

Stress tolerance of a subtropical *Crassostrea virginica* population to the combined effects of temperature and salinity

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ABSTRACT

The combination of salinity and temperature has synergistic effects on virtually all aspects of the biology of estuarine organisms. Of interest were site-specific characteristics in the response of the eastern oyster, *Crassostrea virginica*, from the St. Lucie River Estuary to the interactive effects of temperature and salinity. This estuary, one of the largest on the central east coast of Florida, is strongly influenced by anthropogenic modifications due to management needs to control the patterns of freshwater flow in the St. Lucie River watershed. *Crassostrea virginica* is designated a valued ecosystem component for monitoring the health of this estuary. Our approach used a multidimensional response surface design to study the effects of temperature and salinity on sublethal measures of oyster performance: (1) body condition index as an overall indicator of bioenergetic status and (2) the RNA/DNA ratio as a biochemical indicator of cellular stress. The results showed that there was a greater ability to withstand extreme salinity conditions at lower temperatures. However, there were no site-specific attributes that differentiated the response of the St. Lucie Estuary population from populations along the distribution range. Condition index was a less variable response than the RNA/DNA ratio, and the final models for mean condition index and the RNA/DNA ratios explained 77.3 and 35.8% of the respective variances.

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1. Introduction

Estuaries are among the most biologically productive ecosystems in the world. Yet, because the physical and chemical conditions result in one of the most rigorous habitats, few organisms have successfully colonized estuaries (Nybakken, 1996; Ellis et al., 2006). The St. Lucie Estuary (SLE), which was created by the construction of a permanent inlet between the St. Lucie River and the Atlantic Ocean in the late 1880s, is now one of the largest brackish-water systems on the central east coast of Florida and a major tributary to southern Indian River Lagoon (Sime, 2005; Fig. 1). Historical data, although scarce, show that oyster populations became established and once covered an estimated 1400 acres along the north and south forks of the SLE (Chamberlain and Hayward, 1996; Wilson et al., 2005). Subsequent anthropogenic alterations of

the SLE watershed resulted in extreme alteration of salinity and declines in water quality and overall health of the estuary (SFWMD, 2002; Millie et al., 2004). It has been estimated that the viable oyster habitat in the SLE decreased to approximately 250 acres over the course of a few decades and is now limited to the middle estuary where prevailing salinities are more favorable for oyster habitation (Wilson et al., 2005).

A goal of the Comprehensive Everglades Restoration Project (CERP) and local water management organizations is to create conditions in the SLE that will provide a viable habitat for sustainable populations of fish and invertebrates (SFWMD, 2002). The eastern oyster, *Crassostrea virginica*, has been designated one of the valued ecosystem components (VEC) to monitor the health of the SLE (USEPA, 1987; Barnes et al., 2007); restoration and maintenance of the local population of *C. virginica* are viewed as Ecological Performance Measures of CERP (SFWMD, 2002; Sime, 2005) for monitoring the health of the SLE. Previous studies have shown that specific combinations of temperatures and salinities for successful growth, reproduction, and development are population-dependent (Loosanoff, 1953; Shumway, 1996; Tolley et al., 2005). Hence, generalizations of environmental requirements to previously unstudied oyster populations, such as that of the SLE, while instructive for a general understanding, can be problematic when extrapolated to specific unstudied populations. Thus, there is

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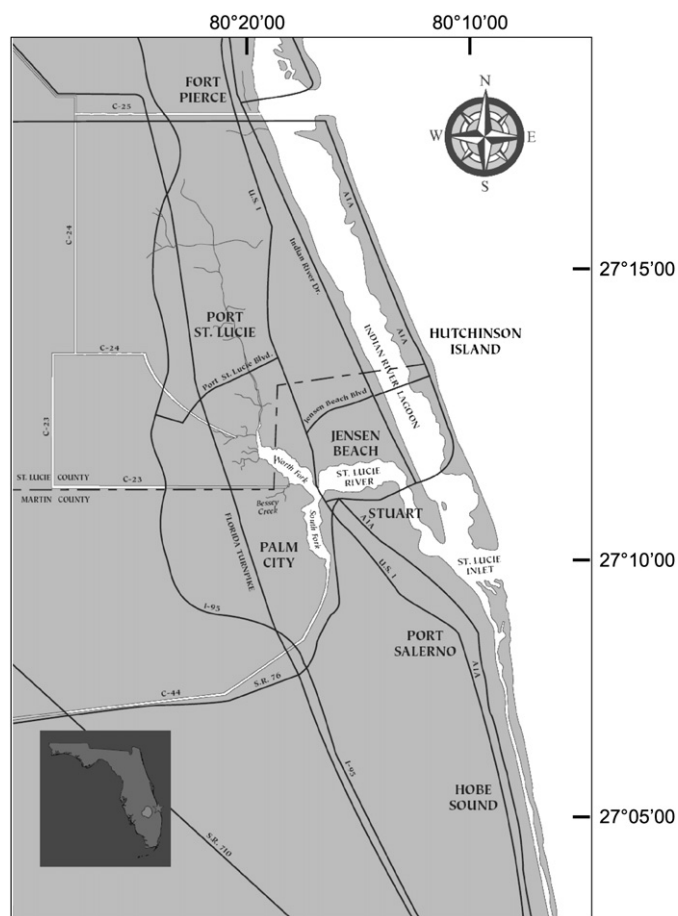


Fig. 1. Map of St. Lucie River System, showing the location of inlet created in the barrier island.

a need to understand better the site-specific effects of differences and changes in environmental salinity and temperature in order to appropriately advise local water management organizations responsible for regulating the flow of freshwater in the SLE watershed.

Variations in temperature and salinity are among the most important factors influencing the biology of marine organisms (Alderice, 1972; Ponce-Palafox et al., 1997). Reduced salinity and increased temperatures are known to influence metabolic and physiological parameters in oysters including heart rate (Feng and Van Winkle, 1975; Bakhmet and Khalaman, 2006), respiration (Shumway and Koehn, 1982), energy acquisition, and growth rate (Bataller et al., 1999) and can affect the overall health of the animal (Tolley et al., 2005; Wilson et al., 2005). While temperature is considered to be the most important modifier of energy flow and hence growth, salinity imposes the greatest additional load on metabolic requirements of aquatic animals.

While much is already reported for the response of *Crassostrea virginica* to temperature and salinity, distinct differences exist along the distribution range warranting site-specific assessments for previously unstudied populations (Brown and Hartwick, 1988; Dame, 1996; for more details see Shumway, 1996). Most studies focused on the effect of a single environmental variable only, relatively few studied the interrelationship of two or more factors, and of this most studies used larval animals only (Robert et al., 1988; Deksheniaks, 1992; Devakie and Ali, 2000). Surprisingly few data are available for adult oysters; of this most are based on field observations or laboratory studies with a very limited temperature and/or salinity range.

The combination of salinity and temperature has synergistic effects (Davis and Calabrese, 1964; Vernberg and Vernberg, 1972; Austin et al., 1993; Shumway, 1996) on virtually all aspects of the biology of oysters. Hence, a multidimensional response surface design that simultaneously analyzes combined effects of salinity and temperature was considered preferable as an experimental approach for studying physiological tolerances in adult oysters. Response surfaces were used to model measures of (1) sublethal stress conditions focused on general oyster performance (body condition index) and (2) a biochemical indicator of cellular stress (RNA/DNA ratio) as a function of salinity and temperature. Results are discussed in the light of (1) specific adaptations of this so far unstudied oyster population and (2) future research strategies to better understand the recruitment pattern of the small but stable oyster population of the SLE with the aim to optimize the restoration process.

2. Material and methods

2.1. Animals and maintenance condition

Eastern oysters, *Crassostrea virginica*, were laboratory-cultured offspring of parental stock obtained in March 2002 from the St. Lucie Estuary, Florida (Fig. 1). Specimens were reared and maintained in the Aquaculture facilities of the Harbor Branch Oceanographic Institution (HBOI, Ft. Pierce, Florida) under environmental conditions (temperature: 26–29 °C; salinity: 28–30). Oysters were fed with cultured phytoplankton species and highly filtered seawater to prevent or limit infection with the protozoan parasite *Perkinsus marinus*, commonly known as Dermo. Throughout the experiments infection levels were below 1 Dermo spore/g tissue as determined with the whole-body burden method. Prior the experiments oysters were transported in coolers to Florida Atlantic University's Gumbo Limbo Marine Science Center (Boca Raton, Florida), where they were maintained in temperature controlled flow-through aquaria for an initial acclimation period of 1 week before use in the experiments. Experiments started in May 2004 and were finished in December 2004, with animals being 30 months old. The mean shell length and tissue wet mass of oysters used in our experiment were 43.76 mm (± 5.98) and 9.15 g (± 2.66), respectively.

The experimental flow-through system consists of five separate identical units, each of which could be independently maintained at controlled conditions of temperature and salinity. Filtered (25- μ m) ocean saltwater and carbon-filtered freshwater entered a 122-L insulated temperature controlled mixing reservoir from which water of desired temperature and salinity was delivered to three treatment tanks (64 L insulated cone-shaped tanks) (Fig. 2). Temperature and salinity of each system were continuously monitored and recorded, variation of temperature and salinity was less than 0.1 °C and 0.1, respectively. Each tank was aerated to provide oxygen for the oysters and to facilitate water movement and mixing. During experimental studies oysters were fed *ad libitum* with a prepared algal diet (Shellfish Diet 1800, Reed Mariculture), diluted to yield a suspension of 100,000 cells per liter.

2.2. Experimental design

A modified central composite inscribed (CCI) response surface design was used to describe the response of the local *Crassostrea virginica* population to a range of salinity and temperature combinations. A CCI design is a scaled down classic central composite design (CCD) with each factor level of the CCC design divided by $\alpha = 1.414$ to generate the CCI design (Khuri and Cornell, 1996). Both require five levels of each factor. The response surface analysis using full quadratic model in salinity and temperature was used in our study.

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