

# ON THE DEVELOPMENT OF A TECHNOLOGICAL SOLUTION FOR LONG-TERM PRESERVATION OF THE COMMERCIAL QUALITIES IN LIVE OYSTERS

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**Abstract:** *The Pacific Oyster has adapted well to the southern Brazilian environmental conditions, where the combination of factors allows harvest in less than eight months. Although convenient for most of the year, it is during summer that water temperature can exceed 30 °C, a summer condition that leads to high mortality rates. According to the literature, if spawning is not triggered, a substantial reduction in mortality can be achieved. Studies previously presented at CYTEF 2020 showed that confining oysters at temperatures close to those of basal metabolism can inhibit the effects of gametogenesis. Further research was conducted in order to verify the possible outcomes of long term confinement of late autumn harvested oysters, presenting high glycogen reserves. This article reports the experimental results of those tests, regarding the preservation of quality attributes and the reduction of mortality rates, when confining oysters under temperatures similar to those of basal metabolism regime.*

**Keywords:** Crassostrea gigas, mortality rates, Product development, Summer mortality

## 1. INTRODUCTION

The oyster farming in Brazil is found almost exclusively in its southern state of Santa Catarina. The state accounts for over 95 % of the national production of bivalve mollusks, having the Pacific oyster as the second largest production [1]. Santa Catarina has impressive conditions for oysters farming: an appropriate physical geography, good quality water, a convenient water temperature regime, and the abundance of nutrients [2]. The species farmed in its coast, *Crassostrea gigas*, is well adapted to the environmental conditions, where the combination of the aforementioned features allows the local producers to harvest once a year. Even though water temperatures are considered to be appropriate most of the year, it is during the summer that they can rise up to 30°C. Such conditions lead to high levels of stress, weight loss and death rates up to 55% [3][4][5].

Spawning is a process that demands a significant amount of the oysters' energy reserves. After spawning the *Crassostrea gigas* presents very low glycogen levels, which can explain its fragility to react against adverse conditions and reduce its capability to perform the physiological functions of adaptation, such as to combat biological attacks. As demonstrated in the article "On the development of a technological solution to mitigate summer mortality in Brazilian oysters' crops: reporting experimental results", presented in CYTEF 2020, if the spawning is not triggered, a substantial reduction in the mortality rates can be achieved [5].

The above scenario drove the development of a technological solution, in order to overcome the aforementioned problem. This article presents the experimental results of additional tests on the effects of prolonged confinement of Pacific oysters harvested in late autumn, presenting high levels of glycogen reserves and superior organoleptic properties.

## 2. MATERIALS AND METHODS

### 2.1. Study site and experimental description

Tests were conducted at the Nixxen Comércio de Frutos do Mar marine farm, located at a protected bay area in the south of Florianópolis island, Brazil, with GPS coordinates: 27°44' S; 48°33' W [4]. The experiment set was built and operated in an isolated area in the marine farm, next to the cultivation site.

The experiment started when 1 mm seeds, from the Marine Molluscs Laboratory of the Federal University of Santa Catarina, were sent to the sea in April/2020 and became adult at late spring of the same year, after consecutive stages of management. On 04/28/2021, a 600 adult oysters population was accommodated in five trays, in a controlled environment tank, with population density of 120 individuals per tray. An equivalent population of oysters remained at sea hosted in three lanterns, at 1.5-m depth, with 300 individuals equally distributed in five out of six compartments of each, for the reestablishment of the described populations' density.

The population was submitted to controlled environmental conditions for periods of up to 60 days, until 07/27/2021, observing the mortality rates, and the maintenance of desirable characteristics assumed as those presented at the beginning of the test.

After a 24-hour acclimatization period, the oysters remained at the range from 10 to 12 °C throughout the experiment. After the reference sample, at least five subsequent weekly observations were carried out to evaluate the following characteristics: oyster height, defined as the longest length measured on its main axis [6]; total weight; drained meat weight and; and mortality rates in the period under analysis.

### 2.2. Experimental apparatus

The experimental apparatus was developed by performing of the informational and conceptual design phases of product development methodology proposed by Back *et al.* [7], which results indicated the need of the following subsystems: a thermally insulated tank where oyster trays and circulating water are housed; hyper aeration subsystem, for water aeration and removing of suspended organic matter; mechanical filtration subsystem; biological filtration; water renewal, and; a water cooling subsystem.

The cooling technology to be applied was defined based on the work of Brown & Domanski [8], as well as the desired environmental conditions were determined based on the studies of Le Gall & RAILLARD [9]. The temperature range between 10 and 12 °C was established, with a 10 % volumetric daily water renewal. No other water parameters were controlled.

### 2.3. Measurement procedures and statistics

Oysters' weight and height were respectively measured by means of a digital electronic balance and a mechanical caliper with resolutions of 0.001 kg and 0.00001 m, respectively. Temperatures in the tank were measured by a digital electronic thermostat, with resolution of 0.1°C for the -10 to 100 °C temperature range, and a minimum of three measurements per day were recorded. The measurement uncertainties were identified considering the uncertainties associated with the measurement system ( $MI_{ins}$ ), and those associated with the measurement process ( $MI_{rep}$ ) [10]. The measurement uncertainty (MI) is given by: equation:

$$MI = t (MI_{ins}^2 + MI_{rep}^2)^{1/2} \quad (1)$$

Where: t stands for the Student t-distribution coefficient or coverage factor;  $MI_{ins}$  and  $MI_{rep}$ , the components of uncertainty due to the measurement system (instruments) and to the repetitions (process) made for its determination. All measurement uncertainties were calculated for 95% probability coverage of the uncertainty interval ( $\pm MI$ ), safeguarding the degrees of freedom for each analyzed variable.

A set of statistical tests was chosen according to the requirements of each data format: Pearson Correlation Coefficient was used to identify correlation levels between variables; t-Student test was applied to identify the behavior of parametric variables; Kruskal-Wallis test, to the analysis of non-parametric data and the Wilcoxon-Mann Whitney as a non-parametric alternative to the t-test when the premises of normality and homogeneity of data couldn't be assured [11][12].

## 3. RESULTS

The mean temperature of the water in the tank was  $11 \pm 1$  °C throughout the experiment, with a maximum of  $17.2 \pm 1$  °C observed in the last week of tests. Circumstantial temperature elevations were promptly mitigated to minimize their effects on test results.

### 3.1. Oysters' height

Figure 1 presents the results for oysters' height in seven independent observations.

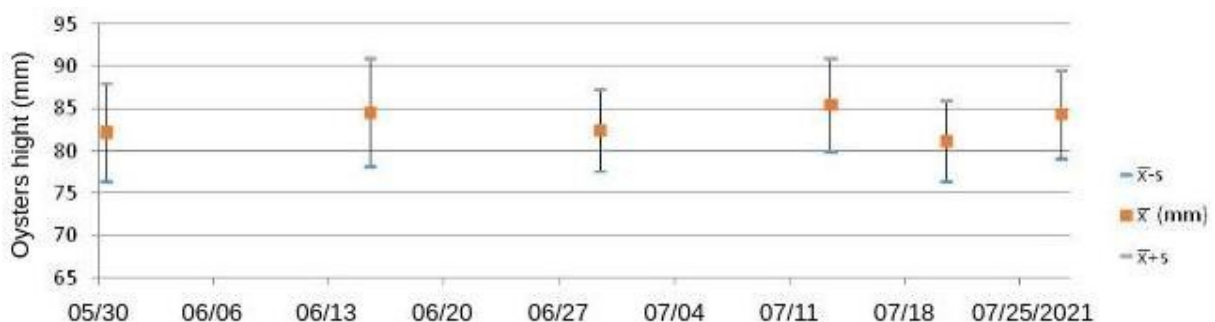


Figure 1: Oysters height (mm).

The oyster shell is formed by a calcareous structure that accompanies the growth of the mollusc and tends to remain stable during periods of little or no growth, never decreasing in size [ 6]. This assumption suggests evaluating a possible increase in oyster size by formulating the following hypotheses:

$H_0$ : There are no height differences between samples.

$H_1$ : There was growth in the batch ( $\mu_1 - \mu_2 < \delta$  ( $\delta=0$ ); single tailed).

The critical region limit ( $t_{\alpha} = -1.686$ ) is obtained from the t-Student table for 38 degrees of freedom and significance  $\alpha$  of 5%, single-tailed. The values of the combined standard deviation and of the coefficient  $T_{calc}$  for 30 and 58 days, are respectively (1.738; -0.154) and (1.788; -1.190), demonstrating, within a significance level of 5 %, that the basic hypothesis  $h_0$  was accepted, that is, it was not possible to identify a significant growth in the observed periods.

### 3.2. Oysters' total weight

The analyses for data distribution normality and variance homogeneity were respectively supported by the Shapiro-Wilks and Bartlett tests, which results upheld the aforementioned requirements. In accordance with the objectives of this research, the total weight of the oysters is expected to remain stable during the testing period. In that sense, weight loss is not expected in the population, which has driven the formulation of the following hypotheses:

$h_0$ : No 'total weight' differences were observed.

$h_1$ : There was weight loss in the samples ( $\mu_1 - \mu_2 > \delta$  ( $\delta = 0$ ) - single tailed).

The critical region limit ( $t_{\alpha} = 1.686$ ) is obtained from the t-Student table for 38 degrees of freedom and significance  $\alpha$  of 5%, single-tailed. The values of the combined standard deviation and of the coefficient  $T_{calc}$  for 30 and 58 days, are respectively (3.887; 0.488) and (3.552; -0.732), demonstrating, within a significance level of 5%, that the alternative hypothesis  $h_1$  was rejected. The results indicate that there was no weight loss for both 30 and 58 days of confinement. Figure 2 presents the results for the variable 'Total weight' throughout the experiment.

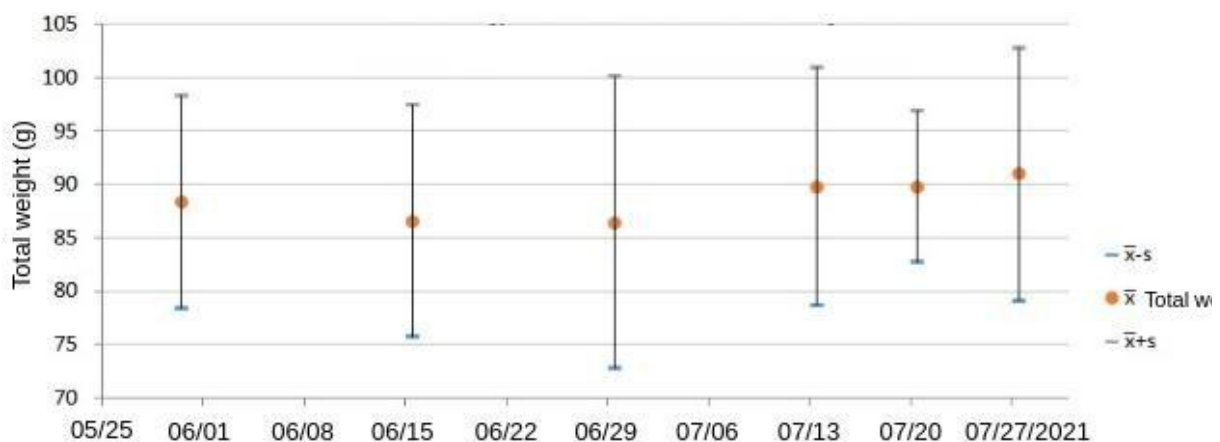


Figure 2. Total weight (g)

### 3.3. Oysters' meat weight

Analogously to the 'total weight' variable, the population is expected to be maintained in similar conditions to those at the beginning of the experiment, and a severe reduction in meat weight is not acceptable. In this way, the basic and alternative hypotheses are formulated in an identical manner to the previous case.

$h_0$ : No 'meat weight' differences were observed.

$h_1$ : There was meat weight loss in the samples ( $\mu_1 - \mu_2 > \delta$  ( $\delta = 0$ ) - single tailed).

The obtained  $t_{\alpha}$ , for 38 degrees of freedom and a significance of 5 %, single-tailed, is 1.686. The values of the combined standard deviation and of the coefficient  $T_{calc}$  for 30 and 58 days

are, respectively, (1.003; -0.698) and (1.058; -0.709) and determine the acceptance of the basic hypothesis, rejecting the alternative  $h_1$ . The results indicate that there was no loss of meat weight for any of the analyzed periods. Analogously, it is demonstrated that the oysters kept, at least, the same initial characteristics. Figure 3 presents the results for the variable 'Meat weight' throughout the experiment.

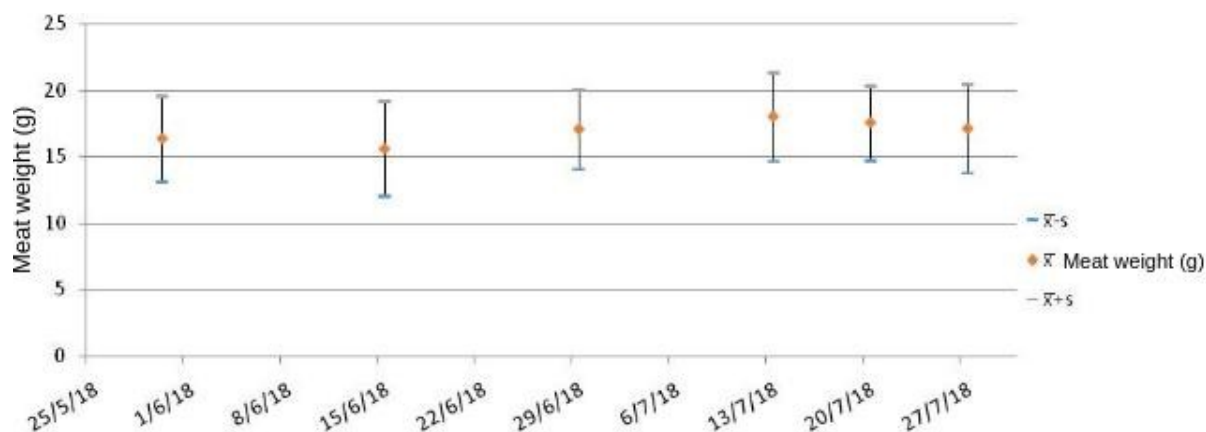


Figure 3. Meat weight (g)

### 3.4. Mortality rates in the confined lots.

A single oyster died during the entire experiment, and it is noteworthy that this death occurred within the first 15 days of confinement. Although, for scientific rigor, a total mortality rate of 0.17% should be reported, it is suspected that the cause of death may be due to factors preexisting to the test period, since in the 45 days that followed this event no further occurrences were recorded and there is no evidence that the confined population suffered losses in any of the observed variables, as attested by the results presented above.

Additionally, a 60 oysters' sampling was taken from the confinement module and returned to the sea, where they remained for further 15 days in order to evaluate its behavior. All survived and showed external signs of good health.

## 4. DISCUSSION

Temperatures in the lodging tank remained stable within the intended range of 10 to 12 °C, with an accessed mean of  $11 \pm 1$  °C. Eventual peaks of temperature did not present noticeable consequences over the observed variables. Results for oyster height are coherent with the expected behavior for temperatures to which the population was submitted [9]. The meat weight remained stable in the confined population, which is also consistent with the expected deliveries from the confinement system. The low mortality rate obtained in this experiment indicates that oysters harvested at this stage of culture, characterized by high glycogen reserves, have better chances of withstanding long periods of confinement under near basal metabolism conditions, when comparing with the reported results for late spring harvested oysters [5].

## 5. CONCLUSION

This work aimed at the evaluation of adult *Crassostrea gigas* behavior when submitted to long term confinement under temperatures equivalent to that of basal metabolism induction. In addition to the mortality rates throughout the tests, other quality related attributes were analyzed for confinement periods of up to 60 days, with an average sampling period of 7 days. Results show that the tested independent lots presented good tolerance to the designed confinement regime. Statistical analysis led to the validation of the proposed solution by revealing the preservation of desirable commercial characteristics and the achieve-

ment of low mortality rates. The mortality rate of 0.17 % obtained for the population housed in the designed technical system suggests that oysters presenting high glycogen reserves are suitable to be kept in confinement for prolonged periods, keeping their main commercial characteristics, and organoleptic parameters, stable.

This work extends research reported in CYTEF 2020 [5] by indicating stages of oyster cultivation that tend to respond best to prolonged confinement. The suitability of the proposed technical system for long-term conservation of live oysters was again verified, indicating the opportunity for further research to verify its possible impact on current cultivation practices, as well as its application to face red tides occurrence in the cultivation areas.

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