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Short Communication

Valve Activity in Cultured Oysters Exposed to Sudden Increases in Salinity

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Abstract

The submergence of live cultured *Crassostrea virginica* in high salinity seawater (> 28 parts per thousand, ppt) is a practice the seafood industry may consider for enhancing taste and marketability. We investigated the response of triploid *C. virginica,* cultured in mesohaline areas of Chesapeake Bay, to sudden exposure to elevated salinity in artificial seawater. Valve activity and duration of valve closure was recorded for three levels of salinity—14 parts per thousand (ppt), 22 ppt, and 28 ppt—in aerated 17-liter aquariums for 72 hours. We recorded valve activity in the first 12 hours of exposure and monitored mortality for 72 hours. Valve activity was influenced by salinity and length of exposure. Higher salinity waters had less valve activity initially but all treatments exhibited strong valve activity within 72 hours. We observed no mortality after the 72-hour period. The research showed that *C. virginica* resumes valve activity within a couple of hours of being submerged in artificial seawater with a range of 14 to 28 ppt salinity. Aquaculturalists seeking to enhance taste through the use of short-term saltwater baths may not need an acclimation step before exposing *C. virginica* to aerated artificial seawater.

Keywords: Salinity; Oysters; Acclimation; Aquaculture

Introduction

Aquaculture production of eastern oysters, *Crassostrea virginica* (Gmelin 1791), in the Chesapeake Bay (herein referred to as the Bay) has shown considerable growth in recent years. Due to an increase in local production of oysters, growers in Maryland are seeking new ways to distinguish their product from competitors.

One method that has been proposed is to place live oysters in a saltwater bath for 24 hours to enhance taste prior to marketing. Growers currently improve taste by harvesting market-sized oysters from 5-15 parts per thousand (ppt) mesohaline water of the Chesapeake Bay and transporting and submerging them in Chincoteague Bay in Virginia where salinities range from 23 to 36 ppt [1]. The re-submergence of harvested shellfish to new locations is known in the industry as relaying. In this case, it is used to increase the salt content of the meat but in other regions, the process is used to purge shellfish of harmful contaminants and pathogens

[2]. Oyster farmers claim that mortality rates in cultured Bay oysters are negligible during the short-term relaying process (Johnny Shockley, co-owner of oyster farm, pers. com., 2012).

Salinity and temperature are influential environmental factors on the life cycle, physiology, and growth rates and feeding in *C. virginica* [3]. When submerged in environmental conditions outside of their optimal range, oysters may remain closed for a period of time, restricting their contact to the outside environment. Glastnoff [4] stated that reduced salinity resulted in partial or complete valve closure and a decrease in water flow through gills. Similarly, Loosanoff [5] observed that valve closure lasted approximately 6 hours when oysters were exposed to lower salinities. Fisher and Newell [7] found that increases in ambient salinity slow the oyster defense system by reducing the locomotive rate of haemocytes and that this affect is greater during greater swings in salinity. More recently, Méthé et al [7] recorded stress responses in *C. virginica* exposed to downriver (high salinity) to upriver sites (low salinity) and found greater stress responses in oysters exposed to lower salinities. This was attributed to differences in osmotic equilibrium caused by a combination of environmental factors, not simply salinity alone.

Some studies have documented mortality induced by osmotic stress, especially when exacerbated by *Perkinsus marinus* infection, a protozoan parasite enzootic in Bay oyster populations [8,9]. An earlier study found that sudden reductions in salinity induced valve closure that lasted for prolonged periods (19.3 ± 1.2h) in *C. virginica* from the Gulf Coast, USA [10]. However, few studies are available regarding how a sudden increase in salinity affects valve activity.

In this study, we investigated the resumption of pumping in cultured *C. virginica* from a mesohaline region of the Bay after being placed in artificial seawater (ASW) baths at salinities of 14 ppt, 22 ppt, and 28 ppt for 12 hours by recording valve movement and duration of closure. We then recorded mortality after 3 days of exposure to ASW.

Materials and Methods

Oysters

Specimens of cultured *C. virginica* were provided from a bottom-cage oyster farm in the mesohaline zone of the Chesapeake Bay (38°18'N, 76°13' W) in December 2012. During the harvest, oysters were removed from cages and placed on a conveyor belt that moved them through a tumbler machine and power-wash. Farm operators provided 135 randomly selected individual oysters for our experiment. Upon delivery, oysters were stored overnight at 5°C before being placed in artificial seawater aquariums for the experiment. Salinity at harvest was 15.5 ppt (MDDNR, 2012).

Artificial Seawater

We made artificial seawater from de-chlorinated municipal drinking water and reef salt (Crystal Sea Marinemix-™, Marine Enterprises International, Baltimore, MD, USA). We prepared three levels of salinities (14 ppt, 22 ppt and 28 ppt) for 9 aerated aquariums so there were three replicates of each salinity treatment. The aquariums with salinities of 14 ppt were our control aquariums, as the salinity was similar to that at the aquaculture site in the Bay during harvest. Each aquarium was given 17 l of artificial seawater at a stable temperature. We then randomly arranged the aquariums on a lab bench in a lab set to room temperature.

We placed 15 oysters on the bottom of the aquariums arranged on a grid that was labeled on two axes—numerically and alphabetically—in order to mark individual oysters during the experiment (Figure 1).

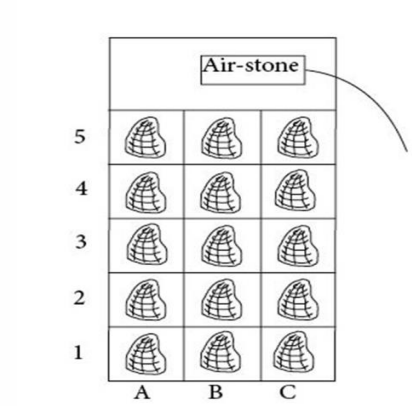

Figure 1. Each aquarium was arranged as above so that each oyster was individually labeled for observations during the study.

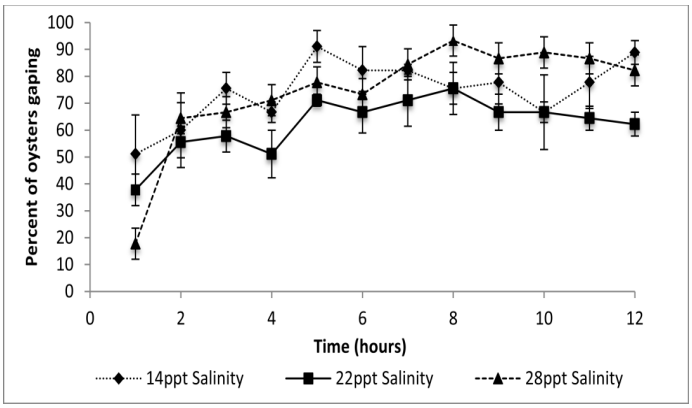

Figure 2. The percentage of oysters visibly gaping over the course of first 12 hours. No difference was detected in resumption of filtration between oysters in differing salinity regimes.

We recorded whether each individual had open valves each hour from 9:00 am until 9:00 pm for 12h of exposure. Valves were checked by sight.

Mortality

We held the oysters in the aquariums for an additional three days (72h) to determine whether exposure to artificial seawater would cause mortality. At 72 hours, we recorded the amount of oyster deaths. Oysters were considered dead if gap-

ing shells did not close upon touch.

Statistics

We analyzed the number of open valves per aquarium in a one-way ANOVA for hour 1, hour 6 and hour 12 to determine the effect of salinity and time held in aquariums using SAS 9.2 software. The frequency of observed open valves by treatment was tested using a one-way ANOVA. We determined the mean length of time to valve opening after initial exposure and the difference among the means was tested for significance in a one-way ANOVA. In both cases alpha (α) was set at 0.05.

Results and Discussion

Frequency of open valves

Figure 2 shows the percent of oysters with visibly open valves during each hour of exposure. Salinity and duration of exposure had a significant effect of the number of valves open during the experiment. We observed more oysters with open valves in the 14 ppt salinity treatment. The number of oysters with open valves was not significantly different between the 22 ppt and 28 ppt seawater treatment. Duration of exposure also had an effect; more open valves were observed as more time passed while the oysters were exposed to the artificial seawater.

Our results showed that 70% of cultivated oysters were able to open their valves within the first eight hours of exposure to a sudden change in salinity, within the range of 14 ppt to 28 ppt at 18^0C. It may be that the quick resumption of valve activity was related to aerobic respiration after a period of stress in which oysters were harvested, cleaned and transported by refrigerated truck to the laboratory.

In their natural habitat, oysters experience sudden increases in salinity when the tides bring in cooler, oligotrophic water from the seas. These waters are typically low in food resources for oysters [11], so resumption in valve activity may not be related to a response to an environmental cue that signals increased food resources. In our experiment oyster were not fed and so any resumption of valve activity was not triggered by the presence of food or by the need for respiration.

We noted that only 2 of the 135 individuals were never observed with open valves. Both of these individuals were held in the 22 ppt artificial seawater. It is possible that these individual had open valves during periods that we did not observe, such as at night or between observations. Besides these exceptions, there appeared to be no adverse effects of placing cultured Bay oysters at salinities ranging from 14 ppt to 28 ppt at this temperature.

Time to Valve Opening

The mean time to valve opening upon submergence into a new salinity regime was 2.47 ± 0.16 hrs with no significant difference between the salinity treatments. Therefore, salinity levels did not affect duration of valve closure once oysters were exposed to a new salinity regime.

Our results contrast with Hand & Stickle [10] in that we observed markedly lower durations of valve closure. The discrepancy could be related to the fact that in their study, salinity rapidly dropped from 20 ppt to 10 ppt instead of being slightly decreased for the 14 ppt treatment and raised for the 22 ppt and 28 ppt treatments in our study. A significant decline in salinity may be more detrimental to the oyster physiology than significant increases. Fresh water flooding during periods of higher than average water temperatures has often resulted in mass mortality of oysters in the wild [12]. Lower salinities may also lead to an osmotic imbalance between the haemolymph and the haemocyst interior, resulting in a stressed lysosomal membrane [13], which adversely affects the immune response of the organism. Exposure to higher salinities may not have adverse physiological affects unless oysters are exposed to predators and parasites associated with higher salinity waters such as the boring sponge, and common protozoan parasites [12,14].

Mortality

At the end of the 3-day study (72h), we did not observe any mortality of oysters in any of the salinity regimes. Therefore the temporary storage of live oysters in ASW to enhance taste may not constitute a shelf-life concern or a loss of product for marketing. This result concurs with Méthé et al [7], where low mortality rates were observed in oysters transferred to sites with differing salinity, similar in range to this experiment (7 to 25 ppt). We did not measure the overall condition of the oysters that may have been affected by the changes in salinity regime. This study could have been improved by the use of the condition index after Lawrence and Scott [15].

Experimental Design

The experiment would have benefited from continuous monitoring equipment rather than visual assessments of valve activity. With continuous monitoring, a more accurate assessment of valve activity could have been made. Additionally, salinity treatments used in this experiment were determined in consultation with aquaculturalists seeking to enhance taste, rather than to learn about oyster physiology. In order to learn more, a further study could expose oysters to extreme salinities that are outside of the organisms' optimal range of 14 ppt to 28 ppt [12] and by examining condition of oysters after exposure as mentioned above.

Conclusion

For aquaculturalists that seek to enhance saltiness of cultured oysters, we conclude that an acclimation step is not needed to prevent mortality, when given ample oxygen supply, and salinity and temperature are maintained within the ranges of this experiment (14 ppt to 28 ppt and 18^0C). Finally, it is possible that cultured oysters could be held in salt water baths for as little as 8 hours since the majority (>50%) of oysters resume filtration within the first 3 hours of exposure.

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Highlights

- Oysters submerged in 14 ppt (parts per thousand) artificial seawater were observed with valve activity more often than in 22 or 28 ppt treatments.
- Salinities between 14 and 28 ppt had no effect on the mean time to valve opening upon submergence.
- Resumption of valve activity occurred within 2.47 ± 0.16 hrs across the treatments.
- Submergence in artificial seawater without an acclimation step did not cause any mortality in oysters.

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