# Growth, survival and physiological condition of hatchery-reared American oyster larvae (*Crassostrea virginica*) in relation to water treatments and diet

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## Résumé

Les larves de bivalves rencontrent souvent de fortes mortalités pendant leur développement larvaire. Cette étude examine la performance (survie, croissance et changements dans les classes de lipides et d'acides gras) de l'huître Américaine, de l'œuf jusqu'aux stades post-larvaires, en fonction des traitements d'eau. L'eau de mer a été traitée à l'ozone avec ou sans UV suivant une expérience factorielle 2×2. Les larves ont été nourries avec un mélange de *Pavlova lutheri* (MONO), *Isochrysis galbana* (TISO) and *Chaetoceros gracilis* (CHGRA). CHGRA a été introduite au jour 2 post-fertilisation (pf) ou au jour 7 pf. Les résultats préliminaires montrent une intéraction significative ozone × UV × diète sur la survie des larves. L'utilisation de l'eau de mer traitée à l'UV réduit la survie des larves d'huître ayant reçu la diète ternaire au jour 7 pf tandis qu'il n'y a pas d'effet sur la survie des larves ayant reçu la diète ternaire au jour 2 pf. Par contre, l'utilisation de l'eau de mer ozonée réduit la survie des larves d'huître recevant la diète ternaire.

## Introduction

- Bivalve larvae often experience high mortalities during their protracted larval development due to outbreak of bacteria in the seawater.
- The larval rearing of bivalve larvae often rely on the use of treated seawater such as UV or ozone.

## Objective

To determine the effects of seawater treatment (UV and ozone) on growth, survival, metamorphosis and lipid composition of oyster larvae as a funtion of diet. Here, we hypothesized that the introduction of CHGRA could have influenced the bacterial community profile of the seawater, which has a strong influence on the effect of water quality on the survival of oyster.

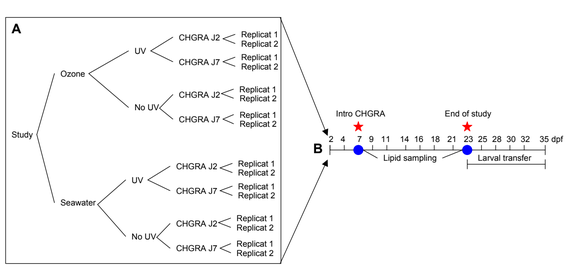
## Material and Methods

REARING CONDITIONS

- Male and female were induced to spawn by raising the temperature.
- Larvae were reared at 20°C in 25-29 ppt.
- Larvae were fed a mixture of *Pavlova lutheri* (MONO), *Isochrysis galbana* (TISO) and *Chaetoceros gracilis* (CHGRA).
- Seawater was plated on marine agar and TCBS for bacterial quantification.

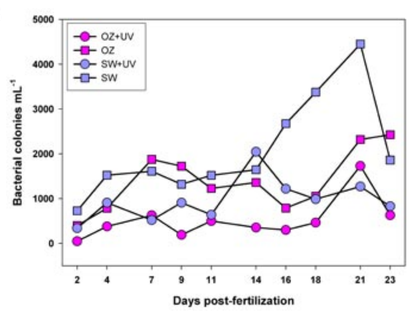
LIPID ANALYSIS

- Lipid were extracted following Folch[1].
- Lipid classes were separated by TLC on Chromarods-SIII and quantified with a Iatroscan MK-VI[2].
- Neutral and polar lipids were separated by SPE on deactivated silica.
- Methylated fatty acids were quantified by GC-FID.



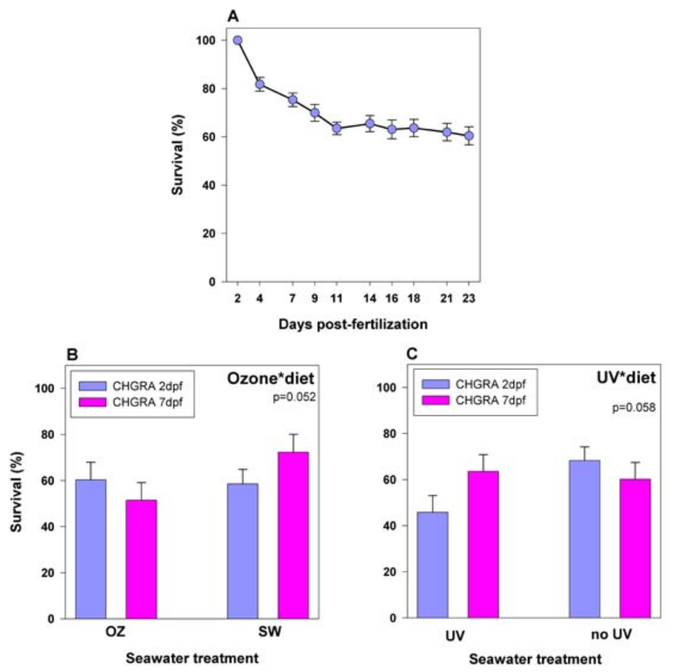

Experimental design. A) the seawater was treated with ozone and/or with UV following a 2×2 factorial experiment. Larvae were fed a mixture of *Pavlova lutheri* (MONO), *Isochrysis galbana* (TISO) and *Chaetoceros gracilis* (CHGRA). CHGRA was introduced either at day 2 post-fertilization (dpf) or at 7 dpf. B) Larvae were sampled at 7 and 23 dpf for lipid analysis and every two days for growth and survival measurements.

## Results



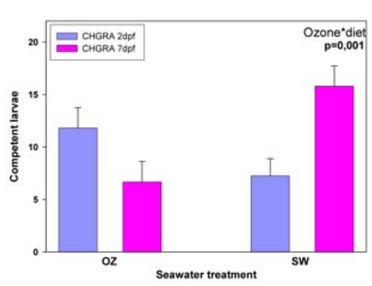

Concentration of bacteria as expressed as number of colonies per mL of seawater as a function of time for each water treatment.

- There was no *Vibrio* detected in the seawater irrespective of the treatments (data not shown).
- The treatment ozone+UV (OZ+UV) showed the lowest bacterial concentration.
- Ambiant seawater (SW) showed the highest concentration of bacteria at 16 dpf and decreased at 23 dpf.



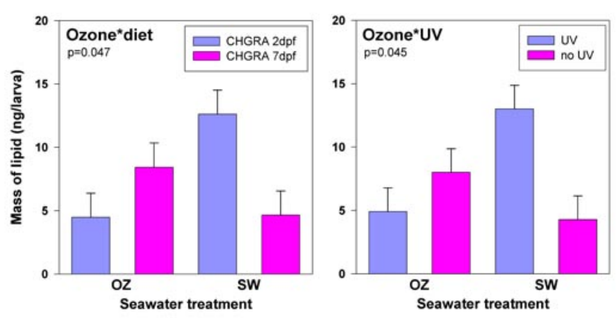

A) Survival of oyster larvae as a function of time, irrespective of treatment. B) Survival of oyster larvae as a function of ozone × diet. C) Survival of oyster larvae as a function of UV × diet (mean ± SE).

- There was no effect of treatment on growth rate of oyster larvae (18.9-25.2 um day$^{-1}$, data not shown).
- Survival varied as a function of UV × diet and ozone × diet.
- Similarly, survival was lower in ozone treated seawater (59%) compared to that in untreated control (62%).
- The top-performing larvae were obtained with untreated seawater + CHGRA at 7 dpf (72% survival at 23 dpf).
- Overall, survival was lower in UV treated seawater (57%) compared to that in untreated control (62%).




Cumulative percentage (mean ± SD) of competent larvae transfered in downwellers for settlement as a function of diet and seawater treatments.

- Percent of competent larvae increased as a function of time and varied as a function of ozone × diet.
- As observed for larval survival, % of competent larvae was lower in ozone treated seawater (9%) compared to that in untreated control (12%). The top-performing treatment based on the yield of competent larvae was untreated seawater + CHGRA at 7 dpf (26% of competent larvae at 35 dpf).




Absolute concentration (mean ± SD) of triacylglycerols as a function of ozone × diet and ozone × UV at 23 dpf.

- Overall, the concentration of TAG increased from 0.2 at 7 dpf to 7.6 ng larvae$^{-1}$ at 23 dpf, thus reflecting the accumulation of storage lipids to fulfill the energetic needs of oyster for metamorphosis.
- Pre-metamorphic larvae fed CHGRA at 2 dpf reared in untreated seawater showed the highest level of TAG.
- Interestingly, the top-performing larvae (based on the number of competent larvae), reared with untreated seawater + CHGRA at 7 dpf, showed the lowest level of TAG. Therefore, our results suggest that varying level of TAG between 4-12 ng larvae$^{-1}$ did not increase the performance of oyster larvae.

## Conclusions

- Our study show that there was no effect of seawater treatments on growth rate of oyster larvae.
- In contrast, larval survival at 23 dpf and % of competent larvae at 35 dpf were lower in ozone treated seawater compared to that in untreated control. The top-performing treatment based on the yield of competent larvae was untreated seawater + CHGRA at 7 dpf.
- The level of TAG varied as a function of seawater treatment, but it seems not correlated with the performance of oyster larvae.
- Overall, despite the fact that UV and ozone are widely used in hatchery cultivation of bivalve larvae, our study cleary showed that these desinfection techniques did not improve the performance of oyster. Our hypothesis is that disinfection may disturb the balance between microbial communities, or favour proliferation of opportunistic bacteria or unpredictable development of bacterial communities. Thus, there is a need for better microbial control during intensive larval production. The use of probiotics has proven advantageous in domestic animal production and microbial management may also have a potential in aquaculture of bivalve larvae[3]. Better control of host–microbe interactions is a prerequisite for stable production of marine larvae in intensive systems.

## Literature cited

[1] Folch J., Lees M., and Sloane-Stanlez G.H. 1957. Journal of Biology and Chemistry 226: 497-509.

[2] Parrish C.C. 1999. Lipids in freshwater ecosystems. M.T. Arts and B.C. Wainman (Eds). New York, Springer-Verlag. pp. 5-20.

[3] Olafsen, J.A. 2001. Interactions between fish larvae and bacteria in marine aquaculture. Aquaculture 200: 223-247.

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