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Research Article

The Effect of UV Irradiation on Rearing Water Quality, Growth, and Survival of European Lobster (*Homarus gammarus*, L.) Larvae

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The successful establishment of European lobster (*Homarus gammarus*) land-based production is hampered by the high mortality rate during larval development, which can be partially explained by the proliferation of pathogens in the communal tanks commonly used for larval rearing. Therefore, disinfection water treatment could improve the low survival rates frequently recorded in *H. gammarus* farming. In this study, we evaluated the effect of UV irradiation on the microbial abundance and physicochemical quality of the culture water, as well as on *H. gammarus* larvae growth and survival reared on a flow-through system for a period of 15 days. Results showed that UV treatment significantly decreased the microbial abundance and reduced the variation in water parameters but did not improve physicochemical water quality (turbidity, ultraviolet transmission, nitrogenous compounds, biochemical, and chemical oxygen demand). Moreover, contrary to the expectations, UV irradiation did not improve but slowed down larvae growth. We initially hypothesized that UV disinfection would enhance the rearing water quality by inactivating bacteria, including potential pathogens, and hence, benefit larvae growth and survival. Our findings suggest that UV disinfection can stabilize the rearing environment but does not benefit *H. gammarus* larvae rearing, at least in a flow-through system set-up. This could be due to UV disinfection eliminating not only potential harmful pathogens but also other microbial groups important for the establishment of a healthy gut microbiota supporting lobster larvae growth.

1. Introduction

The European lobster (*Homarus gammarus*) is among the most valuable shellfish products (Holthuis, 1991). However, *H. gammarus* landings rarely exceed 5000 tonnes per year [1], being insufficient to satisfy the market demand, which has led to an increasing interest in the production of *H. gammarus* in land-based aquaculture systems [2]. The establishment of a viable production of *H. gammarus* would assist the market supply [3, 4] and support the stock-enhancement initiatives aiming at the release of juveniles into natural habitats to improve recruitment [5]. Currently, land-based farming of *H. gammarus* is accomplished by rearing larvae hatched from wild-caught ovigerous females in communal tanks during their pelagic phase (stages I to III) [6], which lasts 2 to 3 weeks under optimal rearing temperature (18°C–20°C) regimes [7].

One of the major issues hampering the viability of *H. gammarus* farming is the low survival rate during the larval pelagic phase, which rarely exceeds 20% [8–11] and is typically much lower, 1–5% until postlarval stage IV (personal observation). Higher survival rates have been reported in studies where *H. gammarus* larvae were reared individually [10, 12], but such a strategy is only operable in small-scale experimental trials. A single female can hatch a high number of larvae per spawning season, up to 15000 individuals according to Goncalves et al. [13], which would be neither practical nor feasible to cultivate individually. The high mortality rate during larval development is, to a great extent, explained by the species strong cannibalistic behavior [11]. However, the rearing conditions under intensive culture can contribute considerably to the problem. Despite the limited scientific literatures, a few diseases caused by parasites and pathogens have been found to affect homarid

lobsters. They include *Gafkaemia*, caused by the Gram-positive bacterium *Aerococcus viridans* var. [14]; the shell disease syndrome, associated with the bacteria *Vibrio* spp. [15]; and the filamentous bacterial disease caused by *Leucothrix mucor* [16]. The last one (*Leucothrix mucor*) is of particular concern for larval *H. gammarus* rearing. This bacterium can proliferate rapidly in the rearing tanks and foul larvae appendages limiting movement and feeding and, consequently, declining larval performance and survival [2]. Even if the levels of disease documented in wild populations are low, they can be problematic in aquaculture as infections can be brought on because of increased rearing temperatures, high stocking densities, cannibalistic attacks leading to injury, and high organic loading [17].

The proliferation of microbes in aquaculture is often controlled by implementing water disinfection in the rearing systems. The disinfection treatment maintains the total microbial abundance at a low level, which also decreases the probability of pathogens in the rearing tanks. Two of the most efficient antimicrobial methods used in aquaculture are ozonation and UV irradiation. Ozone eliminates bacteria directly by damaging cell walls [18] or indirectly by oxidizing organic matter [19]. UV irradiation directly inactivates the bacterial DNA, causing the bacteria to die or loose function [20]. Ozone has been reported to be more efficient in the elimination of bacteria from water than UV disinfection [21] but, when applied in seawater systems, it produces toxic residuals (e.g., bromine and bromate) that may disrupt the normal moulting physiology in lobster species [22]. Conversely, UV disinfection does not generate toxic by-products, and is less costly and complex than ozone, but its efficiency can be severely reduced if high amounts of particles in the water are encountered [20, 23]. The effect of UV irradiation on the culture of European lobster larvae has been previously assessed in semiclosed [9] and closed [12] recirculating aquaculture systems (RAS) but the results are, to some extent, conflicting. Attramadal et al. [12] determined that UV disinfection may disrupt the stability of the microbial environment, reducing *H. gammarus* larvae survival. On the other hand, Middlemiss et al. [9] showed that the use of UV in combination with ozone was efficient at controlling the levels of pathogens in the culture water but it was not sufficient to significantly increase survival rates in the larviculture of *H. gammarus*.

In this study, we investigated the effect of UV disinfection on the microbial abundance and physicochemical water quality, as well as *H. gammarus* larvae growth and survival reared on a flow-through system (FTS) during the most critical period of its cultivation (stages I to III). In comparison to RAS, FTS can promote unstable and unpredictable environments with empty niches and high resources available per bacterium, favoring the selection of opportunists [12]. This is due to FTS having high and unstable nutrient loads as well as low hydraulic retention times in the rearing tanks [24]. We, therefore, hypothesized that continuous exposure of the rearing water to UV irradiation in an FTS would eliminate bacteria, which, in turn, would benefit the rearing water quality and improve *H. gammarus* larvae growth and survival.

2. Materials and Methods

2.1. Larval Rearing Regime and Systems. The experiment was conducted at the facilities of the National Institute of Aquatic Sciences (DTU Aqua), Section for Aquaculture, in Hirtshals, Denmark. Lobster larvae were obtained from three ovigerous females captured along the Skagerrak coast in North Jutland, Denmark, and reared as described in detail by Goncalves et al. [13]. Briefly, newly hatched larvae were collected from broodstock tanks with a net and transferred to six 46 L cylindroconical transparent acrylic tanks, which were part of a flow-through system. Each tank was seeded with 407 larvae over two consecutive days (a density of 8.8 larvae L^{-1}) keeping the same daily distribution in all tanks. All six tanks used were equipped with a bottom seawater inlet kept at a constant flow of 5–7 $L{\cdot}h^{-1}$ and an outflow filter (0.7 mm mesh size). Strong aeration was provided at the bottom using airstones to facilitate larvae maintenance in the water column. Additionally, a recirculation pump (Tunze, Germany) was installed in each rearing tank, allowing an internal recirculation flow of 60 $L{\cdot}h^{-1}$. In three tanks, the internal flow water was forced through a procrystal UV-C 11 W sterilizator (JBL, Germany) and irradiated prior to entering the rearing tank (UV-treatment) resulting in an UV dose of 577 $mWs.cm^{-2}$. The remaining three tanks were not equipped with UV reactors and were used as controls (CTRL-treatment). A diagram of both treatment set-ups is presented in Figure 1.

Larvae were fed with thawed minced Antarctic krill (*Euphausia superba*) (Akudim A/S, Denmark) distributed 5 times per day: manually at 8:00 AM and 1:00 PM and by automatic dispensers controlled by a BT300-2J peristaltic pump (Longerpump, UK) at 6:00 PM, 11:00 PM, and 4:00 AM. The Antarctic krill amount supplied to the tanks was adjusted to larval size and density during the experiment resulting in a progressive reduction due to the high observed mortality (Table 1). Water temperature, salinity, and dissolved oxygen levels were monitored daily and kept constant throughout the experimental period ($20.0 \pm 0.3^{\circ}C$, 33–36 PSU, >97% dissolved oxygen).

2.2. Growth and Survival. Lobster larvae and postlarvae body weight, carapace length, and development stage were determined for 10 individuals per tank at 0, 4, 8, 12, and 15 days poststocking (DPS), unless survival was below 10 individuals, as it was the case in one of the UV tanks by 15 DPS. Wet body weight was recorded to the nearest 0.001 g after gently blotted drying each individual lobster with a paper towel. Larvae and postlarvae were observed and photographed with a stereomicroscope (MC125 C, Leica, Germany) equipped with a digital camera (MC190 HD, Leica, Germany). Carapace length, the distance from the base of the eye socket to the posterior edge of the cephalothorax, was measured using ImageJ 1.52 n software (University of Wisconsin, USA). Each individual development stage was determined according to Rötzer and Haug (2015). On the same sampling day, all six tanks were emptied, and the number of live individuals was counted for an estimation of the survival rate.

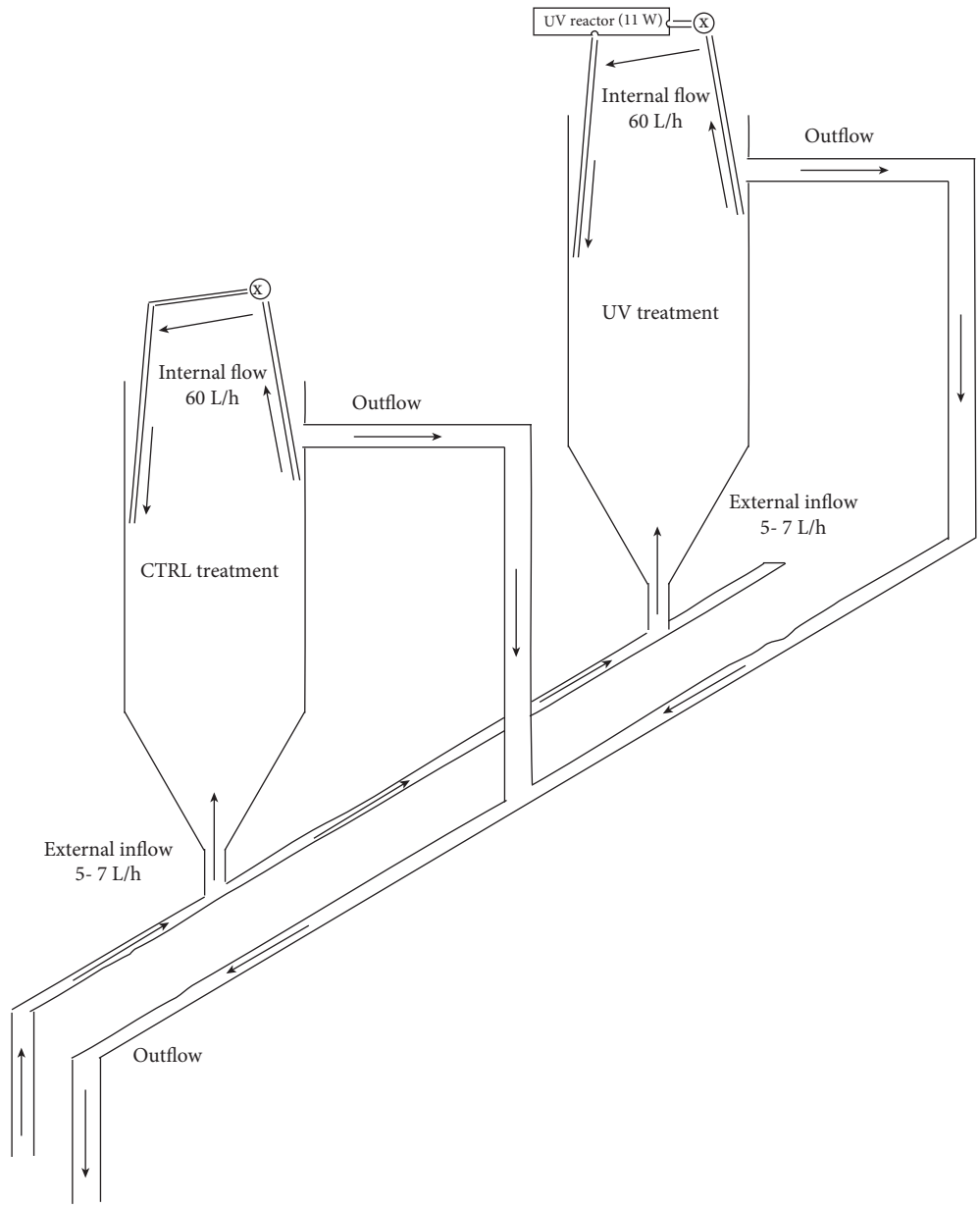

Figure 1: Schematic representation of the systems used in the experiment with European lobster larvae (*Homarus gammarus*). Arrows indicate the direction of the water flow.

Table 1: Food quantity supplied (g per tank) during the experimental period.

0–4 DPS	5–9 DPS	10–15 DPS
16.7	8.3	6.7

DPS: days poststocking.

2.3. Culture Water Sampling and Analysis. Water samples were collected over the experimental period following the schedule summarized in Table 2. Before the first feeding in the morning at 8:00 AM, a 2 L sample was collected near the outlet filter of each rearing tank. Each sample was split into homogeneous subsamples for the analysis of several parameters, as indicated in Table 2. All analyses were performed in duplicate.

The pH was measured using a Hach HQ40d portable multimeter (Hach Lange, USA). Total ammonia-, nitrite-, and nitrate-nitrogen concentrations were determined spectrophotometrically according to DS (1975), ISO 6777 (1984), and ISO 7890-1 (1986), respectively. Total chemical oxygen demand (COD) and 5-day biological oxygen demand (BOD_5) were measured in unfiltered water samples following the standard methods ISO 6060 (1989) and ISO

Table 2: Water quality parameters sampled, analytical methods, and frequency of testing for each.

Parameters	Method of analysis	Frequency of testing
Dissolved oxygen	OxyGuard probe	Recorded daily
Temperature	OxyGuard probe	Recorded daily
Salinity	Seawater refractometer	0, 2, 4, 6, 8, 10, 12, 14, and 15 DPS
pH	Hach 2100Q probe	0, 2, 4, 6, 8, 10, 12, 14, and 15 DPS
Total ammonia nitrogen	Spectrophotometry	0, 2, 4, 6, 8, 10, 12, 14, and 15 DPS
Nitrite nitrogen	Spectrophotometry	0, 2, 4, 6, 8, 10, 12, 14, and 15 DPS
Nitrate nitrogen	Spectrophotometry	0, 2, 4, 6, 8, 10, 12, 14, and 15 DPS
Turbidity	Turbidmeter	0, 3, 7, 11, and 15 DPS
Ultraviolet transmittance	UV spectrophotometry	0, 3, 7, 11, and 15 DPS
Microbial abundance	Flow cytometry	0, 3, 7, 11, and 15 DPS
BOD_5	Potentiometry/O_2 probe	0, 9, and 15 DPS
COD	Spectrophotometry	0, 9, and 15 DPS

DPS: days poststocking.

5815-2 (2003), respectively. Additionally, dissolved COD was measured in filtered samples (0.45 μm filter, Filtropur S 0.2, Starstedt, Germany). Turbidity was determined using a Hach 2100Q (Hach Lange, USA) and ultraviolet transmission (UVT) using a Beckman DU® 530 Life Science UV/Vis spectrophotometer (Bechman Coulter Inc., USA).

The microbial abundance was estimated using flow cytometry. Briefly, 10 mL of rearing water from each tank was filtered through a cell strainer (40 μm FisherBrand, Thermo Fisher Scientific, USA). A 500 μL subsample of the filtrate was labelled with 5 μL of SYBR Green (100×, MilliporeSigma, Germany) and 5 μL of propidium iodide (PI, 600 μM, MilliporeSigma, Germany) and incubated at 37°C for 10 min. At the end of the incubation period, the abundance of total and live cells (cells·mL^{-1}) was measured with a BD Accuri C6 Plus flow cytometer (Becton, Dickinson, and Company, USA).

2.4. Statistical Analysis. All data shown are mean ± 1 SEM unless otherwise specified. Before analyses, normality and homogeneity of variances were tested using the Shapiro-Wilk and Levene's tests, respectively. In instances where the parametric assumptions were met, survival and growth parameters were compared between treatments for each sampling point using a Student's *t*-test; otherwise, a Mann–Whitney test was used. Water quality parameters were compared at the end of the trial (with average values of 7–15 DPS) using the same strategy, i.e., using a Student's *t*-test when parametric assumptions were met, and the Mann–Whitney *U* test when assumptions were not met. Additionally, a principal component analysis (PCA) was performed using the average values (7–15 DPH) of the water quality parameters. The PCA analysis was carried out using R version 3.5.1 software (R Core Team, 2018) and the factoextra version 1.07 package (Kasambara and Mundt, 2020). All other statistical tests were performed with IBM SPSS Statistics 25.0 (IBM Corp., USA) and graphics generated by GraphPad Prism 5.0 software (GraphPad Software, USA).

3. Results

3.1. Growth and Survival. Lobsters reared in tanks exposed to UV irradiation exhibited a trend for higher survival compared to those reared in control tanks until the first half of the trial. Results show that survival was significantly higher (UV = 33.6%, CTRL = 20.8%) 8 days poststocking (DPS) (Figure 2(a), Table 3) but the percentage of larval survival in UV tanks (4.9%) approached CTRL levels (4.8%) towards the end of the experiment (Figure 2(a)). Larvae did, however, grow faster in the CTRL rearing tanks (Figures 1(b) and 1(c)), with significantly increased weight and length observed at 8–12 DPS and 8–15 DPS, respectively (Table 3).

UV irradiation did not significantly affect the development rate (proportion of the predominant larval stage) over the 15-day rearing period (Figure 3). However, the development of successive zoea stages in the UV group slowed down towards the end of the experiment. A small proportion of UV-larvae remained at stage II by days 12 and 15, while in the CTRL group, all larvae developed into stage III in 12 days poststocking.

3.2. Microbial Abundance and Physicochemical Water Quality. The UV disinfection led to significant changes in the microbial abundance of the rearing environment: the abundance of total and live cells was more than 2-fold higher in the CTRL tanks compared to those exposed to UV irradiation (Table 4). However, the impact of UV irradiation on the microbial abundance was not translated into significant changes in the physicochemical water quality parameters, except for nitrite-N levels (6 and 17 μg·L^{-1}), which were significantly decreased in the culture water treated with UV irradiation. The ultraviolet transmission exceeded 95% in both the UV and CTRL treatments. The turbidity was on average lower in the CTRL tanks (0.67 NTU) than in the UV tanks (1.11 NTU), but there was a high variation between replicates and, therefore, no significant differences were detected between both treatments. Neither the chemical (total and dissolved COD) nor the five-day biological (total BOD_5) oxygen demand was affected by UV disinfection. No significant differences were observed in TAN (240 and 266 μg·L^{-1}) and nitrate-N (271 and 269 μg·L^{-1}) levels between the CTRL and UV groups.

A principal component analysis was carried out using the average values (over 7–15 days poststocking) for the microbial abundances and physicochemical variables recorded for each tank to show the differences between the CTRL- and UV-treatments regarding the water quality in the

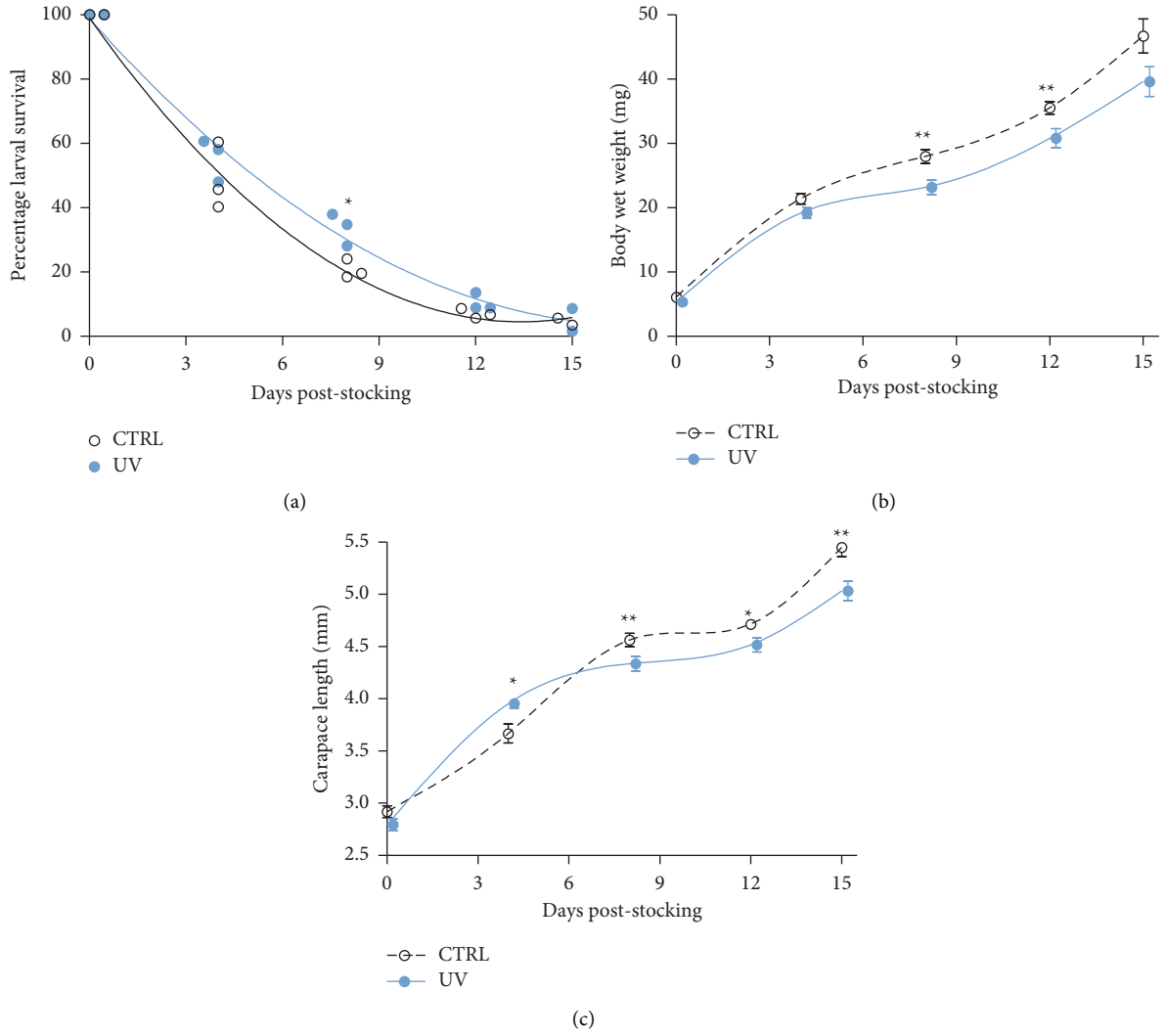

FIGURE 2: *Homarus gammarus* larval response to UV irradiation in terms of survival (panel (a)), body weight (panel (b)), and carapace length (panel (c)). In the upper panel (a), data points represent survival in each replicate tank. In panels b and c it is represented as mean ± 1 SEM, the sample size is equal to all data points ($n = 30$), except for UV at day 15 poststocking ($n = 26$).

rearing environment (Figure 4). Two principal components (PC) explained 73.8% of the variance (PC1 41.0% and PC2 32.8%). From all variables, turbidity, nitrite-N, and live cells showed the highest loadings in PC1 (−0.454, 0.442, and 0.388, respectively). Total cells (−0.474), UVT (0.417), and nitrite-N (0.413) showed the highest loadings in the PC2. The PC1 was the component that separates the CTRL and UV groups, with UV tanks forming a cluster in the left area of the plot, indicative of a positive correlation with turbidity and a negative correlation with nitrite-N and live cell abundance. The control showed a more dispersed distribution within the plot area, indicating greater variability in the water quality of the tanks not exposed to UV irradiation.

4. Discussion

In this study, we found that the continuous exposure of the rearing water to UV irradiation in the present FTS used to cultivate *H. gammarus* larvae successfully decreased the bacterial load in the rearing tanks. However, UV disinfection did not cause significant changes in the physicochemical water quality parameters nor improved lobster larvae survival rates. Moreover, *H. gammarus* larval growth was decreased in the tanks treated with UV. Even though no benefit was determined from the use of UV in *H. gammarus* larviculture, it is worth noting the higher variability in the water quality parameters in the CTRL tanks (Figure 4). Flow-through systems are characterized by low hydraulic retention times with empty niches [24] and, hence, prone to unstable and unpredictable environments favoring selection for opportunistic bacteria [12]. Reducing these bacteria in FTS with UV disinfection can, therefore, promote a more stable rearing environment, capable of sustaining a more stable and reproducible production of larval lobsters over time.

The continuous exposure of the rearing water to UV irradiation in the flow-through lobster larvae culture system resulted in a 52% and 58% reduction of the total and live cell

Table 3: Summary of statistics for testing the effect of UV disinfection on survival and growth of larvae and postlarvae *Homarus gammarus*.

	Student's *t*-test/Mann–Whitney *U* test
Survival	
4 DPS	$t_{(3.4)} = 0.950,\ p = 0.404$
8 DPS	$\mathbf{t_{(3.2)} = 3.819}$, $p = 0.027^{*}$
12 DPS	$t_{(3.2)} = 1.944,\ p = 0.142$
15 DPS	$t_{(2.4)} = 0.042,\ p = 0.970$
Wet body weight	
0 DPS	$t_{(58)} = 0.950,\ p = 0.404$
4 DPS	$U = 325.5,\ n_{ctrl/uv} = 30,\ p = 0.066$
8 DPS	$\mathbf{U = 237.5,\ n_{ctrl/uv} = 30},\ p = 0.002^{**}$
12 DPS	$\mathbf{U = 257.0,\ n_{ctrl/uv} = 30},\ p = 0.004^{**}$
15 DPS	$t_{(54)} = 1.988,\ p = 0.052$
Carapace length	
0 DPS	$U = 333.5,\ n_{ctrl/uv} = 30,\ p = 0.082$
4 DPS	$\mathbf{U = 589.5,\ n_{ctrl/uv} = 30},\ p = 0.037^{*}$
8 DPS	$\mathbf{U = 272.0,\ n_{ctrl/uv} = 30},\ p = 0.008^{**}$
12 DPS	$\mathbf{U = 280.5,\ n_{ctrl/uv} = 30},\ p = 0.011^{*}$
15 DPS	$\mathbf{U = 207.0,\ n_{ctrl} = 30/n_{uv} = 26},\ p = 0.003^{**}$

DPS: days poststocking; rows in bold highlight statistical significance.

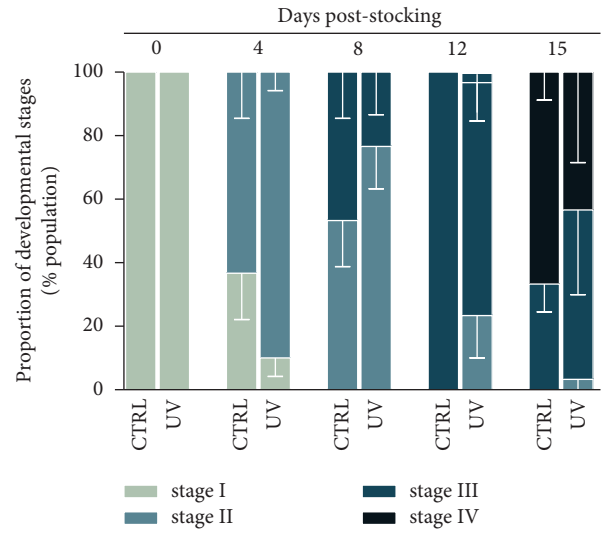

Figure 3: *Homarus gammarus* larval development rate throughout the 15 day rearing period. Bars represented as mean ± 1 SEM ($n = 3$).

abundances, respectively. An UV dose of 577 mWs·cm^{-2} was estimated for the UV reactor used in this trial, which is considerably greater than the 30–35 mWs·cm^{-2} doses typically recommended in commercial aquaculture [25]. Yet, despite the high dose, the degree of bacteria removal was relatively low compared to those reported previously in research studies conducted in recirculating aquaculture systems. For example, de Jesus Gregersen et al. [26] used an UV dose of approximately 100 mWs·cm^{-2}, which resulted in an 88% reduction of bacterial activity. Sharrer et al. [27] achieved 98% and 81% reductions in heterotrophic bacteria counts using doses of 1800 mWs·cm^{-2} and 300 mWs·cm^{-2}, respectively. Hess-Erga et al. [23] achieved 99.9% inactivation of the free-living bacteria with a dose of 350 mWs·cm^{-2}, while a dose of 2120 mWs·cm^{-2} was needed to inactivate the same proportion of particle-associated bacteria, which are more protected and, thus, more resistant to UV disinfection than free-living microorganisms. Indeed, the lower bacteria removal efficiency observed in the current study than in the previous mentioned could be caused by a higher proportion of particle-associated bacteria since the methodology used here to estimate microbial abundance—flow cytometry—mainly targets free-living cells. However, the differences between this and the examples presented before are more likely to be explained by the lower efficiency of UV disinfection in FTS compared to RAS. Even though the hydraulic retention time was kept at a moderate level (7 h–9 h) in the tanks, the number of bacteria and particulate organic matter flushed out of the rearing tanks was likely higher than that in a closed recirculating system. That would already keep the particle concentration at low levels in the rearing tanks, which could limit the additional benefits of UV disinfection. This idea is supported by the lower microbial abundance recorded in our rearing tanks (maximum abundance detected = 9.0×10^6 cells·mL^{-1}) in comparison to a RAS system, where a maximum microbial abundance of 1.1×10^8 cells·mL^{-1} was estimated using the same flow cytometry methodology [28]. Moreover, it cannot be ruled out that new microorganisms were continuously introduced in the rearing tanks through the inlet flow (5–7 L·h^{-1}) of seawater. In this study, the tanks were supplied with seawater directly pumped from the sea, which was only mechanically filtrated by forcing its passage through a sand seabed. No additional disinfection treatment was applied before the water was delivered to the rearing tanks. The initial microbial abundance of 1.9×10^5 cells·mL^{-1} recorded at the beginning of the experiment, before stocking the rearing tanks with larvae, supports this argument.

It was initially hypothesized that, in addition to the direct effect of UV on microbial abundance, the disinfection would also indirectly affect other water quality parameters. Nevertheless, that was not the case for most of the physicochemical parameters measured. One of the most obvious expected side effects of UV disinfection was a reduction in BOD_5. BOD is the amount of oxygen consumed by microorganisms to break down organic matter and, hence, a strong positive correlation between bacterial abundance and BOD is often observed [29]. Likewise, we expected lower

Table 4: Water quality characteristics in the rearing tanks exposed to UVC irradiation (UV) or unexposed (CTRL). Values are given as mean ± 1 SEM over 7–15 days poststocking.

	CTRL	UV	*N*	Statistics
Total cells ($\times 10^6$ cells·mL^{-1})	**3.53 ± 0.65**	**1.70 ± 0.25**	**9**	**$t_{(16.0)} = 2.625^{*}$, $p = 0.018$**
Live cells ($\times 10^6$ cells·mL^{-1})	**1.81 ± 0.23**	**0.76 ± 0.12**	**9**	**$t_{(12.2)} = 4.116^{**}$, $p = 0.001$**
Proportion of dead cells (%)	43.66 ± 5.00	54.41 ± 3.24	9	$t_{(16.0)} = -1.807$, $p = 0.090$
TAN (μg NH_4-N L^{-1})	239.53 ± 40.10	266.40 ± 30.71	15	$t_{(28.0)} = -0.532$, $p = 0.599$
Nitrite (μg NO_2-N L^{-1})	**17.60 ± 3.69**	**6.33 ± 0.53**	**15**	**$t_{(14.6)} = 3.019^{**}$, $p = 0.009$**
Nitrate (μg NO_3-N L^{-1})	271.47 ± 8.02	269.07 ± 7.08	15	$t_{(28.0)} = 0.224$, $p = 0.824$
UVT (% transmission)	95.27 ± 0.54	95.17 ± 0.56	9	$t_{(16.0)} = 0.124$, $p = 0.903$
Turbidity (NTU)	0.67 ± 0.07	1.11 ± 0.25	9	$t_{(9.1)} = -1.656$, $p = 0.132$
Total COD (mgO_2 L^{-1})	11.85 ± 0.55	12.52 ± 0.19	6	$t_{(10.0)} = -1.158$, $p = 0.274$
Dissolved COD (mgO_2 L^{-1})	10.43 ± 1.52	10.87 ± 0.50	6	$t_{(10.0)} = -0.276$, $p = 0.788$
Total BOD_5 (mgO_2 L^{-1})	0.56 ± 0.09	0.79 ± 0.14	6	$t_{(10.0)} = -1.432$, $p = 0.183$

Rows in bold highlight statistical significance. NTU: Nephelometric turbidity unit.

levels of total BOD_5 in the UV tanks because of the lower microbial abundance observed in this treatment. However, no significant differences between the UV and CTRL groups were found. This can be explained by the very low BOD_5 concentration (0.56 mg·L^{-1} and 0.79 mg·L^{-1}) in our systems as compared to typical levels (1–10 mg·L^{-1}) in intensive, flow-through land-based aquaculture systems [30]. Such low levels might have limited the detection of an improvement in the UV treatment. The low total BOD_5 levels recorded in both treatments indicate that the bacteria may have been carbon limited, particularlyin the CTRL tanks, where there was less carbon available per bacterium compared to the UV tanks.

The reduction in microbial abundance in the UV tanks was also expected to be accompanied by a decline in COD as microbial organic matter, i.e., the organic matter that makes up the body of microbes, constitutes part of the COD, in particular the particulate fraction. However, we did not detect any significant changes in total or dissolved COD between the UV and CTRL groups. One possible explanation for this could be the high proportion of dissolved COD (87% and 88% in the UV and CTRL systems, respectively) and, consequently, a small amount of particulate COD. de Jesus Gregersen et al. [26] observed that an UV dose of 100 mWs·cm^{-2} led, in addition to a large reduction in the microbial activity, to a decrease in the COD levels, but the proportion of dissolved COD in their pilot RAS system was considerably lower (approximately 60%) than that in our FTS. Additionally, it could be that organic matter was flushed out of the FTS at a much higher rate than in the system used by de Jesus Gregersen et al. [26]. The higher COD levels (23–54 mg·L^{-1}) in the pilot RAS [26] in comparison to those recorded in our FTS (11.9–12.5 mg·L^{-1}) support the argument. The lack of effect of UV on the water clarity estimated by means of turbidity and UVT is also likely related to the flushing of particles out of the FTS.

Interestingly, the use of UV caused a significant decrease in the nitrite-N levels, while no significant effect was detected on total ammonia-N or nitrate-N. At best, a decrease in nitrate-N could have occurred as nitrate can be chemically transformed to nitrite during exposure to UV irradiation when nitrate levels are high [31]. Despite the higher nitrite-N levels in the CTRL group, all three forms of nitrogenous waste were maintained at low levels in both treatments, suggesting that the moderate water exchange rate in the present FTS was enough to control nitrogenous compounds. Since the nitrification process mainly occurs in the biofilter component of RAS [32], we did not anticipate the presence of nitrifying bacteria in the rearing tanks of none of the two treatments in sufficient numbers to convert ammonia-N into nitrite-N. The relatively low, but still higher, nitrite-N level in the CTRL tanks suggests that the absence of UV disinfection may favor the growth of AOB bacteria, which most likely thrive in biofilms formed on the surface of the rearing tank walls.

The toxicity of nitrite-N is considerably lower than that of ammonia-N; in particular in seawater as chloride blocks the uptake of nitrite [33], but theoretical "safe" limits may underestimate its impact on the growth and physiological condition of crustaceans [33]. An example is the demonstration that nitrite-N levels of 4 mg·L^{-1} reduced the moult increment of *Penaeus monodon* juveniles, causing lower growth after 20 days [34] albeit the "safe" levels would theoretically be 17 mg·L^{-1} [35]. Any negative effect of nitrite-

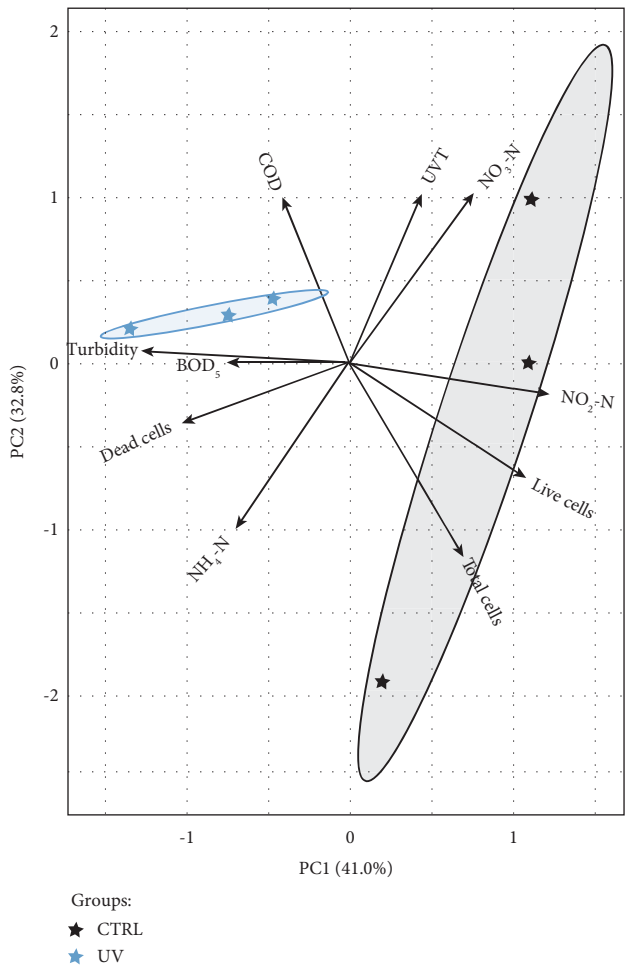

FIGURE 4: Principal component analysis (PCA) of water quality parameters of rearing tanks exposed (UV-treatment) or unexposed (CTRL-treatment) to UV disinfection. The mean values over the period 7–15 days poststocking recorded for the biochemical and microbial water parameters (Table 4) in each tank were used to form the principal components. PC1 separated the water quality horizontally and explained 41% of the variance. The PC2 separated the variables vertically and explained 32.8% of the variance. The contribution of the variables (biochemical and microbial parameters) is represented by the arrows and the stronger the correlation with PC1 or PC2, the longer the arrow is.

N on the condition or growth of *H. gammarus* larvae in this study is, however, unlikely as the levels recorded were extremely low (<18 μg NO_2-N L^{-1}).

Despite the bacterial loading reduction in the culture water, UV disinfection did not confer any obvious advantages in the survival and growth of the *H. gammarus* larvae. Even though UV enhanced survival at an intermediate sampling point during larval rearing, the survival rate was below 9% in all tanks by the end of the experiment, and no significant difference was detected between the CTRL and UV groups. Similar results were obtained by Middlemiss et al. [9], who observed that the combined use of UV and ozone disinfection was effective at reducing bacterial loading in the rearing tanks but without a significant improvement in the *H. gammarus* larvae survival rates. More recently, Attramadal et al. [12] compared the bacterial environment in RAS with and without UV disinfection used to cultivate *H. gammarus* larvae and observed that the survival rate was higher in the RAS without UV than in the RAS with UV. This was explained by reduced regrowth and smaller changes in the microbial community composition in the absence of UV disinfection.

In addition to the lack of effect on survival by the end of the experiment, the use of UV caused a decrease in *H. gammarus* larval growth. In a previous study, *H. gammarus* larvae showed a similar response when exposed to ozonation, i.e., the length and weight of larvae declined in the tanks where the rearing water was disinfected with ozone [11]. The authors suggested that the reduction in growth was potentially caused by the effects of ozone on water chemistry and, hence, larval nutrition and physiology. In this study, since UV disinfection does not generate any toxic residuals as ozone does, changes in water chemistry are unlikely to explain any effect of UV on the larvae growth. Instead, it is more likely that growth changes have been caused by adjustments in water microbiological parameters. Here, we only evaluated the microbial abundance and, therefore, can only speculate on the effect of UV on the microbial community composition. Nevertheless, it is reasonable to assume that a reduction in the total amount of microbes in the UV tanks also caused a shift in the microbial community composition. Even though the target was to eliminate pathogenic bacteria, UV disinfection is not selective and can inactivate both harmful and beneficial bacteria in the community. It has also been demonstrated that the gut microbiome of aquatic invertebrates is flexible and can be modulated by medium-associated microbes [36, 37]. In turn, changes in the gut microbiome community can influence digestive enzyme activity and the subsequent digestion of the host, which contributes to its growth [38]. Bearing these arguments in mind, we suggest that the higher growth performance of CTRL larvae is a consequence of a more diverse and beneficial microbial community in the CTRL rearing tanks. Recent findings that *H. gammarus* reared in a sea-based container culture system presented a more diverse gut microbiome conferring benefits to the health and growth of their hosts than those reared in a land-based culture system [39] support our hypothesis.

5. Conclusions

The use of a high UV dose (577 mWs·cm^{-2}) successfully reduced the microbial abundance in the rearing tanks of a flow-through system culturing *H. gammarus* larvae. However, no significant impact was detected in several other biochemical water parameters, including organic matter content, water clarity, and waste nitrogenous compounds, except for the higher nitrite-N level in the UV tanks. In general, the water quality was maintained in fairly good conditions in both treatments, which might have limited the additional benefits of UV disinfection. While no effect of UV on survival rate was detected, surprisingly, larval growth decreased in the rearing tanks exposed to UV irradiation. The impact of UV disinfection on the culture water and

lobster larval microbiome composition should be considered in future studies as medium-associated microbes may play an important role in shaping the host gut microbiome, with consequences for their health and growth.

Data Availability

The research data generated during the current study will be made public by the corresponding author via the DTU data respiratory on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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