PRINT ISSN 1119-8362
Electronic ISSN 2659-1499
Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja
J. Appl. Sci. Environ. Manage.
Vol. 28 (10) 3235-3243 October, 2024

Efficient Low-Technology Apparatus for Sterilising Seawater for Mariculture Research

[1,2]ABUBAKAR, F; [1]BOATENG, A; [1,3]ASIEDU, D; [1,4]CHUKU, E; [1]MIREKU, KK; *[1]ACHEAMPONG, E

*[1]*Department of Fisheries and Aquatic Sciences (DFAS), and Centre for Coastal Management, African of Excellence in Coastal Resilience (ACECoR), University of Cape Coast, Ghana.*
[2] *College of Arts and Sciences, University of New Haven, New Jersey, USA.*
[3] *National Institute of Aquatic Resource, Technical University of Denmark, Kemitorvet, Kongens 9 Lyngby, Denmark.*
[4] *Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania.*

**Corresponding author Email: emmanuel.acheampong@ucc.edu.gh*
**ORCID: https://orcid.org/0000-0001-6243-294X*
**Tel: +233 50 492 9936*

Co-authors Email: abubakarfurkan@gmail.com; alexasomeniboateng1234@gmail.com; delas@aqua.dtu.dk; eobengchuku@ucc.edu.gh; kmireku@ucc.edu.gh

ABSTRACT: One problem limiting marine biotechnology in low-income countries is lack of durable, inexpensive equipment to sterilise seawater to develop species for research and application (e.g., in medicine and feed production). Autoclaves flounder in low-income countries due to socioeconomic limitations on the maintenance of the equipment. Hence, the objective of this paper was to propose a bench-top ultraviolet (UV) sterilisation apparatus for seawater. The apparatus was fabricated with polyvinyl chloride materials to hold and release filtered seawater into a sterilisation chamber fitted with UV lamp that emits monochromatic light at 254 nm. These materials are relatively cheap and easy to ensemble from local sources. Tests conducted on polluted, tropical seawater collected from the Elmina fishing harbour, near Cape Coast, Ghana show that the apparatus has a disinfection efficiency of 99.6% in 60 minutes, which is similar to the efficiency achieved by autoclave and other expensive methods. The new apparatus can therefore be used to sterilise media for mariculture research and biotechnology applications in resource-constrained environments.

DOI: https://dx.doi.org/10.4314/jasem.v28i10.34

Cite this Article as: ABUBAKAR, F; BOATENG, A; ASIEDU, D; CHUKU, E; MIREKU, K. K; ACHEAMPONG, E. (2024). Efficient Low-technology Apparatus for Sterilising Seawater for Mariculture Research. *J. Appl. Sci. Environ. Manage.* 28 (10) 3235-3243

Dates: Received: 07 July 2024; Revised: 15 August 2024; Accepted: 19 August 2024 Published: 05 October 2024

Keywords: **M**ariculture research; Seawater sterilisation; Marine microbiology; Ultraviolet light; Coliform bacteria

The cost of importing scientific equipment and spare parts is one principal factor limiting scientific research in low-income countries (Öman *et al*., 2006; Vose and Cervellini, 1983). These countries import the bulk of their science equipment mostly from the Western countries, often with financial support from international donors and foreign governments (Vose and Cervellini, 1983). Due to lack of funds, the equipment are imported without essential follow-up procedures to maintain and repair equipment (Öman *et al*., 2006). As a consequence, research facilities in low-income countries have become "graveyards" of equipment that require minimal repairs (Musar, 1993).

A case in point in marine aquaculture is related to autoclaves – conventional machines for sterilising culture media and other laboratory supplies (Dion and Parker, 2013). Autoclaves are relatively easy to maintain in western countries. However, they fail when transferred to low-income countries, as "it is too

**Corresponding author Email: emmanuel.acheampong@ucc.edu.gh*
**ORCID: https://orcid.org/0000-0001-6243-294X*
**Tel: +233 50 492 9936*

expensive" and "economically not viable" for manufacturers/suppliers to provide repair and maintenance services in low income countries (Huijs, 2014). So in place of autoclaves, other methods of sterilisation are used (Table 1). Some of these methods, including the use of microwave ovens and chemical disinfectants (e.g. chlorine), impact adversely on the quality of culture media: they alter pH, and contaminate media with harmful halogens (Keller *et al*., 1988; Price *et al*., 1989). Hence, they are useful when combined with methods for decontaminating samples after sterilisation (Tisserat *et al*., 1992).

Table 1: Summary of water sterilisation methods, their application and limits (Chang *et al*., 1985; Kawachi and Noël, 2005; Keller *et al*., 1988; Ludovici *et al*., 1977; Shan *et al*., 2022).

Sterilization Method		**Effective Application**	**Limitation(s)**
Heat Sterilisation	Autoclaving	121°C at 2 atm for 1 hour for 10 litres of seawater	Changes water pH; May result in the formation of precipitates that do not redissolve when seawater is cooled
	Pasteurization	65−80 °C for 8–10 hours followed by quick cooling to 4−10 °C	incomplete sterilization of water
	Tyndallization	60−80 °C for 30 minutes, followed by quick cooling to 4−10 °C; cycle repeated 3 times in 3 days	Time consuming: requires at least 3 days to complete
Chemical sterilisation	Chlorination	1- 5 mL of 5% sodium hypochlorite per litre of seawater for 12 hours	Halogen contamination; changes water pH
	Copperization	0.15 mg copper ions per litre of water	Heavy metal contamination
Electromagnetic sterilisation	Microwaving	Microwave at 700-W for 10 minutes for 1.5 litre of seawater in a Teflon bottle	Changes water pH
	Ultraviolet (UV) irradiation (254 nm)	For 60 minutes for 1.4 litres of seawater (***based on results from experiments in this report***)	Degrades ultraviolet sensitive plastics

The application of ultraviolet light, particularly at shorter wavelengths (200 – 280 nm) is another method of sterilisation considered as effective replacement for the use of autoclaves (Oppenheimer *et al*., 1997; Song *et al*., 2016). The radiation is easy to produce using low-pressure mercury vapour lamps or UV light-emitting diode (Hijnen *et al*., 2006). It works by damaging the DNA strands of organisms (Fraise *et al*., 2008; Balogh *et al*., 2011). UV radiation does not alter pH of culture media; also, it does not contaminate media with harmful chemicals (Mori *et al*., 2007). The method is therefore relatively cheaper to implement, as it does not involve methods for decontaminating samples after sterilisation (Song *et al*., 2016). It is implemented in commercially available units (e.g., from Trojan Industries®) for sterilising solid surfaces, drinking water and wastewater.

However, the application of UV radiation for the disinfection of seawater is poorly described: the literature on diverse sterilization techniques for preparing growth media for marine organisms is vague on the effective UV dose (fluence rate) and treatment duration for sterilising of seawater (Kawachi and Noël, 2005). The current standards for the application of UV radiation were developed specifically for controlling unwanted/pathogenic microbes in freshwater hydroponics and aquaculture systems (Mori and Smith, 2019; Song *et al*., 2016). They do not apply to seawater because the community of microbes are substantially different, both in terms of species composition (Logares *et al*., 2009) and resistance to the impact of UV radiation (Hijnen *et al*., 2006). A UV sterilisation protocol, peculiar to seawater is therefore needed, especially as microbes in salty marine waters have genetic mutation that confers increased resistance to the impact of UV radiation (Davies and Evison, 1991; Gourmelon, 1995). Past attempts to address this question were limited to seawater from high latitude areas with microbes adapted to cold temperatures (9 – 10 °C; Kelly, 1961). So at the moment, there is no data demonstrating effectiveness of UV radiation on microbes adapted to warmer oceans of the tropics , where oceanographic conditions (e.g. upwelling, increased pollution) support different diversity of marine microbes (Baldwin *et al*., 2005; Raes *et al*., 2018), and low-income countries have urgent need for inexpensive method to sterilise seawater to develop culturable species to advance marine biotechnology for environmental and biomedical applications (Thompson *et al*., 2017). Therefore, the objective of

this this paper was to propose a simple bench-top apparatus for sterilising seawater with UV radiation. The ultimate goal is to provide a low-cost method that is easy to ensemble from locally available materials to supply sterile media for marine research and biotechnology applications in resource-constrained environments. The apparatus was therefore fabricated with polyvinyl chloride materials and UV lamp that are easy to obtain from local suppliers. We have demonstrated the effectiveness and standards for using the apparatus based on tests conducted on polluted, tropical seawater collected from the Elmina fishing harbour, near Cape Coast, Ghana.

MATERIALS AND METHODS

Development of the Sterilisation Apparatus: The layout of the UV light apparatus we propose for seawater sterilisation is shown in Fig. 1. It is based on the design for bench scale devices provided by Bolten and Linden (2003). The apparatus is made up of a polyethylene canister (volume ≈ 20 L) connected to a cylindrical water treatment chamber (total volume ≈ 7 L) with a UV lamp horizontally suspended in it. The water treatment chamber was built using rigid, opaque polyvinyl chloride (PVC) pipe (outside diameter: 12 cm, wall thickness: 0.3 cm), considered chemically inert and resistant to UV radiation (Arthur *et al*., 2020; Saad *et al*., 2012). A low pressure UV lamp of 230 V 50 Hz, which emits monochromatic light at 254 nm was used. Technical data available from the manufacturer (Philips Electronics®) indicate that the amount of energy for operating the lamp is 4.32 Kwh/day.

Previous research suggest that the germicidal power of UV lamps is determined by dose of radiation they deliver (Song *et al*., 2016). This dose is the product of exposure time and irradiance (fluence rate), measured relative to the surface of the treatment water with the help of probes such as radiometer and actinometer (Bolten and Linden, 2003). These details are not explicitly included in the design of the present apparatus, as our target is an appropriate technology that is affordable and locally autonomous particularly with regard to calibration and maintenance. The installation of the UV lamp in the present apparatus was therefore fixed, through a slot opened on the top on the treatment chamber (Fig.1). Due to this design, incident irradiance of UV varies with volume of water in the treatment chamber of the apparatus. The germicidal power of the propose apparatus was therefore determined via experiments involving different volumes of seawater and UV light exposure periods as described in the Section below. Two polyethylene valves regulate the volume of water in treatment chamber: one valve regulates the flow of water from the polyethylene canister into the chamber; the other is an outlet valve that is opened only when water treatment is completed.

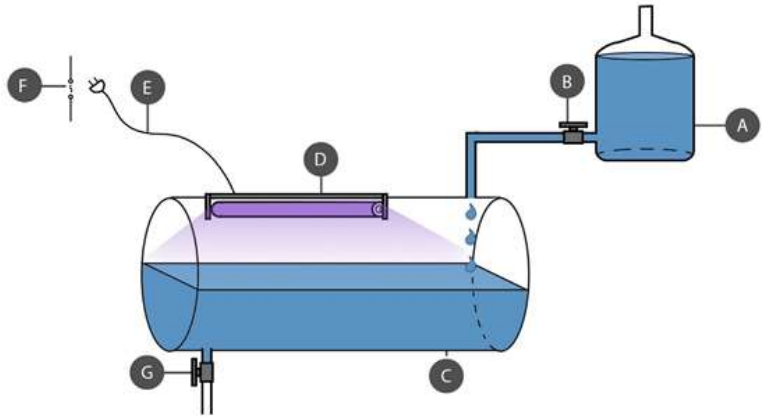

Fig 1: Sketch of the UV sterilization apparatus made up of a polyethylene canister (A) to hold and release filtered seawater into sterilization chamber (C) made using rigid *p*olyvinyl chloride pipe and a 230 V 50 Hz lamp (D) that generates 254 nm ultraviolet radiation to sterilise the filtered water. The lamp requires 4.32 kwh of energy per day, which can be derived from a direct or an alternating current sources (F) via a power cable (E) protected in a plastic tube. Polyethylene valves, B and G, control water flow into and out of the sterilisation chamber, respectively.

Sterilisation of Seawater: The proposed apparatus was tested on seawater (34‰ salinity) collected from Elmina fishing harbour, near Cape Coast, Ghana (5°06'01.8"N 1°16'58.5"W). It was tested under normal laboratory conditions. After sampling, and in following with established protocols (Kawachi and Noël, 2005; Creswell, 2010), the water was first filtered through a 20 µm mesh sieve, and then through a Millipore® sterile filters (pore size = 1.5 µm) to remove suspended particulates and larger-sized microbes. After these filtrations, samples of the water in triplicate (1 mL each) were collected into sterilized Petri dishes with nutrient agar for incubation as described below to determine microbial load of the

raw water before sterilisation. Another set of triplicate samples (1.0 L each) were collected into polycarbonate-coated Duran® bottles (total volume: 1200 mL) for treatment in an autoclave (model LS-60HJ) at 121 °C under 2 atm for 60 minutes. After autoclaving, samples (1 mL each) were collected from each of the bottles into sterilized Petri dishes for incubation and microbial load determination. The microbial load of these samples were used as the baseline for evaluating the effectiveness of the new UV sterilisation apparatus. The standard for using the new apparatus was determined by exposing different volumes of the filtered seawater (800, 1000, 1200 and 1400 mL) to UV light for different durations (10, 20, 40 and 60 minutes). The maximum treatment duration was set at 60 minutes, in accordance with the duration for sterilising water with autoclave machines (Table 1). Triplicates (1 mL each) of the water were collected at the end of each treatment into sterilized Petri dishes for incubation and microbial load determination as described below. All collection of samples for incubation was done using a pipette (Thermo Fisher Scientific) with tips sterilised in an autoclave (model LS-60HJ) at 120 ˚C under 15 psi for 15 minutes, in keeping with previous report (Sowah, 2019).

Seawater Incubation for Microbial Load Determination: To avoid contamination of samples, the incubation was done on work benches sprayed with 70% ethanol. The samples were first diluted to 10^{-3} with sterile normal saline; they were then incubated in nutrient agar prepared as described by Jannasch and Jones (1959). The incubation was done at 37 °C for 24 hours using the pour plate method described by Cheesbrough (2006). After incubation, total number of viable bacteria colonies in the samples were counted using a bacteria counter (Stuart scientific SC5 colony counter). These counts were taken to represent the microbial load of the treated water; they were converted into Colony Forming Unit (CFU) using Equation 1.

$$CFU = \frac{No.CC \times DF\ Used}{SV} \quad (1)$$

Where SV =sample volume; DF used= dilution factor used; No. CC = number of colonies counted

Data Analysis: Based on previous report (Pulleritis *et al*., 2020), microbial load of the water treated with the present apparatus was expected to decrease linearly with increasing duration of UV exposure. For the present experiment, the significance of the linear relationship between microbial load and duration of UV radiation was based on coefficient of determination (R^2) of the relationship, in line with previous studies (Dion and Parker, 2013). The relationship was considered significant where R^2 was ≥ 0.5 at 95% significant level. This assessment was done for each of the four different volumes of seawater treated in the present experiment. To determine the optimum volume of water that could be treated effectively by the new apparatus, slopes describing the relationship between UV exposure and microbial load of each volume of water were compered. A one-tail T-test based on the standard error of the slopes was used for this comparison (Andrade and Estévez-Pérez, 2014). The critical p-value for this test was Bonferroni corrected by dividing the number of comparison performed. The volume giving the steepest slope was considered as the optimum volume for achieving maximum sterilisation of seawater with the new apparatus. To ascertain the effectiveness of the new sterilisation apparatus, microbial load of water treated in the apparatus was compared with the load in water treated in autoclave (model LS-60HJ). A two-sample t-test, assuming autoclave as the standard sterilisation method, was used for the comparison. The critical p-value for this analysis was taken to be 0.05.

RESULTS AND DISCUSSION

Our results show that the new apparatus can effectively sterilise seawater with UV irradiation (Fig. 2 and 3). The equipment was tested on seawater collected from a Ghanaian harbour polluted with high concentration ($1 - 4 \times 10^4$ CFU.mL^{-1}) of coliform and other pathogenic bacteria (Obodai *et al*., 2010; Takyi *et al*., 2022).

The microbial load of the raw seawater freshly collected from field was $1.8 \times 10^5 \pm 1.3 \times 10^4$ CFU. mL^{-1}, in agreement with results from previous investigation of the harbour (Obodai *et al*., 2010). It was reduced significantly (by ≈ 70%) after Millipore filtration (t-test comparison of filtered and unfiltered water: t-observed = 16.15, df = 4, p = 8.6E-5). On average, the microbial density left after the filtration of the water was $5.3 \times 10^4 \pm 4.1 \times 10^3$ CFU.mL^{-1} (Fig 3). This concentration is over 400 times higher than the microbial contamination limit of 100 CFU.mL^{-1} recommended by environmental health authorities (Zappalà *et al*., 2012).

Hence, a significant proportion of the microbial community within the test water was small enough to physically pass through 1.5 µm pore filters. Such filterable microbes are usually nano-sized organisms such as coliform bacteria (e.g. *Escherichia coli*) and archaea (e.g. *Nanoarchaeum equitans*) ubiquitous in diverse range of marine habitats (Ghuneim *et al*., 2018). Fig. 2 shows changes in the microbial load of seawater treated using the new apparatus. Significant

(67 – 99%) decline in microbial load of the water was observed during the first 10 minutes of treatment (Fig. 3B).

Table 2: T-test comparing slopes of linear regression between UV light exposure and microbial load of seawater treated at different volumes (Fig. 2). For each test, *df* = 20; the critical p-value was Bonferroni corrected by dividing the 0.05 by 6, the number of tests performed. P-values showing significant differences are italicised.

Volume (mL) of Water Treated		Comparison of Slopes	
		T-observed	P-value
800	1000	3.84832	*0.001*
800	1200	2.73251	0.013
800	1400	5.1642	*<0.001*
1000	1200	9.367	*<0.001*
1000	1400	50.274	*<0.001*
1200	1400	15.352	*<0.001*

This observation agrees with results from previous experiments investigating the response of microbes to UV radiation (Chevremont *et al*. 2012; Vilhunen *et al*., 2009). *Escherichia coli*, for example, becomes deactivated in less than a minute after exposure to UV radiation similar to the wavelength (254 nm) used in the present apparatus (Chevremont *et al*., 2012). *Vibro parahaemolyticus*, a bacterium common in seas and in estuaries, is also deactivated within 6 minute of UV exposure (Nakahashi *et al*., 2014).

Based on these previous observations and our results, it can be said that the new apparatus proposed in the present report demonstrates the germicidal power of UV radiation. The microbial load decreased linearly with increasing duration of UV radiation (R^2 = 0.65 – 0.86), irrespective of the volume of seawater treated in the apparatus (Fig. 2). The slopes representing the rate of disinfection were significantly different, depending on the volume of water treated (Table 2). Hence, the effectiveness of the proposed apparatus varies, depending on the volume of water treated.

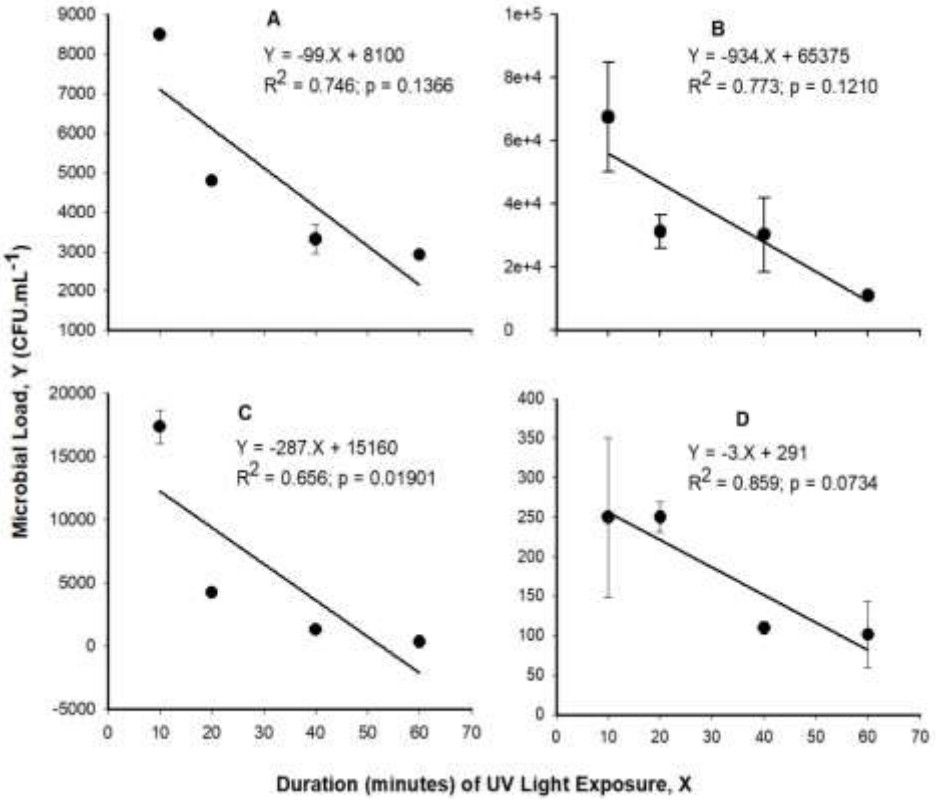

Fig 2: Microbial load (mean ± SD) of seawater subjected to UV radiation of 254 nm over different durations. Subplots A, B, C and D represent results when the volume of water treated was 800, 1000, 1200, and 1400 ml, respectively.

A post hoc comparison of results from the present experiment suggests that highest (≈ 99.6%) reduction in microbial load could be achieved when the volume of the treated water was 1400 mL (Fig 3). This rate of disinfection was significantly (5 – 20 %) higher than the rate obtained when the volumes of the treated water was reduced (One-way ANOVA comparison of water treated for 60 minutes; F_3 = 1124.747, p = 7.7E-11); it compares with the disinfection rate of 99% reported by studies on seawater sterilisation using sophisticated UV equipment (Kelly, 1961) and autoclave machines (Jorquera *et al*., 2002).

Disinfection by the new apparatus brought down the microbial load of the treated seawater to 101± 42 CFU.mL^{-1}, a low level statistically similar to the microbial load of seawater treated by autoclave in the present experiment (Fig. 3) and previous studies (Jorquera *et al*., 2002). This high level of disinfection was achieved when UV exposure and volume of test water was 60 minutes and 1400 mL, respectively (Fig. 2 and 3).

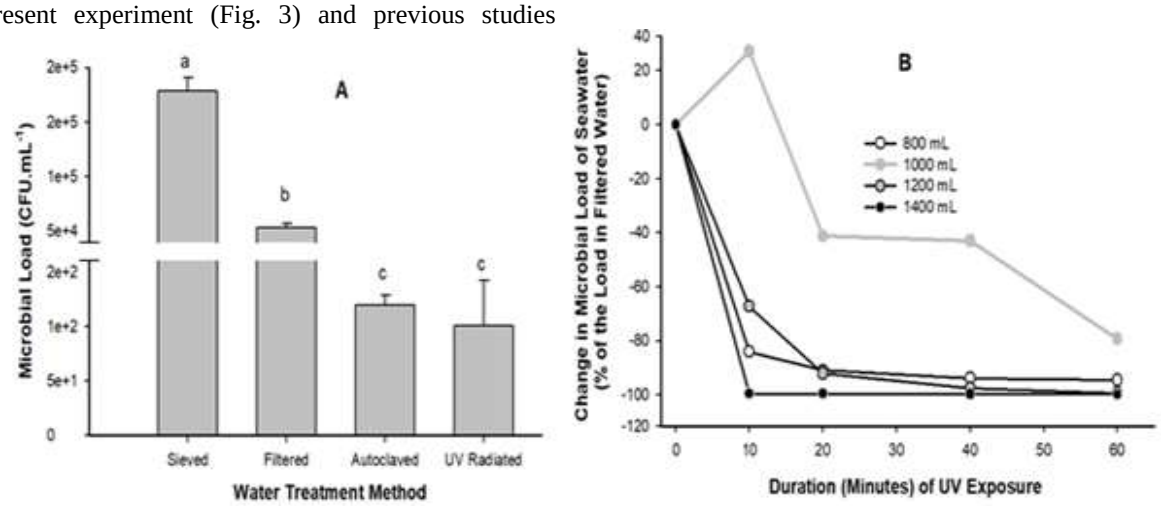

Fig. 3: Comparison of microbial load (mean ± SD) of seawater treated with different methods. Mean values that are significantly different are indicated by different alphabets (Tukey HSD *post hoc* test at $p < 0.05$ after one-way ANOVA).

The microbial load left in the water treated on these standards compares with microbial load of water for culturing marine organisms (Mahadevaswamy and Venkataraman, 1981; Makridis *et al*., 2006; Rong *et al*., 2022). Makridis *et al*. (2006) for example cultured two species of microalgae (*Tetraselmis chuii* and *Chlorella minutissima*) in media with microbial contamination of over 10,000 CFU.mL^{-1}. For marine zooplankton, microbial water quality of cultures used as live feed is estimated to range between 1000 – 4000 CFU.mL^{-1} (Rong *et al*., 2022), which is >10 times higher than the CFU of water treated for 60 minutes by the present apparatus (Fig. 3). Therefore, the apparatus in this report (Fig. 1) could be used to disinfect seawater to culture marine organisms. Previous studies have noted that the effectiveness of UV radiation varies from microorganism to microorganism. Some microbes can overcome UV radiation through "dark repair" processes after sterilisation (Song *et al*., 2006 and references therein). Others (e.g. bacteria) produce spores that can survive and grow after exposure to UV radiation (Setlow, 2006). Naked viruses are also more resistant to UV irradiation than enveloped viruses (Watanabe *et al*., 1989). These issues were not investigated in the present experiment and must be considered by subsequent research. In addition, the UV lamp used in proposed apparatus is fragile and contain toxic mercury, which is hazardous to the environment and requires proper disposal (Song *et al*., 2016). The lamp also has a relatively short lifetime of about 5,000 hours (Schalk *et al*., 2006). It was preferred for the proposed apparatus because it is cheaper than UV light-emitting diodes (LEDs) that are environmentally friendly (no mercury) and durable, with lifetime longer than 100,000 hours (Ibrahim *et al*., 2014).

Conclusion: Experiments conducted as part of this study suggest that the proposed UV sterilisation apparatus is most effective within an hour of UV exposure with low volume of water to be treated. The parameters obtained has been recommended as standards for using the new apparatus. The low-pressure UV lamp in the proposed apparatus could be replaced with UV LEDs, which are more environmentally friendly, where affordable.

Acknowledgement: This work was financed with grants from Denmark's development cooperation, DANIDA (Hotspot project, grant number: 17-M03-DTU) and United Kingdom Research and Innovation (UKRI) Global Challenges Research Fund (GCRF) (One Ocean Hub Project, grant number: NE/S008950/1).

Conflict Of Interest: We, the authors, declare that we have no known competing interests or personal relationships that could have influenced the work reported in this paper.

Data Availability Statement: The data presented in this report are available upon request from the corresponding author.

REFERENCES

Andrade, JM; Estévez-Pérez, MG (2014). Statistical comparison of the slopes of two regression lines: A tutorial. *Anal. Chim. Acta* 838: 1 – 12. DOI: https://doi.org/10.1016/j.aca.2014.04.057

Arthur, EK; Gikunoo, E; Agyemang, FO; Azeko, ST; Andrews, A; Twenewaa, A (2020). Material Selection for Water Pipes by the Multi-Objective Decision-Making Method: The Case of Alternative Materials for PVC Pipes. *J. Sci. Technol.* 5(1): 29 – 42.

Baldwin, AJ; Moss, JA; Pakulski, JD; Catala, P; Joux, F; Jeffrey, W H (2005). Microbial diversity in a Pacific Ocean transect from the Arctic to Antarctic circles. *Aquat. Microb. Ecol.* 41(1): 91 – 102. DOI: https://doi:10.3354/ame041091

Balogh, TS: Velasco, MVR; Pedriali, CA; Kaneko, TM; Baby, AR (2011). Proteção à radiação ultravioleta: recursos disponíveis na atualidade em fotoproteção. *An. Bras. Dermatol. 86*: 732 – 742. DOI: https://doi.org/10.1590/S0365-05962011000400016

Bolton, JR; Linden, KG (2003). Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *J. Environ. Eng.* 129(3): 209 – 215. DOI: https://doi.org/10.1061/(ASCE)0733-9372(2003)129:3(209)

Chang, JC; Ossoff, SF; Lobe, DC; Dorfman, MH; Dumais, CM; Qualls, RG; Johnson, JD (1985). UV inactivation of pathogenic and indicator microorganisms. *Appl. Environ Microb.* 49(6): 1361 – 1365. DOI: https://doi.org/10.1128/aem.49.6.1361-1365.1985

Cheesbrough, M (2006). *District laboratory practice in tropical countries, part 2*. Cambridge University Press.

Chevremont, AC; Farnet, AM; Coulomb, B; Boudenne, JL (2012). Effect of coupled UV-A and UV-C LEDs on both microbiological and chemical pollution of urban wastewaters. *Sci. Total Environ.* 426: 304e310. DOI: https://doi.org/10.1016/j.scitotenv.2012.03.043

Creswell, L., 2010. *Phytoplankton culture for aquaculture feed*. Southern Regional Aquaculture Center.

Davies, CM; Evison, LM (1991). Sunlight and the survival of enteric bacteria in natural waters. *J. Appl. Bacteriol.* 70(3): 265 – 274. DOI: https://doi.org/10.1111/j.1365-2672.1991.tb02935.x

Dion, M; Parker, W (2013). Steam sterilization principles. *Pharm. Eng.* 33(6): 1 – 8.

Fraise, AP; Lambert, PA; Maillard, JY (2008). *Russell, Hugo and Ayliffe's principles and practice of disinfection, preservation and sterilization*. John Wiley and Sons.

Gourmelon, M (1995). *Etude de la Lumière Visible comme Facteur Limitant de la Survie de Escherichia coli en Milieu Marin*. Univ. Rennes I, p. 144.
DOI: https://archimer.ifremer.fr/doc/00105/21577/

Ghuneim, LAJ; Jones, DL; Golyshin, PN; Golyshina, OV (2018). Nano-sized and filterable bacteria and archaea: biodiversity and function. *Front. Microbiol.* 9: 1971.
DOI: https://doi.org/10.3389/fmicb.2018.01971

Hijnen, WAM; Beerendonk, EF; Medema, GJ (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo) cysts in water: a review. *Water Res.* 40(1): 3 – 22.
DOI: https://doi.org/10.1016/j.watres.2005.10.030

Huijs, J (2014). *Facing the standards gap: an autoclave for the rest of us*. IET. P. 1 – 4. DOI: https://doi.org/10.1049/cp.2014.0791

Ibrahim, MA; MacAdam, J; Autin, O; Jefferson, B (2014). Evaluating the impact of LED bulb development on the economic viability of ultraviolet technology for disinfection. *Environ. Technol.* 35(4), 400 – 406. DOI: https://doi.org/10.1080/09593330.2013.829858

Jannasch, HW; Jones, GE (1959). Bacterial populations in sea water as determined by different methods of enumeration 1. *Limnol. Oceanogr.* 4(2) 128 – 139.
DOI: https://doi.org/10.4319/lo.1959.4.2.0128

Jorquera, MA; Valencia, G; Eguchi, M; Katayose, M; Riquelme, C (2002). Disinfection of seawater for hatchery aquaculture systems using electrolytic water treatment. *Aquaculture* 207(3-4), 213-224. DOI: https://doi.org/10.1016/S0044-8486(01)00766-9

Kawachi, M; Noël, MH (2005). Sterilization and sterile technique. *Algal culturing techniques*, 65 – 81.

Keller, MD; Bellows, WK; Guillard, RR (1988). Microwave treatment for sterilization of phytoplankton culture media. *J. Exp. Mar. Biol. Ecol.* 117(3): 279 – 283. DOI: https://doi.org/10.1016/0022-0981(88)90063-9

Kelly, CB (1961). Disinfection of sea water by ultraviolet radiation. *Am. J. Public Health N.* 51(11): 1670 – 1680.

Logares, R; Bråte, J; Bertilsson, S; Clasen, JL; Shalchian-Tabrizi, K; Rengefors, K (2009). Infrequent marine–freshwater transitions in the microbial world. *Trends Microbiol.* 17(9): 414 – 422. DOI: https://doi.org/10.1016/j.tim.2009.05.010

Ludovici, PP; Phillips, RA; Jeter, WS (1977). Comparative inactivation of bacteria and viruses in tertiary-treated wastewater by chlorination. *Disinfection: water and wastewater*. Ann Arbor Science Publishers, Ann Arbor, Michigan, USA, p. 359 – 390.

Luiten, EE; Akkerman, I; Koulman, A; Kamermans, P; Reith, H; Barbosa, MJ; Detmer, S; Wijffels, RH (2003). Realizing the promises of marine biotechnology. *Biomol. Eng.* 20(4-6): 429 – 439. DOI: https://doi.org/10.1016/j.biotechadv.2012.03.006

Makridis, P; Costa, RA; Dinis, MT (2006). Microbial conditions and antimicrobial activity in cultures of two microalgae species, *Tetraselmis chuii* and *Chlorella minutissima*, and effect on bacterial load of enriched *Artemia* metanauplii. *Aquaculture* 255(1-4): 76-81.
DOI: https://doi.org/10.1016/j.aquaculture.2005.12.010

Mori, M; Hamamoto, A; Takahashi, A; Nakano, M; Wakikawa, N; Tachibana, S; Ikehara, T; Nakaya, Y; Akutagawa, M; Kinouchi, Y (2007). Development of a new water sterilization device with a 365 nm UV-LED. *Med. Biol. Eng. Comput.* 45: 1237 – 1241. DOI: https://doi.org/10.1007/s11517-007-0263-1

Mori, J; Smith, R (2019). Transmission of waterborne fish and plant pathogens in aquaponics and their control with physical disinfection and filtration: A systematized review. *Aquaculture* 504: 380 – 395. DOI: https://doi.org/10.1016/j.aquaculture.2019.02.009

Musar, A (1993). *Equipment for science education constraints and opportunities*. World Bank.

Nakahashi, M; Mawatari, K; Hirata, A; Maetani, M; Shimohata, T; Uebanso, T; Hamada, Y; Akutagawa, M;Kinouchi, Y; Takahashi, A (2014). Simultaneous irradiation with different wavelengths of ultraviolet light has synergistic bactericidal effect on *Vibrio parahaemolyticus*. *Photochem. Photobiol.* 90(6): 1397 – 1403. DOI: https://doi.org/10.1111/php.12309

Obodai, EA; Nyarko, HD; Amponsah, SL (2010). Effect of depuration on microbial content of mangrove oyster (Crassostr Ea Tulipa) from Benya Lagoon, Ghana. *Ethiopian J. Environ. Stud. Manag.* 3(2). DOI: https://doi.org/10.4314/ejesm.v3i2.59832

Oppenheimer, JA; Jacangelo, JG; Laîné, JM; Hoagland, JE (1997). Testing the equivalency of ultraviolet light and chlorine for disinfection of wastewater to reclamation standards. *Water Environ. Res.* 69(1): 14 – 24. DOI: https://doi.org/10.2175/106143097X125137

Öman, CB; Gamaniel, KS; Addy, ME (2006). Properly functioning scientific equipment in developing countries. *Anal. Chem.* 78(15): 5273 – 5276. DOI: https://doi.org/10.1021/ac069434o

Pullerits, K; Ahlinder, J; Holmer, L; Salomonsson, E; Öhrman, C; Jacobsson, K; Dryselius, R; Forsman, M; Paul, CJ; Rådström, P (2020). Impact of UV irradiation at full scale on bacterial communities in drinking water. *NPJ Clean Water* 3(1): 11. DOI: https://doi.org/10.1038/s41545-020-0057-7

Price, NM; Harrison, GI; Hering, JG; Hudson, RJ; Nirel, PM; Palenik, B; Morel, FM (1989). Preparation and chemistry of the artificial algal culture medium Aquil. *Biol. Oceanogr.* 6(5-6): 443 – 461. DOI: https://doi.org/10.1080/01965581.1988.10749544

Raes, EJ; Bodrossy, L; Van De Kamp, J; Bissett, A; Ostrowski, M; Brown, MV; Sow, SL; Sloyan, B; Waite, AM (2018). Oceanographic boundaries constrain microbial diversity gradients in the South Pacific Ocean. *P. Natl. Acad. Sci. USA* 115(35): E8266 – E8275 DOI: https://doi.org/10.1073/pnas.1719335115

Rong, RL; Gagnat, MR; Attramadal, Y; Vadstein, O (2022). Microbial water quality of the copepod

Acartia tonsa in cultures for use as live feed. *Aquaculture* 560: p.738439.
DOI: https://doi.org/10.1016/j.aquaculture.2022.738439

Saad, NA; Al-Maamory, MH; Mohammed, MR; Hashim, AA (2012). The effect of several service and weathering parameters on tensile properties of PVC pipe materials. *Mater.Sci. Appl.* 3(11). DOI: https://doi.org.10.4236/msa.2012.311114

Schalk, S; Adam, V; Arnold, E; Brieden, K; Voronov, A; Witzke, HD (2006). UV-lamps for disinfection and advanced oxidation-lamp types, technologies and applications. *IUVA news* 8(1): 32 – 37.

Shan, J; Tian, X; Guan, C; Zhang, C; Zhang, Y; Chen, S (2022). Effect of copper ion sterilization on bacterial community in a freshwater recirculating aquaculture system. *Current Microbiol.* 79(2): 1 – 8. DOI: https://doi.org/10.1007/s00284-021-02707-2

Setlow, P (2006). Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals. *J. Appl. Microbiol.* 101(3), 514 – 525.
DOI: https://doi.org/10.1111/j.1365-2672.2005.02736.x

Song, K; Mohseni, M; Taghipour, F (2016). Application of ultraviolet light-emitting diodes (UV LEDs) for water disinfection: A review. *Water Res.* 94: 341 – 349. DOI: https://doi.org/10.1016/j.watres.2016.03.003

Sowah, SA (2019). *Assessment of some water quality parameters of three coastal water bodies towards the culture of oysters in Ghana*. MPhil Dissertation, University of Cape Coast. http://hdl.handle.net/123456789/5021

Takyi, R; El Mahrad, B; Nunoo, FKE; Adade, R; ElHadary, M; Essandoh, J (2022). Adaptive management of environmental challenges in West African coastal lagoons. *Sci. Total Environ.* 838: 156234. DOI: https://doi.org/10.1016/j.scitotenv.2022.156234

Tisserat, B; Jones, D; Galletta, PD (1992). Microwave sterilization of plant tissue culture media. *HortScience* 27(4), 358 – 361. DOI: https://doi.org/10.21273/HORTSCI.27.4.358

Thompson, CC; Kruger, RH; Thompson, FL (2017). Unlocking marine biotechnology in the developing world. *Trends Biotechnol.* 35(12): 1119 – 1121. DOI: https://doi.org/10.1016/j.tibtech.2017.08.005

Vilhunen, S; Sarkka, H; Sillanpaa, M (2009). Ultraviolet light-emitting diodes in water disinfection. *Environ. Sci. Pollut. R.* 16(4): 439e442.
DOI: https://doi.org/10.1007/s11356-009-0103-y

Vose, PB; Cervellini, A (1983). Problems of scientific research in developing countries. *IAEA Bulletin* 25(2): 37 – 40.

Watanabe, Y; Miyata, H; Sato, H (1989). Inactivation of laboratory animal RNA-viruses by physicochemical treatment. *Exp. Anim. Tokyo* 38(4): 305 – 311. DOI: https://doi.org/10.1538/expanim1978.38.4_305

Zappalà, G; Bonamano, S; Madonia, A; Caruso, G; Marcelli, M (2012). Microbiological risk assessment in a coastal marine environment through the use of mathematical models. *WIT Trans. Ecol. Environ.* 164: 3 – 14.