DEVELOPMENT OF A NEW STERILE MICROALGAE DIET TO APPLY IN RELAYING AND DEPURATION OF OYSTER *Magallana gigas*

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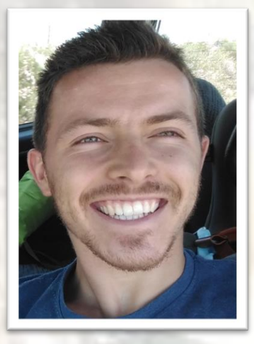

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AQUA 2024

INTRODUCTION

Oysters Prior to commercialization, bivalves reared or harvested in locations with high microbiological contamination require **depuration** for food safety

Depuration Animals are held in tanks of clean seawater with **total absence of food** for 1-3 days or longer in relaying facilities under conditions that maximize the expelling of their intestinal contents (Lee *et al*., 2008)

Problem Animals in starvation and low animal welfare (Ruano *et al*., 2012): ↓ Survival ↓ Physiological condition ↓ Nutrition value (e.g. essential fatty acids)

Solution **Develop microalgae-based diets** for depuration and relaying facilities to efficiently **maintain** the animal's **condition**, **prevent** undesirable **mortality** and onshore microbiological **safety** of bivalves for human consumption. Thermal treatments in microalgae liquid concentrates can reduce considerably their bacterial content (Dan *et al*., 2018) which is essential for the application of microalgae diets in depuration

OBJECTIVE

Design and formulate a new microbiological-safe liquid-concentrated microalgal diet to apply in relaying and improve depuration efficiency of the pacific oyster *Magallana gigas*

METHODOLOGY

Diet formulation and sterilization

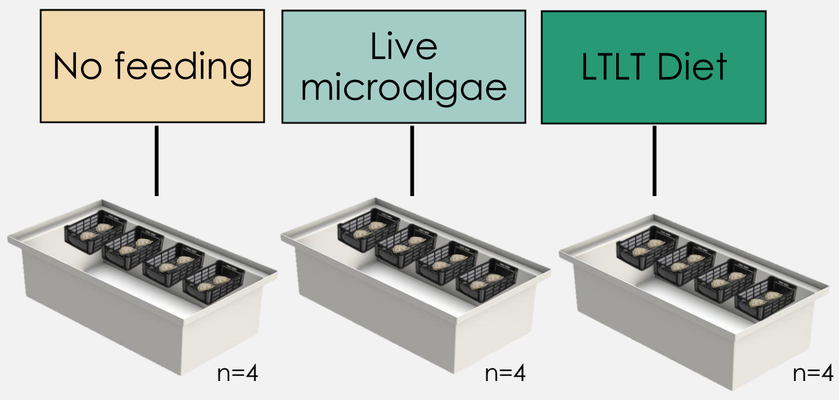
Microalgal liquid concentrate diet

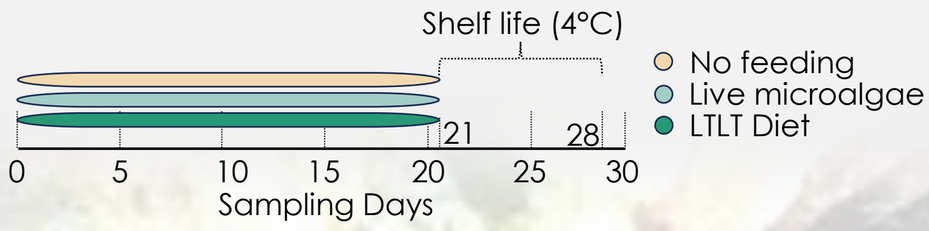
- 8% Dry Weight (**DW**)
- *Tetraselmis* sp., *Schizochytrium* sp., cornstarch

Diet Sterilization	
1. Autoclaved Diet	121°C, 40 min
2. Low-temperature long-time (**LTLT**) Diet	75°C, 60 min, cold shock until 10°C
3. Control Diet	No thermal treatment

Analysis: total bacteria (Tryptic Soy Agar (**TSA**))

Oysters feeding during Depuration and oysters Shelf Life





Dietary treatments	
1. Control	No feeding
2. Live microalgae	3:2 *Tisochrysis lutea* and *Tetraselmis* sp. (3%) (g of microalgae per g of meat dry weight)
3. LTLT Diet	8% (g of diet DW per g of meat dry weight)

Analyses

- Environmental parameters
- Survival
- Condition Index (**CI**)
- Biometric measurements (**wet weight**, **length**)
- Microbiology (**TSA;** Most Probable Number (**MPN**)
- Pigments and product decantation

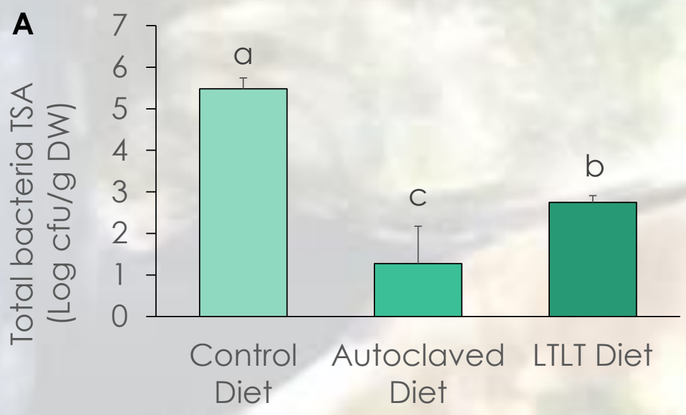
RESULTS AND DISCUSSION

Microbiology

Biometric Measurements

Physiological Condition

Control Diet | Autoclaved Diet | LTLT Diet | Base level | No feeding | Live microalgae



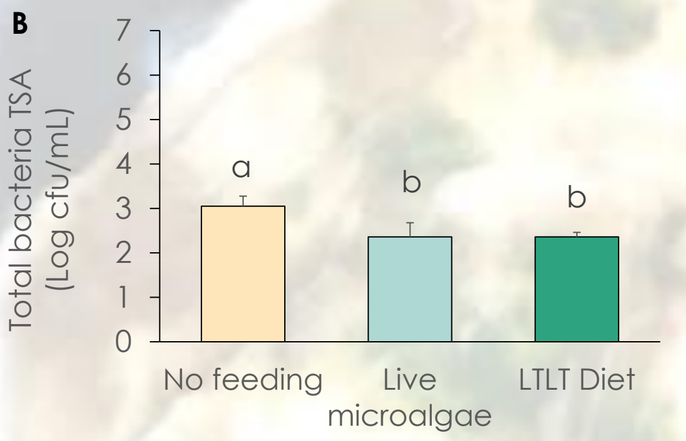
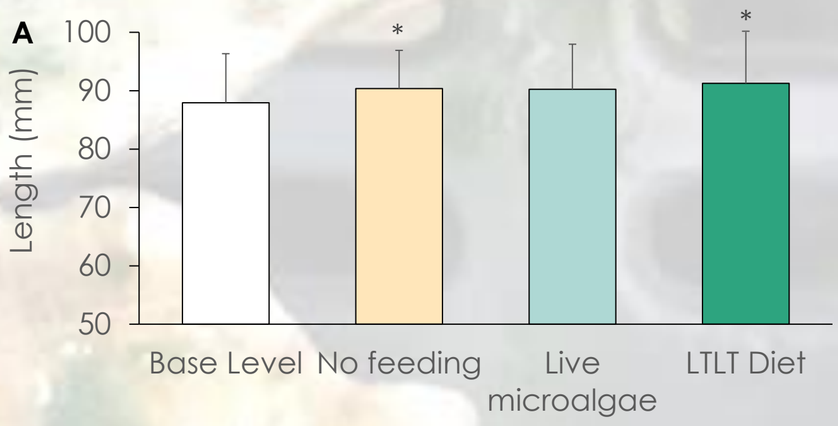


Figure 1: Total Bacteria (TSA) of diets and depuration water (mean ± SD, n = 3): A) microalgal liquid concentrate diet without thermal treatment, or treated with autoclaving and LTLT; B) water from oysters depuration tanks after 3 days of feeding with treatments. Letters represent significant differences (One-way ANOVA, *post-hoc* Tuckey, $p < 0.05$)



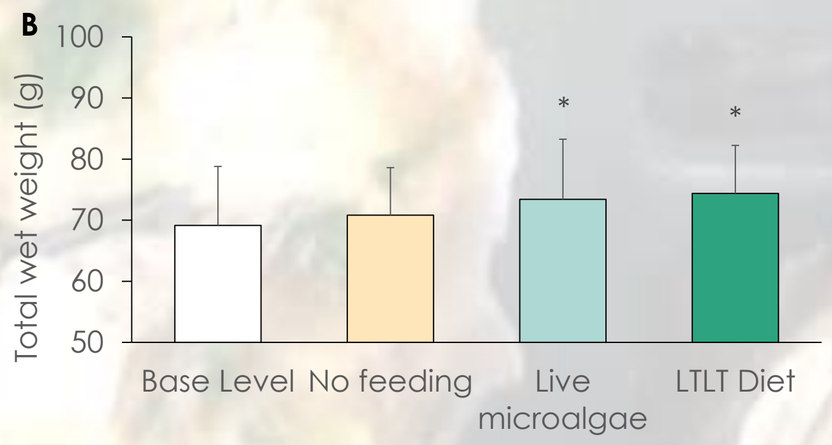
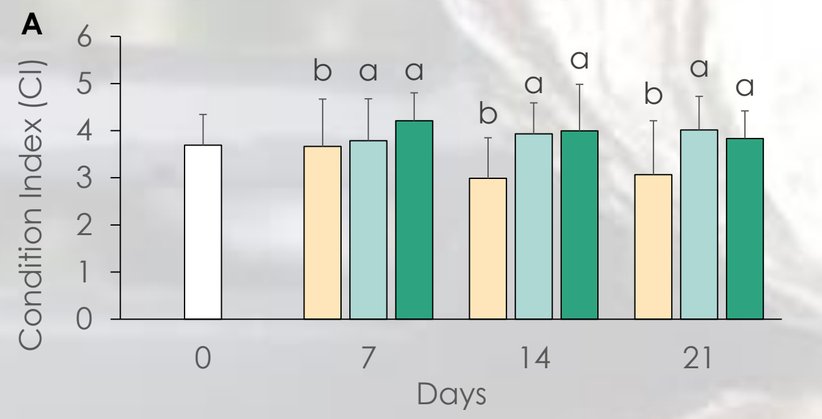


Figure 2: *M. gigas* oysters biometric measurements after 21 days of depuration for each dietary treatment and comparison with the base level (0 h) (mean ± SD, n = 50): A) valve length (mm) and B) total wet weight (g). Asterisk represents significant differences in comparison to base level (independent samples T-test, $p < 0.05$).



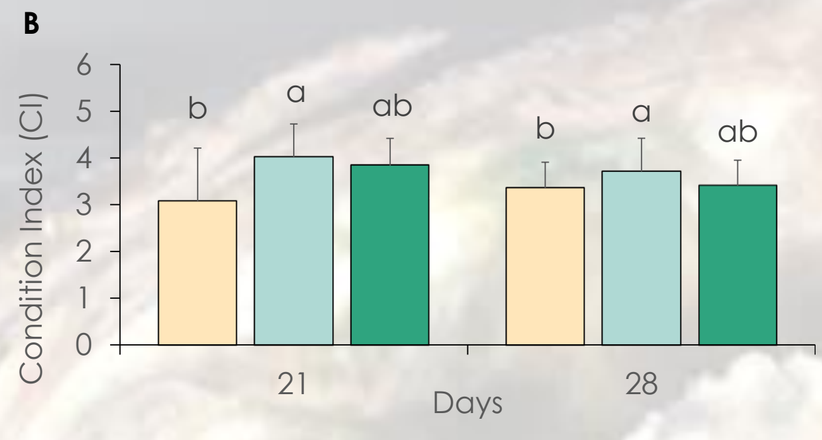


Figure 3: *M. gigas* oysters Condition Index (CI) of each dietary treatment: A) oyster CI at base level (0 h) and throughout the depuration trial (mean ± SD, n = 10); and B) oysters CI after 7 days of shelf life (4 °C) (day 28) in comparison with the last day of feeding at depuration (day 21). Letters represent significant differences between treatments (Two-way ANOVA, *post-hoc* Tuckey, $p < 0.05$).

- **Thermal treatments significantly reduced** the product's **bacterial content** compared to the control. **LTLT diet** was chosen due to **significant lower decantation** in the water column along with a **minor decrease** of **carotenoids** and **Chlorophyll *a*** useful for bivalve nutrition
- *E. coli* was <18 MPN/100 g within the first 18 hours of depuration
- Oysters fed with **LTLT diet** had **significantly higher length** and **total wet weight** at the end of the feeding trial compared to base level
- After 3 days of depuration, the treatment **without feed** showed significantly **higher total bacteria in water** compared to the other treatments
- Oysters CI did not change after 7 days in refrigeration. At the **end of shelf life**, **LTLT diet** showed **no significant differences** compared to the other treatments
- **LTLT diet significantly improved CI compared to control** and had **no significant differences** compared to live microalgae feeding

CONCLUSIONS

- The **thermal treatment** (LTLT) allowed to **reduce** the **bacterial load** and **extend** the diet **shelf life** compared to non-treated diet
- LTLT diet is **nutritionally-balanced** for *M. gigas* during depuration and **adequate** to sustain oysters **physiological condition** during **shelf life**
- The **LTLT diet**, created to feed oysters during relaying and depuration, is an excellent and **better alternative** to improve **growth** and maintain the **animals' physiological condition** compared to standard depuration (no feeding)

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ACKNOWLEDGEMENTS: This project has received funding from Fundo Europeu dos Assuntos Marítimos e das Pescas (FEAMP) and MAR2030 research and innovation programmes under grant agreement No. MAR-021.1.3-FEAMPA-00023 (SNMB-SUL)