Aquaculture 497 (2018) 164–173

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

# Understanding bacterial communities for informed biosecurity and improved larval survival in Pacific oysters

Olivier Laroche[a,b,*], Jane E. Symonds[c], Kirsty F. Smith[a], Jonathan C. Banks[a], Hannah Mae[c], John P. Bowman[d], Xavier Pochon[a,e]

[a] Coastal and Freshwater Group, Cawthron Institute, Private Bag 2, Nelson 7042, New Zealand
[b] School of Biological Sciences, University of Auckland, Auckland 1010, New Zealand
[c] Aquaculture Group, Cawthron Institute, Private Bag 2, Nelson 7042, New Zealand
[d] Centre for Food Safety and Innovation Centre, University of Tasmania, Hobart, Tasmania 7005, Australia
[e] Institute of Marine Science, University of Auckland, Warkworth 0941, New Zealand

ARTICLE INFO

*Keywords:*
16S ribosomal RNA gene
High-throughput sequencing
Larvae oyster rearing
Microbiome
Metabarcoding
UV treatment

ABSTRACT

Bacteria are ubiquitous in all marine habitats and can be beneficial or detrimental to the survival and growth of shellfish raised in aquaculture. To reduce the exposure of cultured shellfish to potential pathogens, antibiotics can be used. However, risks associated with this practice have led to the development of alternative techniques such as ultraviolet (UV) light exposure for seawater disinfection. In this study, we used 16S rRNA gene metabarcoding to measure the effect of low (50 mJoules/cm$^2$) and high (200 mJoules/cm$^2$) UV treatment of seawater on the bacterial communities present in hatchery rearing water and in Pacific oyster *Crassostrea gigas* larvae. 81 samples were collected between 13 and 29 March 2017 for high-throughput DNA sequencing of bacterial communities, including differentially-treated seawater, the microalgae fed to the oysters and oyster larvae (fertilized eggs, early veliger larval stage [D-larvae], and pre-settlement stage) samples. Differences in larval mortality between low and high UV treatments were also assessed. We found that the two UV treatments influenced the overall bacterial community diversity and affected its composition in both seawater and oyster larval samples. While alpha-diversity was mostly driven by the UV treatment, we found that the microbiome composition was primarily affected by temporal changes in the water source. Compared to the bacterial microbiome associated with seawater, the oyster larvae microbiome changed more significantly in response to both UV treatments and sampling dates with marked shifts in the dominant bacteria associated with fertilized eggs (class *Gammaproteobacteria*, families *Rhodospirillaceae* and *Pelagibacteraceae*), the D-larvae (family *Alteromonadaceae*), and the pre-settlement larvae (families *Flavobacteriaceae* and *Rhodobacteraceae*). As larval development progressed, an increasingly complex bacterial community structure was observed. These bacterial community changes were likely driven by multiple factors, including the microbiome associated with microalgal cultures, which increased in complexity over time. Despite the clear response of bacterial communities to both low and high UV treatments, no significant effect was observed in relation to the oyster larval mortality rate. These results suggest that increasing the UV treatment to 200 mJoules/cm$^2$, if required for more efficacious disinfection and biosecurity, would not be detrimental to Pacific oyster larval survival. Further research is required to increase understanding of the dynamics of functionally critical bacterial taxa and their successive roles in post-larval development of Pacific oysters.

## 1. Introduction

The United Nations Food and Agriculture Organization (FAO) has identified disease outbreaks as a significant restraint to the expansion of aquaculture industries (Subasinghe et al., 2001; FAO, 2016a, 2016b). The advent of new diseases in shellfish raised in aquaculture has increased interest in disease prevention, and in risks of disease introduction via hatchery stock transfer.

Antibiotics are used to reduce pathogen concentrations in hatchery-reared oyster spat in many countries. However, there are concerns about excess use of antimicrobial agents (e.g., especially antimicrobials that have been identified by the United Nations as “Critically Important

* Corresponding author at: 98 Halifax St East, Nelson 7010, New Zealand.
*E-mail address:* Olivier.Laroche@cawthron.org.nz (O. Laroche).

https://doi.org/10.1016/j.aquaculture.2018.07.052
Received 16 April 2018; Received in revised form 24 July 2018; Accepted 27 July 2018
Available online 30 July 2018
0044-8486/ 

Antimicrobials for Human Medicine", in food production (Defoirdt et al., 2011). The United Nations has also identified the unregulated use of antimicrobials in food production as an important risk factor in the development of antimicrobial resistance, and suggested improved biosecurity as a practical measure to reduce the need for antimicrobials in food production (FAO, 2016b).

There are several methods to control pathogenic bacteria in aquaculture that do not require the use of antimicrobial agents used in human medicine, for example bacteriophages (viruses of bacteria; Nakai and Park, 2002), bacterial growth inhibitors (Defoirdt et al., 2011) and probiotics (Defoirdt et al., 2007). Treatment of rearing water with ultraviolet (UV) light is also used to reduce mortality in oyster hatchery stocks (e.g., Ford et al., 2001), as UV light penetrates cell walls of microorganisms and kills a wide range of parasites, bacteria and viruses (Liltved, 2001). However, a variety of factors may affect UV's disinfecting ability, including turbidity, exposure time to the UV light and its intensity (Becker and Speare, 2004). For this reason, the treatment is usually reported as a UV dose corresponding to the product of UV light intensity by residence time (contact time in the reaction chamber). Depending on the target organisms, the UV dose required for the inactivation of microorganisms can vary substantially (from 2 to 230 $mJ/cm^2$; Summerfelt, 2003). Liltved (2001) reported the inactivation of most fish pathogens at 30 $mJ/cm^2$, while much higher values are needed for viruses such as the infectious pancreatic necrosis virus (122 $mJ/cm^2$). To the best of our knowledge, no optimal UV treatment values have been determined for oyster larvae rearing and none have shown detrimental effect of a high UV dose (e.g. > 50 $mJ/cm^2$). Nonetheless, there is some concern that altering the microbiome of the incoming water through disinfection may affect the development of spat by inactivating probiotic strains (Karim et al., 2013; Li et al., 2017). Indeed, microbiota have been shown to support host fitness through defense mechanisms against pathogens (McFall-Ngai et al., 2013). For example, some bacterial species (e.g., *Pseudoalteromonas*) can inhabit the oyster hemolymph (a tissue with immune function analogous to vertebrate blood) and produce antimicrobial compounds that prevent colonization by external pathogens and disease (Defer et al., 2013; Desriac et al., 2014). Higher UV doses can be more efficacious at killing potential pathogens in shellfish hatchery production (Brown and Russo, 1979). However, it is important to understand the influence increasing the UV dose has on larval survival before increased doses are implemented commercially. It remains to be determined what UV dose is required to treat seawater effectively in oyster hatchery systems, while still maintaining good spat survival.

Advances in high-throughput sequencing technologies have improved the characterization of microbiomes at unprecedented scale and resolution (Caporaso et al., 2011). For example, a number of recent studies have used 16S rRNA gene metabarcoding to characterize oyster microbiomes from various organs, including stomach, intestine, gills, mantle and hemolymph (King et al., 2012; Wegner et al., 2013; Lokmer and Wegner, 2015). Lokmer et al. (2016) reported high abundances of members of class *Gammaproteobacteria* in the oyster's gut, gill, and mantle, and interactions between the oyster hemolymph microbiome and the surrounding environment, which can have an effect on oyster mortality rates. Due to these possible interactions, investigation of the effect of different UV treatments on microbiomes associated with hatchery water supply and oyster larvae during rearing operations seems particularly sensible.

We used 16S rRNA gene metabarcoding to examine the effect of two doses of UV treatment of hatchery influent seawater on the bacterial communities present in the water and in the Pacific oyster, *Crassostrea gigas*, from fertilized eggs (FE) to pre-settlement stages. We also report the effect of the two doses of UV treatment on oyster larval mortality. We hypothesized that 1) a higher level of UV treatment on incoming seawater would affect the composition and reduce the diversity of the bacterial community in the inlet and larval rearing water, and those associated with oyster larvae, and 2) that a higher UV treatment would influence oyster larval mortality.

## 2. Material and methods

### 2.1. Experimental design

Untreated seawater from Tasman Bay was supplied to the oyster hatchery at the Cawthron Aquaculture Park (CAP) (41°11′29.2″S 173°21′01.9″E). Commercial Pacific oyster (*C. gigas*) broodstock were held at CAP from November 23, 2016, and conditioned at 21 °C in a flow-through system supplied with a mixed diet of wild pond microalgae and cultured microalgae (supplied to reach a background cell concentration of 30–50 cells/mL) from March 12, 2017, 15 weeks prior to spawning.

For incubation and larval rearing, seawater was stored in 25,000 L tanks. Prior to use, the water was filtered (1 μm) and heated to 24 °C. For fertilization and incubation the seawater was also aged with 12 μM ethylenediaminetetraacetic acid (EDTA), a chelating agent, in order to sequester metal ions. For this trial either a high (200 $mJ/cm^2$) or low (50 $mJ/cm^2$) dose UV treatment was applied to the seawater post-filtration and the water was delivered to separate header tanks before being supplied to the larval rearing units. For a schematic of the global experimental design used see Fig. 1.

Gametes were collected on 13 March 2017 by manually stripping sperm from two males and eggs from four females to be used for fertilization. The fertilized eggs were pooled and split into two groups before stocking in 150 L incubators at a density of 125 embryos per mL.

The fertilized eggs were incubated overnight in either low or high UV-treated static seawater with gentle aeration (Fig. 1C). The next day (14 March 2017) the resulting D-larvae were evenly distributed among 12 individual 2.5 L flow-through tanks (Cawthron Ultra Dense Larval rearing systems [CUDLs]) for larval rearing (Ragg et al., 2010; Supplementary Fig. S1). Each CUDL received approximately 500,000 D-larvae. Six CUDLs received low UV treated seawater and six received high UV treated water for the duration of the trial (Fig. 1). Larval rearing temperature averaged 23.46 °C ( ± 0.10 °C) during the trial.

The larvae were reared for 16 days and the experiment was terminated when they reached the pre-settlement stage on 29 March 2017. During the trial, microalgae was supplied continuously to the CUDLs according to standard operating procedures developed for oyster rearing at CAP. A mixed diet was provided at equal quantity to both high and low UV treatments with daily adjustment according to increasing larval demand; target feeding rates per CUDL varied from 70 algal cells per μL at the start of the trial to 90 algal cells per μL at the end of the trial. Four different species of microalgae were used to feed the larvae during the trial. From day 0 (14 March 2017) to day 4 of larval rearing, a mix of *Chaetoceros calcitrans* and the golden-yellow haptophyte *Tisochrysis lutea* (*Isochrysis* sp., 'Tahitian strain', T-*Iso*; Bendif et al., 2013) was fed. On day 5 *Chaetoceros muelleri* was added to the mix and on day 9 (i.e. 22 March 2017), *Pavlova lutheri* was also added.

### 2.2. Sampling

One L seawater samples were collected from the influent water, before UV treatment (control, Ctl), and from the two header tanks after high and low UV treatment on 13, 14, 20, and 29 March 2017 (Fig. 1B). Three one mL samples of the fertilized eggs were collected and rinsed to remove sperm (13 March 2017; Fig. 1C). Three one L samples of the D-larvae-water mixture were collected from the low and high UV treated groups prior to stocking in the CUDLs (14 March 2017; Fig. 1C). One 50 mL sample of the larvae-water-algae mixture was collected from each of the 12 CUDLs during the trial (20 March 2017) and on the final day of the trial (29 March 2017; Fig. 1C). Finally, 100 mL of microalgal culture samples were collected for DNA extraction on 14, 20, and 29 March 2017.