raceway, a submerged case of 60 chambers (each 75 × 37 mm and 75 mm deep) with highly perforated walls and floor was mounted with clamps to the wall of the raceway. The chambers had walls protruding 1 cm above the water surface and no roof.

In both RAS, the used rearing water was collected a reservoir (100 L) and treated in a skimmer (80 L, Helgoland 500) before entering two moving bed biofilters in series (230 L each) filled (30 % of the volume) with biofilm carriers (K1, Kaldnes, Norway). After the biofilters, the water was passed through a degasser (50 L, 50 cm vacuum). In the RAS-UV a UV-reactor (A10-PE, Wedeco, Germany) treated the whole recirculated water flow immediately before it entered the rearing tanks. One week before introduction of lobster larvae, water was circulated in the two RAS and Biofilter 1 of each system was fed fish feed granulate (3 g day$^{-1}$) and Biofilter 2 of each system was fed ammonium chloride (3 g day$^{-1}$) to increase and stabilize the populations of heterotrophic and nitrifying bacteria, respectively. To secure a similar microbiological start of the experiments for both RAS, half of the biofilm carriers of RAS-ND Biofilter 1 (by volume) was exchanged with the same volume of carriers from RAS-UV Biofilter 1 right before the start of each experiment. Similarly, half of the biofilm carriers of RAS-ND Biofilter 2 was exchanged with the same volume of carriers from RAS-UV Biofilter 2. The day before transferring larvae to the systems in the 2013 experiment, the raceways of the two RAS were emptied of water, then the UV-treatment in the RAS-UV was turned on before they were slowly filled. The day before transferring larvae to the systems in the 2014 experiment, the raceways were emptied, then the surfaces were disinfected (Virkon S) and rinsed with freshwater before the UV-treatment in the RAS-UV was turned on and then the raceways were filled slowly with water from the respective RAS.

Temperature, oxygen and pH was measured with an YSI Multiparameter. Salinity was measured using a refractometer. Increasing salinity was observed in the RAS due to evaporation during the experiments. Freshwater was added in Biofilter 1 to compensate for this effect.

European lobster (*Homarus gammarus*) larvae (Stage I, one day post hatch (dph)) from Norwegian wild caught lobster carrying eggs), were obtained at Norsk Hummer AS in Tjeldbergodden and shipped to Trondheim. A total of 360 larvae were used in each experiment. From the start of the experiment one larva was stocked in each of the 60 compartments of each system. The number of days it took for the larvae to reach Stage IV differed between the experiments, due to a higher

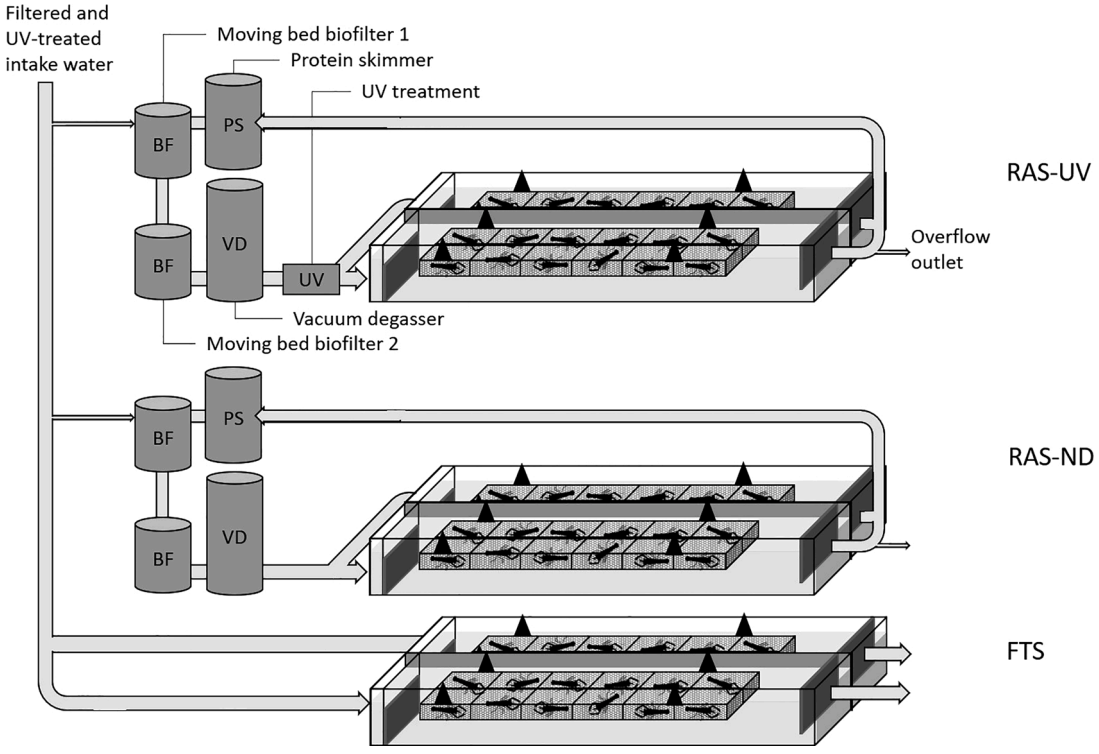


**Fig. 2.** Overview of the three systems for water treatment used in the two independent first feeding experiments with European lobster larvae (*Homarus gammarus*) in 2013 and 2014. Each system contained two replicate raceways with water inlet in one end and the outlet in the other. In each raceway, a submerged case of 60 chambers with highly perforated walls and floor was mounted with clamps to the wall of the raceway. RAS-UV was a recirculating aquaculture system (RAS) consisting of a protein skimmer, two moving bed biofilters in series, a vacuum degasser and a UV-system treating the entire flow of recirculated water just before it entered the raceways. RAS-ND was similar to the RAS-UV, except that it did not contain UV-treatment of the recirculating water flow (ND = no disinfection). All systems received the same intake water: the two RAS at a rate of 10 % new water volume of system volume day$^{-1}$ and the flow through system (FTS) at a rate of 300 % new water volume of system volume day$^{-1}$.

average water temperature in the 2013 experiment than in the 2014 experiment (Table 1). Thus, the 2013 experiment was terminated 14 dph, and the 2014 experiment was terminated 21 dph.

Adult stages of the copepod *Acartia tonsa* grown on *Rhodomonas baltica* were used to feed the lobster during the 2013 experiment and the first week of the 2014 experiment. Each lobster compartment was fed twice a day with copepods to a final concentration in the total rearing water of 7 copepods $mL^{-1}$ of raceway water volume. The level of feeding was based on previous successful cultivation of lobster larvae in the research group (Evjemo, unpublished data). In similar feeding experiments with marine fish larvae, the copepod density normally ranges between 5–10 copepods $mL^{-1}$ (Øie et al., 2015). During the last two weeks of the 2014 experiment *Artemia* sp. (EG cysts; INVE, Belgium) was used to feed the lobster. *Artemia* nauplii were hatched each day and transferred to an ongrowing tank with *Rhodomonas baltica*. This resulted in a mixture of *Artemia* of increasingly different sizes during the experiment. *Artemia* from the ongrowing tank were fed twice daily in equal portions to each individual lobster compartment with a syringe to a final concentration of to the lobster compartments to a concentration of 3 *Artemia* $mL^{-1}$ of raceway water volume. Live feed organisms were washed with filtered seawater on a sieve before they were fed to the larvae. Raceway bottoms were siphoned for debris three times a week.

### 2.2. Microbial community analyses

Samples for characterization of the composition of the microbial communities in water were collected from the inflowing water to and at the end of each raceway. Water and larvae were sampled at 4, 8 and 12 dph (Stage IV reached) in the 2013 experiment, and at 7, 14 and 21 dph (Stage IV reached) in the 2014 experiment. The water samples were prefiltered (120 µm) and then 40 mL of sample was filtered through sterile 0.2 µm 2.5 $cm^2$ hollow fibre syringe filter for aqueous solutions (DynaGard, Microgon Inc., California). Biofilm samples were collected from raceway walls with a sterile swab at the end of the 2014 experiment. The filters and swabs were stored at -20 °C. Four random larvae were sampled from each replicate raceway for each of the three sampling days of each experiment. L arvae were sampled in the morning, before feeding, into a cup of seawater kept on ice and then quickly rinsed from salt in a cup of milli-Q water before they were placed individually in cryotubes, snap-frozen in liquid nitrogen, and then stored at −20 °C. Data for survival was corrected for the sampling of larvae. To compare treatments for both experiments combined, the survival was normalized so that the survival of the best performing replicate raceway(s) represented 100 % for each experiment, and the normalized survival of the other raceways were calculated accordingly.

DNA was extracted from larvae, water, and biofilm samples by using the Powersoil DNA isolation kit from MoBio Laboratories. In this kit, the lysis step includes a bead-beating step. The hollow fibres from the Dynaguard filters were directly transferred into the lysis tubes included in the kit. To ensure complete DNA extraction from the whole larva samples, the frozen larvae were homogenized individually by using a glass rod to mechanically grind the exoskeleton of the larva, and then transferred to the lysis tubes.

The concentration of total DNA in the extracts was quantified with a NanoDrop spectrophotometer (ND-1000 Spectrophotometer, NanoDrop Technologies). The DNA extracts were stored at −20 °C. An approximately 200 bp fragment encompassing the v3 region of the 16S rRNA gene was amplified using a nested PCR protocol to avoid amplification of Eukaryotic DNA (Bakke et al., 2011). The primers 338 F (5′-actcctacgggaggcagcag-3′) and 518R (5′-attaccgcggctgctgg-3′) were used for the internal PCR reaction. PCR products were analysed by DGGE as described by Muyzer et al. (1993). DGGE was performed with the INGENYphorU DGGE system (Ingeny), using 8% acrylamide gels with a denaturing gradient of 35–55 % (where 100 % correspond to 7 M urea and 40 % formamide), 0.5 times TAE electrophoresis buffer, at 100 V and 60 °C for approximately 18 h. After electrophoresis, DGGE gels were stained with Sybr Gold (1:10 000 dilution, Molecular Probes) for a minimum of 1 h, rinsed with MilliQ water, and visualized and photographed in a GenBox geldoc system (Syngene). The pictures of DGGE gels are supplementary presented in Supplementary Material S1: water 2013, S2: water and biofilm 2014, S3: larvae 4 dph 2013, S4: larvae 8 dph 2013, and S5: larvae 12 dph 2013.

The DGGE gel images were analysed with the software program Gel2K (Svein Norland, Dept. of Biology, University of Bergen, Norway), which converts band profiles to histograms. Peak areas (raw data available in Supplementary Material S6), reflecting the intensities of the DGGE bands, were exported to Excel spreadsheets and used for further statistical analyses.

### 2.3. Statistical analysis

Mean ± standard error (SE), standard deviation (SD) or coefficient of variation (CV) is presented. Statistical analysis was performed in SPSS (SPSS Inc., USA). One-way ANOVA/t-test or Kruskal-Wallis/Mann-Whitney test was used depending on the homogeneity of variance of the variables. Comparisons of survival was done by chi-square tests, and Bonferroni corrected when appropriate. Statistical analyses of the microbial community composition were performed using the program package PAST version 2.04 (Hammer et al., 2001). Percent normalized peak area data from the DGGE gels were used to calculate diversity indices for each sample; band richness (S), Shannon's diversity index (H'), diversity numbers ($e^{H'}$), and diversity number based evenness ($e^{H'}/S$). For comparison of DGGE profiles, Bray-Curtis similarities were calculated from square root transformed, normalized peak area data (Bray and Curtis, 1957). Non- metric multidimensional scaling (NMDS; Taguchi and Oono, 2005) based on Bray-Curtis similarities was used for comparing microbial communities among samples. All NMDS plots were based on one single DGGE gel. One-way PERMANOVA based on Bray-Curtis similarities was used to test for differences in community composition between groups of samples (Anderson, 2001).

## 3. Results

### 3.1. Chemical water quality

Salinity, oxygen, and temperature differed slightly between treatments and experiments (Table 1). Salinity and temperature were higher during the 2013 experiment compared to the 2014 experiment. In the

**Table 1**
Water quality (salinity, oxygen, temperature, total ammonia nitrogen (TAN), nitrite nitrogen ($NO_2$-N) and nitrate nitrogen ($NO_3$-N)) measured in the rearing water of the lobster larvae in the 2013 and 2014 experiment. Letters indicate significant differences between treatments.

| | Salinity | | Oxygen | | Temperature | | TAN | | $NO_2$-N | $NO_3$-N |
|---|---|---|---|---|---|---|---|---|---|---|
| | ppt | | mg $L^{-1}$ | % | °C | | µg $L^{-1}$ | | µg $L^{-1}$ | mg $L^{-1}$ |
| Year | 2013 | 2014 | 2013 | 2014 | 2013 | 2014 | 2013 | 2014 | 2013 | 2013 |
| RAS-ND | $38.2 \pm 1.1^{a}$ | $34.8 \pm 0.4^{a}$ | $7.8 \pm 0.1^{a}$ | $97.1 \pm 2.4$ | $20.0 \pm 0.5^{b}$ | $17.1 \pm 0.5^{b}$ | $73 \pm 31$ | $30 \pm 14$ | $5.3 \pm 1.6$ | $1.0 \pm 0.1^{a}$ |
| RAS-UV | $37.8 \pm 1.3^{a}$ | $34.7 \pm 0.8^{a}$ | $7.6 \pm 0.1^{b}$ | $96.7 \pm 2.2$ | $20.7 \pm 0.4^{a}$ | $17.7 \pm 0.4^{c}$ | $87 \pm 28$ | 30 | $4.5 \pm 2.7$ | $0.9 \pm 0.1^{a}$ |
| FTS | $37.2 \pm 0.5^{b}$ | $36.2 \pm 0.9^{b}$ | $7.8 \pm 0.1^{a}$ | $95.5 \pm 3.0$ | $19.3 \pm 0.5^{c}$ | $16.3 \pm 0.4^{a}$ | $58 \pm 29$ | $35 \pm 21$ | $3.2 \pm 0.8$ | $0.7 \pm 0.1^{b}$ |
| ANOVA | $p = 0.002$ | $p < 0.001$ | $p < 0.001$ | $p = 0.244$ | $p < 0.001$ | $p < 0.001$ | $p = 0.273$ | $p = 0.928$ | $p = 0.169$ | $p = 0.007$ |

2014 experiment pH was 8.2 in the two RAS and 8.0 in the FTS ($p = 0.002$). No significant differences were found in the concentrations of total ammonia nitrogen (TAN) or nitrite nitrogen ($NO_2$-N), but the concentration of nitrate nitrogen ($NO_3$-N) was slightly higher in the two RAS than in the FTS (Table 1, only measured in the 2013 experiment).

### 3.2. Microbial communities in larvae, water and biofilm

An NMDS ordination of the microbial profiles of the incoming water, biofilm and rearing water in replicate raceways in each system for the two independent experiments (Fig. 3), indicate that the three systems represented significantly different microbial communities in the rearing water in both experiments. In the 2013 experiment, the microbial profiles for all sample dates closely group according to system in the NMDS plot (Fig. 3, upper panel). In the 2014 experiment, however, the water microbiota was more similar between treatments at the first sample point (7 dph) than at the two last sample points (Fig. 3, lower panel), which indicated a diverging development between the systems. Statistical comparison confirmed that all three systems had significantly different microbial community compositions of the rearing water in the 2013 experiment (PERMANOVA of Bray-Curtis similarity, Bonferroni corrected $p < 0.037$). In the 2014 experiment the differences in the rearing water microbiota were significant for uncorrected p-values from PERMANOVA of Bray-Curtis similarity, but only marginally significant for Bonferroni corrected p-values ($p = 0.068$ for RAS-UV vs RAS-ND, $p = 0.072$ for RAS-ND vs FTS, and $p = 0.079$ for FTS vs RAS-UV). Fig. 3 indicates that in the 2014 experiment, the microbial composition of the rearing water was more similar to that of the incoming water than to that of the biofilm for all three systems. For comparisons at the same day, average Bray-Curtis similarity ± SD for comparisons of rearing water with incoming water was 0.51 ± 0.15, 0.73 ± 0.09, and 0.63 ± 0.12 in the RAS-ND, RAS-UV and FTS, respectively, and with biofilm 0.33 ± 0.04, 0.35 ± 0.01, and 0.26 ± 0.03 in the RAS-ND, RAS-UV and FTS, respectively.

Average Bray-Curtis similarities for comparison of the microbial community composition of the water between the three systems (Fig. 4, Supplementary Material S7) indicates that the stability over time of the microbial composition of the incoming water to tanks was higher in the

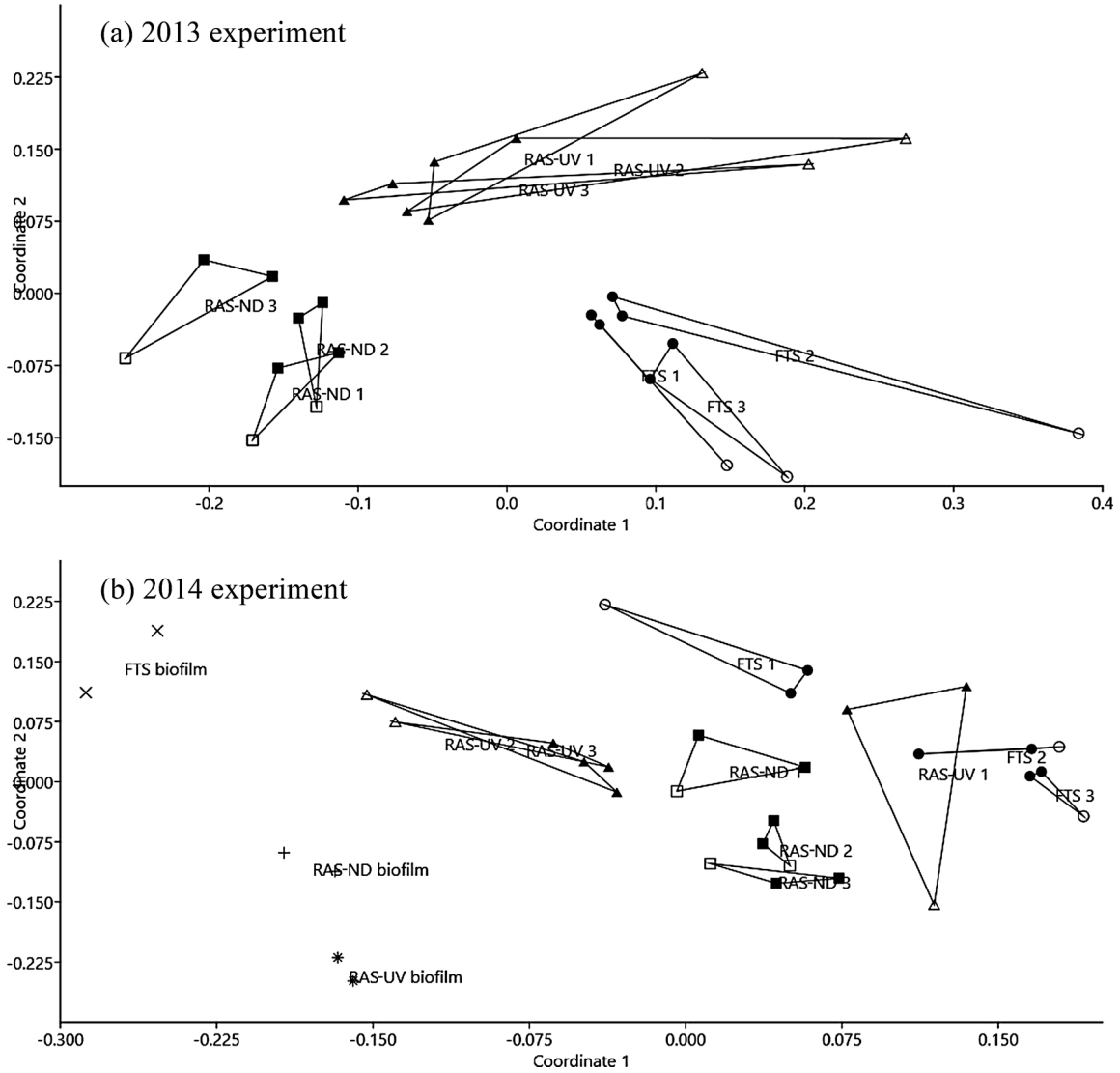


**Fig. 3.** Non-metric Multi Dimensional Scaling (NMDS) based on Bray-Curtis similarities of square root transformed abundance data from microbial profiles (PCR/DGGE) of incoming water (open symbols), rearing water (filled symbols) from three sample times (1, 2, and 3) in the recirculating system without disinfection (RAS-ND, squares), the recirculating system with UV-treatment (RAS-UV, triangles), and the flow through system (FTS, circles) in the (a) 2013 experiment (upper panel, Gel S1), and (b) 2014 experiment (lower panel, Gel S2). Lines connect the points representing the water microflora in each treatment at each sampling date. The lower panel also include samples of biofilm of the raceways in the RAS-ND (+), RAS-UV (*) and FTS (×) at the end of the 2014 experiment.

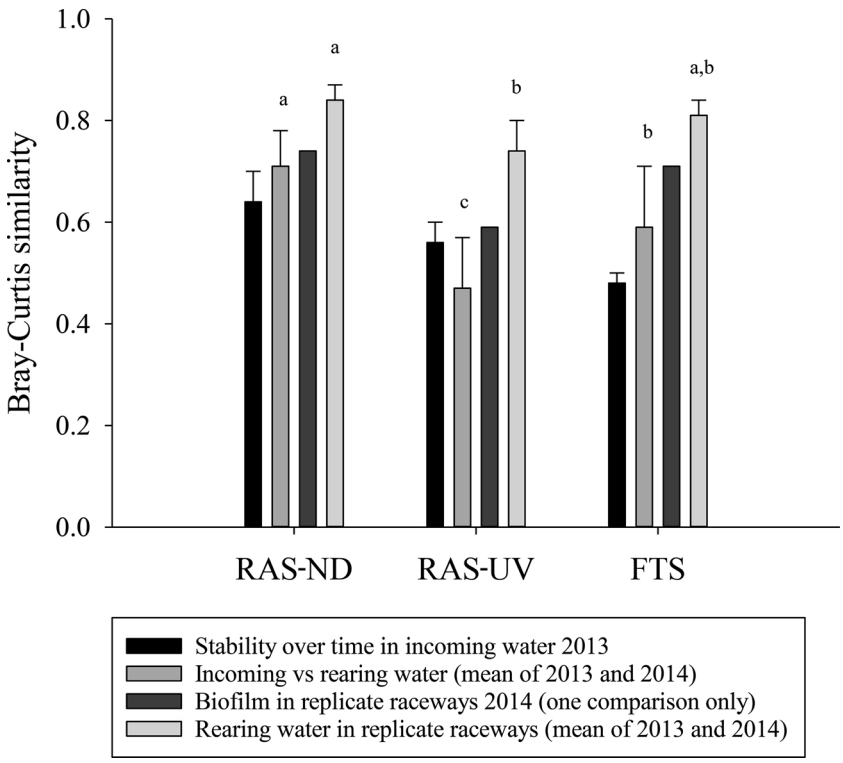


**Fig. 4.** Average Bray-Curtis similarities (± SD) for the three systems comparing the microbial community composition 1: of the incoming water to each raceway over time for the 2013 experiment, 2: between the incoming water and the rearing water in each system (averaged for the two experiments), 3: between the biofilm in each replicate raceway of each system for the 2014 experiment, and 4: between the rearing water in each replicate raceway of each system (average for the two experiments). Letters indicates statistical significance between systems.

RAS-ND, followed by the RAS-UV, and lowest in the FTS. These differences were not statistically significant (2013 experiment only, $p = 0.078$). For both experiments, the microbiota of the rearing water of the RAS-ND was more similar to that of the incoming water than it was in the other systems. Furthermore, for both experiments, the microbiota of the rearing water was more similar to that of the incoming water in FTS than in RAS-UV ($p < 0.001$, Fig. 4). The similarity of the rearing water microbiota between replicate raceways was highest in RAS-ND, followed by the FTS, and lowest in the RAS-UV in both experiments ($p = 0.015$, Fig. 4). In addition, the biofilm microbiota appeared to be more similar between replicate raceways for RAS-ND and lowest for RAS-UV in the end of the 2014 experiment (Fig. 4). However, this could not be tested statistically, because there was only one biofilm sample available from each raceway.

All three systems showed significantly different larval microbiota for the 2013 experiment (PERMANOVA of Bray-Curtis similarity, Bonferroni corrected), at all sampling days except for comparison of FTS and RAS-ND at 4 dph ($p = 0.059$). An NMDS plot of the microbial profiles of individual larvae and rearing water in replicate raceways in each system during the 2013 experiment (Fig. 5, Bray-Curtis similarities in Supplementary Material S8) indicates that the microbiota of the larvae in RAS-ND and RAS-UV was different from that of the larvae in the FTS. In this context it should be noted that the FTS was also the system that showed the lowest survival during the 2013 experiment (Fig. 6).

No clear trends stood out from the diversity indices calculated for the microbial profiles of the DGGE gels (band richness (S), diversity numbers ($e^{H'}$), and diversity number based evenness ($e^{H'}/S$), Supplementary Material S9). The DGGE band richness of the rearing water was significantly higher ($p = 0.037$) and the evenness was significantly lower ($p = 0.011$) in RAS-ND than in the FTS for the 2013 experiment. A somewhat similar pattern was observed for the rearing water samples on the three larvae gels from the same experiment, with higher band richness in the RAS-ND ($p = 0.090$) and significantly higher evenness in the FTS compared to the other treatments ($p < 0.001$). The evenness of the microbial composition was higher in the incoming water of the RAS-UV than the RAS-ND ($p = 0.044$) in the 2014 experiment. However, because the incoming water to the RAS-UV was sampled in the immediate outflow from the UV-treatment, most of the bands probably represented bacteria that had been inactivated. There were only two samples of the biofilm from each system, but they indicate higher DGGE band richness, diversity and evenness in the biofilm of RAS-ND and FTS compared to the RAS-UV in 2014, but due to low power we could not detect statistically significant differences ($p = 0.093$, 0.091 and 0.125, respectively). For larvae, the only significant difference found in microbial diversity indices was a higher diversity at 8 dph in RAS-ND compared to FTS ($p = 0.025$). Although not statistically significant, there were also indications that the band richness was higher in the larvae of the RAS-ND ($p = 0.062$) for the same date.

### 3.3. Survival of larvae

On average, the survival to Stage IV lobsters was highest in the RAS-ND in both experiments (Fig. 6, Bonferroni corrected $p = 0.002$ for replicate raceways and both years together). Mean survival (± SD) to Stage IV was 31 ± 6 %, 22 ± 16 %, and 8 ± 0 % in the RAS-ND, RAS-UV, and FTS, respectively, in the 2013 experiment, and 61 ± 7 %, 38 ± 12 %, and 52 ± 12 % in the 2014 experiment. The survival of larvae in RAS-ND was on average 43 and 275 % higher in 2013, and 64 and 18 % higher in 2014, compared to RAS-UV and FTS, respectively. For replicate raceways together, the survival was significantly higher in RAS-ND compared to FTS in 2013 and compared to RAS-UV in 2014 (Bonferroni corrected $p < 0.001$). Survival of larvae to Stage IV was significantly higher in the 2014 experiment, than in the 2013 experiment ($p = 0.014$). As predicted, for both years seen together, there was less variation in survival between the replicate raceways in the RAS-ND (CV 39 %) compared to the RAS-UV (CV 49 %) and the FTS (CV 87 %). The RAS-UV was the only treatment that showed a statistically significant difference in mortality between replicate raceways ($p = 0.007$ in 2013). Variation in survival (CV) between replicate raceways within the same treatment was 74 %, 19 %, and 0 % in 2013 for RAS-UV, RAS-ND, and FTS, respectively, and 31 %, 12 %, and 23 % in 2014.

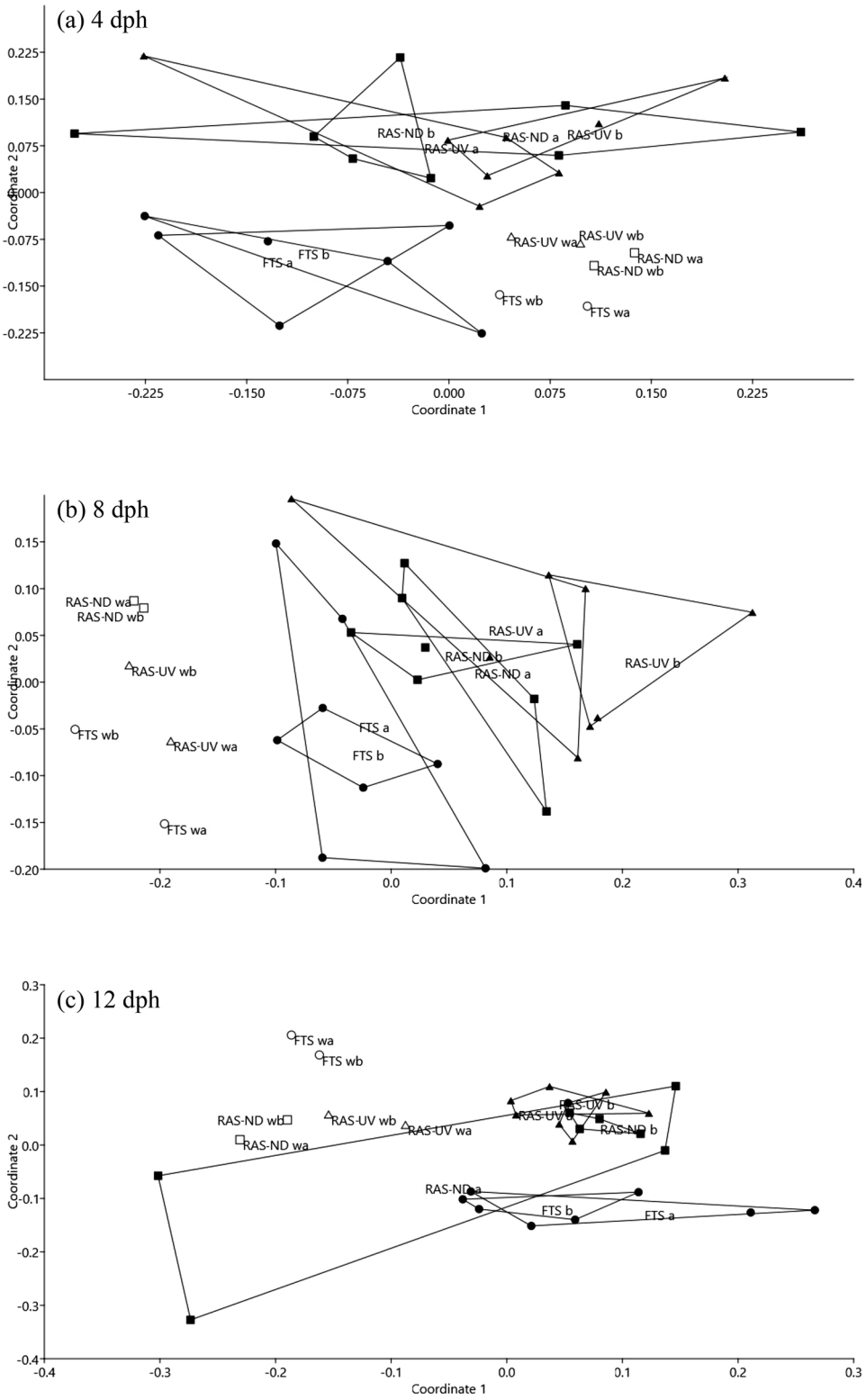


**Fig. 5.** Non-metric Multi Dimensional Scaling (NMDS) based on Bray-Curtis similarities of square root transformed abundance data from PCR/DGGE of the microbial profiles of individual lobster larvae (filled symbols) and rearing water (open symbols) in the two raceways (a and b), respectively, of the recirculating system without disinfection (RAS-ND, squares), the recirculating system with UV-treatment (RAS-UV, triangles), and the flow through system (FTS, circles) at (a) 4 dph (upper panel, Gel S3), (b) 8 dph (middle panel, Gel S4), and (c) 12 dph (lower panel, Gel S5) during the 2013 experiment. Lines connect the points representing the water microflora from individual lobster larvae in each replicate raceway for each treatment.

## 4. Discussion

As predicted, significantly different microbial community compositions developed in the three different systems in both experiments, both in the rearing water and associated with the lobster larvae. This is in accordance with previous studies showing significantly different water microbiota in systems with different water treatment (Attramadal et al., 2012a, b, 2014, Wold et al., 2014) and in marine larvae reared in systems with the same feed and physicochemical conditions, but with different water microbiota (Attramadal et al., 2016; Vestrum et al., 2018). The differences in the microbial community composition between the RAS with and without disinfection were clear, despite the use of DNA-based analyses that underestimate differences in the water microbiota, since bacteria killed by the UV-treatment may still contain DNA of sufficient quality for PCR. Most of the bacteria in the water entering the RAS-UV raceway were likely inactivated, since practically

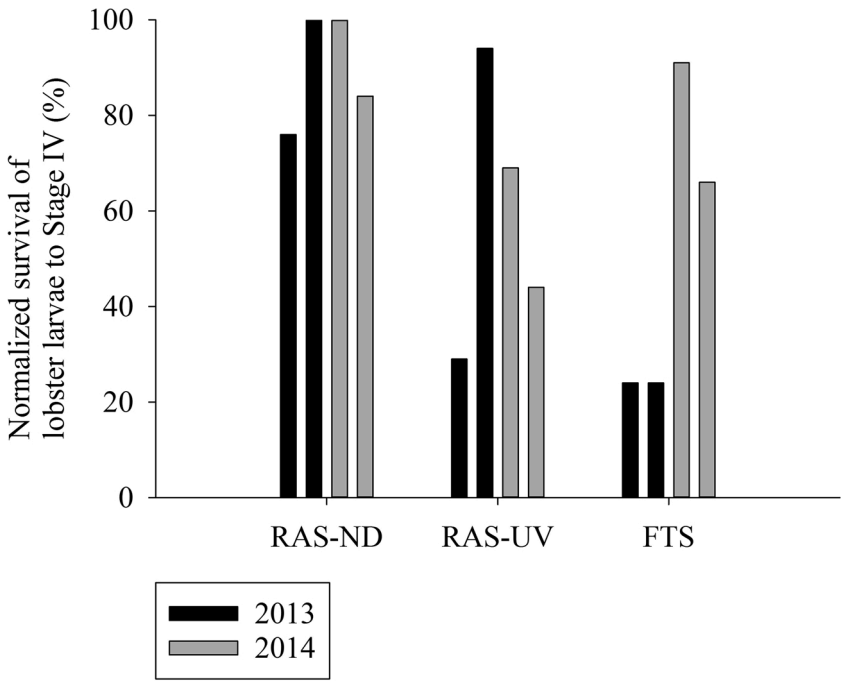


**Fig. 6.** Normalized survival of lobster larvae to Stage IV in the two replicate raceways of each of the recirculating system without disinfection (RAS-ND), the recirculating system with UV-treatment (RAS-UV), and the flow through system (FTS) in the 2013 (black bars) and 2014 (gray bars) experiment. Normalization was done for each experiment by relating the results from all the raceways to the best performing replicate that was set to represent 100 %.

all free-living bacteria in the water are inactivated during strong UV-treatment (Hess-Erga et al., 2008).

As predicted, there are several indications that the RAS without disinfection provided a rearing environment with a microbiota that was more stable over time and more reproducible within the system compared to the other systems. This support previous studies (Attramadal et al., 2014, 2016). For example, the microbiota of the rearing water of the RAS-ND was more similar to the microbiota in the inflowing water to the raceways than in the other systems, for both experiments. In addition, the stability of the microbiota of the inflowing water to the raceways was higher over time in the RAS-ND. Further, the microbiota of the rearing water and the wall biofilm was more similar between replicate raceways in the RAS-ND than in the other systems. Because the microbial re-assembly following a perturbation (i.e. disinfection or sudden increase in CC) is partly stochastic, larger differences are expected between the initial water and the rearing water, and between replicate rearing units in FTS and RAS-UV than in systems with a stable microbiota that is not perturbed (i.e. RAS-ND). This is important, because reduced bacterial regrowth and less change of community composition in RAS-ND means an increased control of the microbial community of the tank water. Based on this and previous work (Attramadal et al., 2014, 2016) we propose that the similarity in numbers (i.e. CC) and composition of bacteria between inflowing water and tank water can be used as a meaningful estimate for the control of the microbial environment of the rearing water.

In comparable experiments with lobster larvae feeding on copepods, survival has varied between 50 and 60 % from hatching until Stage IV (Evjemo, unpublished data). The higher survival of larvae in the 2014 experiment compared to the 2013 experiment may be explained by differences in genetics or in the quality of eggs in the two different batches. For sibling groups with similar feeding and physicochemical conditions, a higher and more stable survival of lobster larvae were observed in RAS-ND compared to FTS in 2013, and RAS-UV in 2014. This supports our hypothesis and shows the potential for high survival of good egg groups given beneficial microbial conditions. On average for the two experiments, the use of UV or FTS reduced the survival with 34 and 44 %, respectively, compared to RAS without disinfection. This is in accordance with previous studies showing that the survival of both Atlantic cod larvae and lumpfish juveniles is reduced in RAS with disinfection and in FTS compared to RAS with low or no disinfection (Attramadal et al., 2012c; Dahle et al., 2020). Compared to RAS-ND, relatively large variations in the survival of lobster was seen between replicate rearing tanks in the RAS-UV, as well as in the FTS between the two different years. This supports the hypotheses that the development of the microbial composition and the survival of larvae is more variable for the systems that have high regrowth combined with r-selection when the water enters the rearing tank.

It cannot be ruled out that differences in water quality may also have influenced survival rates. However, it is considered unlikely that physicochemical water quality had a large impact on survival rates in these experiments, as it was always well within acceptable levels for the lobster larvae (Carly et al., 2010), and the variations between treatments and experiments were small (Table 1).

The relatively high survival of larvae in one of the RAS-UV raceways in 2013 may be related to the presence of mature biofilm in the raceway. There were no samples from the tank wall biofilm in the 2013 experiment. In 2014, in contrast to the previous experiment, the biofilm formed during the maturation period was removed, and the RAS-UV raceways were disinfected before filling with UV-treated water at the start of the experiment. There were only two samples of the biofilm from each system in 2014, indicating higher DGGE band richness, diversity and evenness in the biofilm of RAS-ND and FTS compared to the RAS-UV. The presence of a matured wall biofilm may influence the microbial competition and the initial colonising of the disinfected water of the raceway, thus influencing the larvae. This should be further investigated to decide how to best prepare the system for newly hatched larvae, including a strategy for the biofilter, the rearing water and the wall biofilm of the rearing unit, to optimise microbial conditions during the first days with microbial colonisation of the larvae.

Hydraulic retention time (HRT) in rearing tanks and in the total system is important for microbial selection and the effect of disinfection within a system (Bakke et al., 2017). In the presented experiments, HRT was 8 h in the raceways of the RAS and in the total system of the FTS. The HRT for the total system of each RAS was 10 days, which leave time

for bacterial growth in the rearing water. A different development of the microbiota would be expected in systems with low HRT in the rearing tanks (less than one to half an hour), as the time the water body is in the tank at some point will be too short for significant regrowth of the suspended water microbiota. Further, for other disinfection methods, such as addition of chemicals like peracetic acid or hydrogen peroxide, other effects may be expected, because these disinfectants follow the water through the system. Based on the findings of the presented and previous work, RAS is recommended over FTS for the cultivation of marine larvae where the HRT of the rearing tanks exceeds an hour. For the same scenario it is also advised to avoid point-disinfection of the water entering the rearing tanks of the RAS.

## 5. Conclusion

As predicted, for both independent experiments in 2013 and 2014, the three different water treatment systems developed significantly different compositions of the microbiota of the rearing water and larvae. For both experiments, the RAS without disinfection provided a rearing environment with a microbiota that was more stable over time and more reproducible within the system compared to the other systems.The survival of lobster larvae was higher and more stable in RAS without disinfection than in the other two treatments. In the RAS with UV and the FTS, the average survival was reduced by 34 and 44 %, respectively, compared to RAS with no disinfection. Our work support previous studies that have shown that RAS is beneficial for other marine larvae and document the beneficial effects specifically for lobster larvae. Based on the presented and our previous work, RAS without point-disinfection of the recirculating water is recommended for a beneficial microbial environment and mutualistic host-microbe interactions in the cultivation of marine larvae.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aquaeng.2021.102176.

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