

Effect of temperature on growth and survival of *Crassostrea corteziensis* spat during late-nursery culturing at the hatchery

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Received 2 March 2007; received in revised form 20 June 2007; accepted 21 June 2007

Abstract

Nine temperatures (16, 18, 20, 22, 24, 26, 28, 30, and 32 °C) within the natural range of distribution of the Cortez oyster *Crassostrea corteziensis* were tested in a first experiment to determine the optimal temperature for growth and survival. Based on these results, a second study assessed two temperatures above this range (34 and 36 °C) to determine upper median lethal temperature for the species. The species was thermo-tolerant between 16–32 °C, grew faster and larger at 24 to 30 °C, and had optimal growth at 28–30 °C. The lower tolerance of the species appears far from the lowest value tested (16 °C). In contrast, the upper tolerance temperature was near 32 °C, since 100% spat mortality occurred within 96 h at 34 and 36 °C. These results are being used to develop a protocol for large-scale hatchery culture of the species in Mexico.

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Keywords: Mexico; Oyster; Spat; Temperature; Nursery culture; Hatchery

1. Introduction

In marine bivalves, temperature is recognized as one of the fundamental exogenous factors influencing most aspects of the ecology and biology of the species (see reviews by Kinne, 1971 and Shumway, 1982). It limits the general distribution of a species within large geographic areas, as well as specific habitat occupation between diverse communities or populations. Temperature also regulates many physiological functions of organisms, such as cilia activity during filter-feeding, cardiac and growth rates, gonad development and reproductive

output, energy budget, and metabolic activity of enzymes (Widdows, 1973; Bayne et al., 1976; Newell and Branch, 1980; Shpigel et al., 1992; Barber and Blake, 2006). Additionally, changes in temperature affect survival of larvae, juveniles, and adults of most marine bivalve species, not only in wild populations, but also in laboratory experiments (Holland, 1978).

Among the diverse marine bivalve species inhabiting the Pacific Ocean, the Cortez oyster *Crassostrea corteziensis* (Hertlein, 1951), whose distribution covers most of the tropical and subtropical coasts from Mexico to Peru, is of particular interest (Stuardo and Martínez, 1975; Mazón-Suástegui, 1996; Chávez-Villalba et al., 2005). In the states of Sonora and Sinaloa in Mexico, the species has been overexploited and most natural beds show severe signs of depletion or even virtual extinction

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(Hoyos-Chairez and Robles-Mungaray, 1990; Chávez-Villalba et al., 2005). Consequently, the oyster industry in the northwest coastal Mexico has concentrated its efforts on cultivating the Pacific oyster *C. gigas* (Thunberg, 1793). However, this species has a natural temperate distribution, and although it has been successfully introduced to Mexico decades ago, it has suffered large die-offs in recent years. Given this situation, *C. corteziensis* has been receiving great deal of attention because of its relatively low production costs, excellent taste, and high nutritional value. Currently, the incipient fishery developed with this species does not provide the numbers of oysters demanded by the local and national markets, and therefore, scientific improvement of cultivation methods is essential in the short future (Mazón-Suástegui et al., 2001; Chávez-Villalba et al., 2005). So far, there is some understanding of the ecology and biology of the species, including population dynamics (Stuardo and Martínez, 1975), changes in tissue chemical composition (Páez-Osuna et al., 1993), reproductive cycle (Frias-Espicueta et al., 1997), field cultivation (Chávez-Villalba et al., 2005), and more recently, determination of nutritional requirements of broodstock using artificial low-cost products (Leyva-Miranda, 2005). However, other aspects involving physiological regulation of the species still remain unclear.

As part of a broad project aimed to develop a protocol for large-scale hatchery culture of *C. corteziensis* in Mexico, this paper studied the response of spat exposed to a wide range of temperatures during late-nursery culture at the hatchery. The goal was to find the optimum temperature for growth and survival and the upper median lethal temperature during the juvenile stage prior to transfer to the field for growth to commercial size.

2. Materials and methods

The optimum temperature for growth and survival was determined in a first experiment by measuring growth rates of juveniles exposed to a wide range of temperatures occurring in the natural habitat where the species is distributed (Mazón-Suástegui et al., 2001; Chávez-Villalba et al., 2005; Leyva-Miranda, 2005). Upper median lethal temperature was estimated in a second experiment with survival data of juveniles exposed to high temperatures.

2.1. Optimal temperature for growth

Spat used for this study was produced at the hatchery following the methods of Mazón-Suástegui et al. (2001). From this, 810 juveniles (5.0 ± 0.1 mm shell height) were acclimated for one week at a temperature of 26 ± 1 °C and a salinity of 37 ± 1 . During acclimation, juveniles were fed

Isochrysis galbana and *Chaetoceros muelleri* at a 1:1 ratio (cell count) and a density of 80×10^3 cells ml^{-1} . After acclimation, they were divided into nine experimental treatments for exposure to the following temperatures held constant throughout the study: 16, 18, 20, 22, 24, 26, 28, 30, and 32 °C. Experimental treatments at each temperature consisted of triplicate 20-L plastic containers holding thirty specimens placed in plastic mesh bags. They were fed an equal mixture (cell count) of *I. galbana* and *C. muelleri* at 80×10^3 cells ml^{-1} (days 1 through 7) and 100×10^3 cells ml^{-1} (after day 8). Each container used 1- μm filtered and UV sterilized seawater (salinity of 37 ± 1). The containers were drained, washed, and refilled with clean seawater every three days.

At the beginning of the experiment (t_0), initial shell dimensions and wet weight of 100 juvenile oysters were measured. Shell and weight growth rates were estimated by the difference between final and initial values, divided by the experimental time (days). Subsequent measurements of both dimensions were made on days 7 (t_1), 14 (t_2), 21 (t_3), and 28 (t_4), using 10 specimens randomly selected from each container (30 specimens per thermal treatment).

2.2. Temperature tolerance

In the second experiment, the upper temperature tolerance (LT_{50}) of the species was determined by the median lethal dose method (Sprague, 1973); 200 hatchery-reared spat (4.1 ± 0.1 mm shell height) were exposed to constant temperatures of 34 and 36 °C, using the same acclimation procedures and experimental protocol as the first experiment. These temperatures were chosen because no deaths occurred in the range of 16–32 °C in the first experiment. Survival was estimated until all oysters were dead. Death was determined using the criteria of a gaping shell. The upper LT_{50} value and its 95% confidence limits were calculated for the first six days of temperature exposure with a computer program based on the method of Finney (1971), which transforms raw mortality data into probit mortality. The estimated probit line, together with results of a chi-square test for goodness-of-fit and a z-test to compare the two LT_{50} values (at 5% significance) were determined.

2.3. Statistical treatment of data

Group normality (in shell height and wet weight of spat) was initially evaluated with the Kolmogorov–Smirnov test and then with two-way, nested ANOVA for significant differences in growth of spat as a function of temperature (main factor with nine levels) and batch replication (nested factor with three levels) (Sokal and Rohlf, 1981). When

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