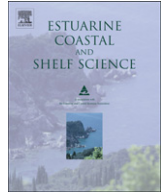




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Stress tolerance of a subtropical *Crassostrea virginica* population to the combined effects of temperature and salinity

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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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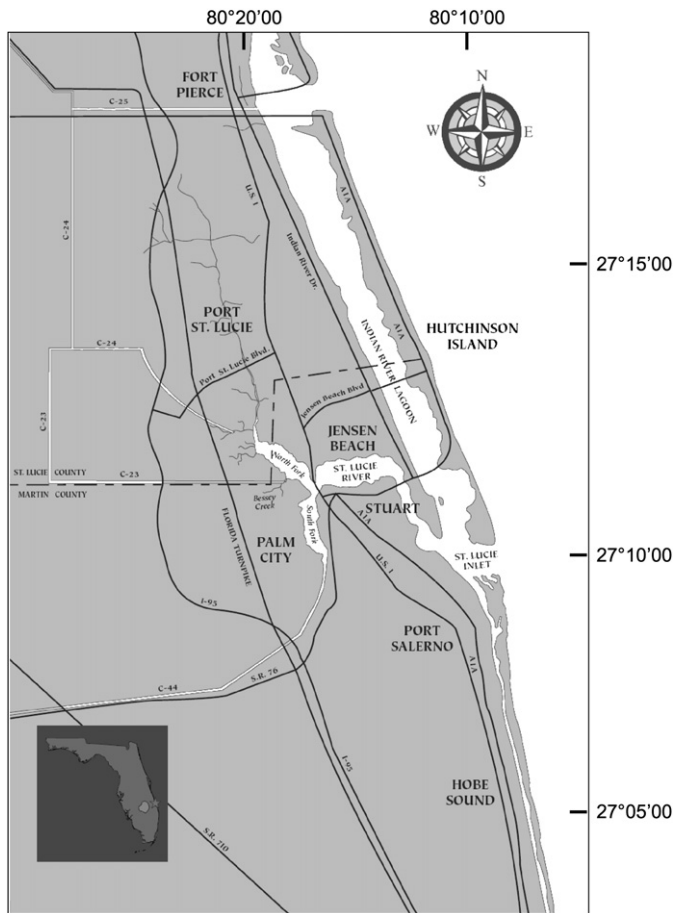


Fig. 1. Map of St. Lucie River System, showing the location of inlet created in the barrier island.

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 (Alderdice, 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 CCD 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.

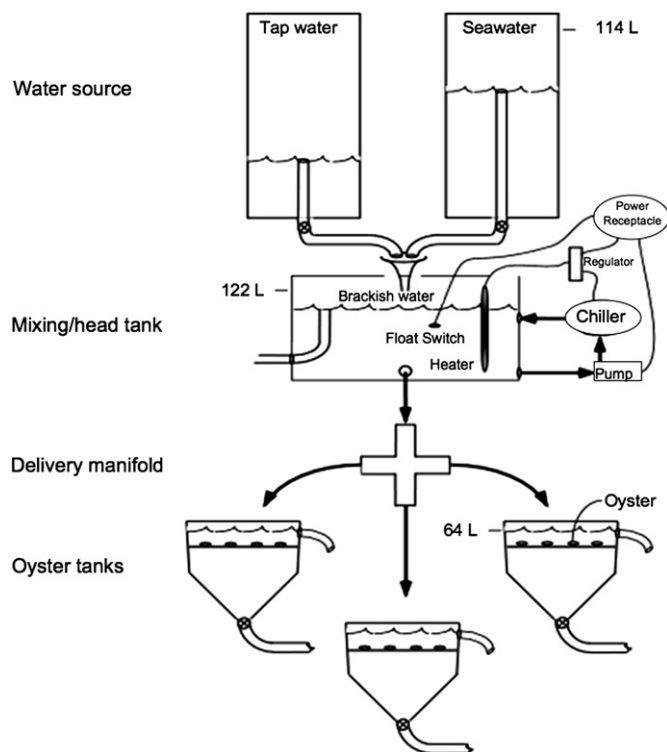


Fig. 2. Schematic representation of the oyster 'Life Support System' at Gumbo Limbo. Three tanks for maintaining oysters are included in a single mesocosm unit (i.e. =one "system"). During this study four systems were used for experimental manipulations, while the fifth system was used to maintain a control group at "Harbor Branch conditions", in order to account for the staggered experimental design.

To simulate the natural variance within the SLE minimum and maximum limits for salinity and temperature were set to 2 and 25 and 15 and 30 °C, respectively. As experimental conditions to satisfy the CCI design, nine combinations of salinity and temperature over this range were identified, five of which were at center point conditions (salinity: 13.5; temperature: 22.5 °C) resulting in 13 experimental combinations to be tested (Table 1).

The flow-through system at the Gumbo Limbo Marine Science Center allowed running of three replicates for each temperature–salinity combination. Thus, the 13 conditions were divided into a total of five experimental blocks conducted directly after each other. For each experiment the same number of oysters was randomly placed in each tank and then subjected to stepwise acclimation to experimental conditions at not more than 2 °C/day and a salinity decrease of 5/day within the first week of transport. Following a stepwise temperature–salinity acclimation oysters were kept at experimental conditions for 2 weeks before being sacrificed and prepared for further analysis.

Each experimental block included up to four systems running at experimental conditions and an additional fifth system set at the actual Harbor Branch culture conditions at the time of transfer to the Gumbo Limbo Marine Science Center. This last group was used as a reference to control for changes from Harbor Branch culture conditions, as well as any seasonal differences that may have resulted from running experiments at different times of the year.

2.3. Body condition index

A common measurement of oyster condition and growth and hence an indicator of general oyster health is the body condition index (BCI). BCI is determined as the ratio of the shell-free soft tissue dry mass (SFDM; 68 °C for 48 h) of the organisms to the shell

Table 1

Experimental conditions (salinity and temperature) identified for use in response surface analysis using the central composite inscribed design and mean values (body condition index, RNA/DNA ratio) per tank. Italicized values = center point measurements

Exp	Sal	Temp [°C]	BCI replicates			RNA/DNA replicates		
			1	2	3	1	2	3
4	2.00	15.00	4.914	5.028	5.163	–	–	–
5	2.00	30.00	4.255	3.501	3.664	0.305	0.217	0.559
7	5.37	22.50	4.111	4.317	3.900	0.286	0.823	0.485
7	13.50	17.20	5.117	5.172	5.176	0.638	1.016	0.686
5 ^a	13.50	22.50	3.615	6.081	4.810	0.594	0.806	0.641
5 ^a	13.50	22.50	4.593	4.268	4.505	0.256	0.536	0.411
6 ^a	13.50	22.50	4.719	4.859	5.255	0.400	0.224	0.567
7 ^a	13.50	22.50	5.570	4.898	5.149	0.372	0.463	0.524
7 ^a	13.50	22.50	5.236	4.592	4.640	0.629	0.691	0.440
6	13.50	27.80	4.654	4.587	4.674	0.695	0.375	0.944
6	21.63	22.50	4.948	4.764	4.933	0.444	0.624	0.745
4	25.00	15.00	6.929	6.412	6.519	–	–	–
5	25.00	30.00	5.109	4.833	5.151	0.781	0.535	0.374
8	2.00	30.00	4.132	3.696	3.446	0.264	0.343	0.286
8	2.00	15.00	6.763	6.391	5.012	0.549	0.532	0.397
8	25.00	15.00	6.910	6.776	5.998	0.355	0.721	0.514
8	25.00	30.00	6.523	6.833	6.422	0.512	0.358	0.362

^a Center point conditions and results.

cavity volume (CV) according to the following formula (Lawrence and Scott, 1982):

$$\text{BCI} = \frac{\text{SFDM}}{\text{CV}} 100 \quad (1)$$

CV is estimated as the difference between whole oyster mass after 60 min air drying (WM_{Total}) and the dry mass of the shell alone (DM_{Shell}) after oven drying (48 h at 68 °C)

$$\text{CV} = WM_{\text{Total}} - DM_{\text{Shell}} \quad (2)$$

2.4. RNA/DNA ratio

The RNA/DNA ratio is a commonly used growth 'biomarker' of stress in marine invertebrates (Vrede et al., 2002; Dahlhoff, 2004; Norkko et al., 2005). A decline in the RNA/DNA ratio is indicative of stress at the cellular level and provides a complementary measure to the whole animal response to stress derived from measure of the BCI. RNA/DNA ratios of gill tissues' measurements were based on a modification of the method of Kaplan et al. (2001). Following lysis, aliquots were processed separately for analysis of either total DNA or total RNA. Detection was based on the use of the fluorescent dyes PicoGreen for detection of DNA and RiboGreen for detection of RNA. Details of the procedure are described below. DNA and RNA samples in black 96-well microtiter plate were analyzed on a Fluoroskan II fluorescence plate reader (MTX Lab Systems, Inc., #P97052). Measurements were made at 480 nm excitation and 520 nm emission. The RNA/DNA ratio was determined from μg RNA/ μg DNA.

2.4.1. Sample preparation

Excised gills were frozen immediately in liquid nitrogen, then transferred to a –80 °C freezer for storage. Approximately 10 mg of gill tissue were digested for 12 h at 55 °C in 100 μl lysis buffer (0.1 M NaCl, 10 mM Tris pH 8.0, 0.2% SDS, 10 μg Proteinase K). Final tissue-to-buffer ratio was 1:10. Following digestion, samples were centrifuged at 4000 \times g for 10 min. The supernatant was divided into two aliquots: one to determine the RNA concentration and the

other to determine the DNA concentration. Lysates were stored at -20°C .

2.4.2. DNA measurement

Ten microliters of the supernatant were placed in a 96-well microtiter plate containing $40\ \mu\text{l}$ TE and $100\ \mu\text{l}$ aqueous working solution of PicoGreen (Quant-iT PicoGreen, Molecular Probes, #P7581). The total DNA concentration was determined fluorometrically using a DNA standard curve (ultra-pure calf-thymus DNA; Invitrogen, #15633-019).

2.4.3. RNA measurement

Ten microliters of the supernatant were placed in a 96-well microtiter plate containing $50\ \mu\text{l}$ RNase-free DNase (Promega: $2\ \mu\text{l}$ enzyme RNase-free DNase and $48\ \mu\text{l}$ $1\times$ RNase-free DNase buffer from $10\times$ stock). After an incubation of 30 min at 37°C to allow digestion of DNA, $40\ \mu\text{l}$ TE and $100\ \mu\text{l}$ aqueous working solution of RiboGreen (Quant-iT RiboGreen, Molecular Probes, #R11490) were added. Total RNA was determined fluorometrically using a RNA standard curve (ribosomal RNA standard from 16S and 23S rRNA *Escherichia coli*; stock concentration $100\ \mu\text{g/ml}$; Quant-iT RiboGreen RNA Assay Kit, Molecular Probes).

2.5. Statistical analysis

The flow-through system at the Gumbo Limbo Marine Laboratory allowed running of three replicates for each temperature–salinity combination. Each replicate was the average of 5–10 oysters. All data were checked for outliers beyond the 95% confidence limits of an t -distribution, $r_A > r(95)$, using Nalimov's test (Noack, 1980). In order to account for the staggered experimental design and related temporal effects a “fixed-block” model was used to analyze for block effect. On reanalysis, we found that the block effect was not significant and could be excluded from the final response surface model.

Using intermediate iterations, we tested the model for non-significant parameters, and omitted these from the final model. To investigate the effect of salinity and temperature on BCI and RNA/DNA ratio, a two-factor analysis of variance (ANOVA) was initially used for preliminary analysis. Data were analyzed with the JMP 5.0.1 software package and a response surface model was finally constructed. As RNA/DNA ratios were included into the experimental design to replace shell growth measurements starting experiment 5, we added a final experimental block (experiment 8; Table 1) to cover the necessary conditions. Overall 51 mean tank data for BCI and 45 for RNA/DNA ratios were analyzed.

3. Results

3.1. Body condition index

After exclusion of four outliers the final regression model describing mean BCI containing 47 observations was

$$\text{BCI} = 11.862 - 0.592(\text{Temp}, ^{\circ}\text{C}) + 0.00272[(\text{Sal}) \times (\text{Temp}, ^{\circ}\text{C})] + 0.0106[(\text{Temp}, ^{\circ}\text{C}) \times (\text{Temp}, ^{\circ}\text{C})]$$

BCI of *Crassostrea virginica* was significantly affected by temperature ($F = 29.39$; $P < 0.001$) and the squared temperature term ($F = 19.27$; $P < 0.001$). An interaction between salinity and temperature was also detected ($F = 72.35$; $P < 0.001$). The model captures 77.3% of the variability in mean BCI with an adjusted $R^2 = 75.7\%$ and a standard deviation of 0.433; around 60% of the observed variability can be explained by the interaction of temperature and salinity. The response surface plot derived from the final model is shown in Fig. 3, which shows that estimated BCI (the

fitted values) increased as salinity increased and temperature decreased. Highest mean BCI occurred at salinities between 15 and 25 and temperatures below 17.5°C (Fig. 3). Very low rates of BCI (< 4.5) were observed at temperatures above 25°C and salinities below 5.

3.2. RNA/DNA ratio

After exclusion of outliers the final model for mean RNA/DNA ratios contained 39 observations. The regression was:

$$\text{RNA/DNA ratio} = 0.5096 - 0.0087(\text{Temp}, ^{\circ}\text{C}) + 0.0291(\text{Sal}) - 0.0008[(\text{Sal}) \times (\text{Sal})]$$

The RNA/DNA ratio was significantly affected by salinity ($F = 8.95$; $P = 0.005$), temperature ($F = 4.48$; $P = 0.041$) and the squared salinity term ($F = 5.53$; $P = 0.024$). No interaction was detected between temperature and salinity. The model explained 35.8% of the variability in mean RNA/DNA ratio with adjusted $R^2 = 30.3\%$; an analysis of variance shows that half of the observed variance can be explained with the salinity square term. The response surface model for RNA/DNA ratios (Fig. 4) exhibited general correspondence with that for BCI, with highest stress at high temperature and low salinity conditions. The highest RNA/DNA ratios, considered beneficial, were seen at salinities above 10 and temperatures below 22.5°C , due to lower salinity- and temperature-related stress. The highest decrease in RNA/DNA ratios was at temperatures above 25°C .

4. Discussion

4.1. Temperature–salinity-tolerance of oysters from the SLE

Crassostrea virginica dwells in an estuarine habitat that exposes them to a wide range of environmental stresses including high and low temperatures, very little or no oxygen for extended periods of time, and rapid salinity changes of 10 or more. Of all biotic factors that can affect the biology of estuarine organisms, the synergistic effects of temperature and salinity are likely the most predominating ones (Shumway, 1996). As pointed out in a number of studies (e.g. Brown and Hartwick, 1988; Ponce-Palafox et al., 1997;

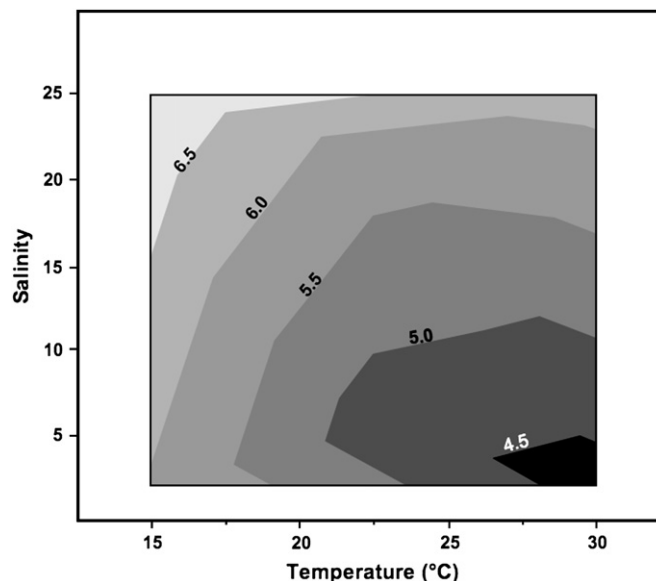


Fig. 3. *Crassostrea virginica*. Response surface plots with isobars estimating the body condition index (interval size is 0.5 units) at a range of temperature and salinity levels. The model is best described by the equation given in Section 3.1.

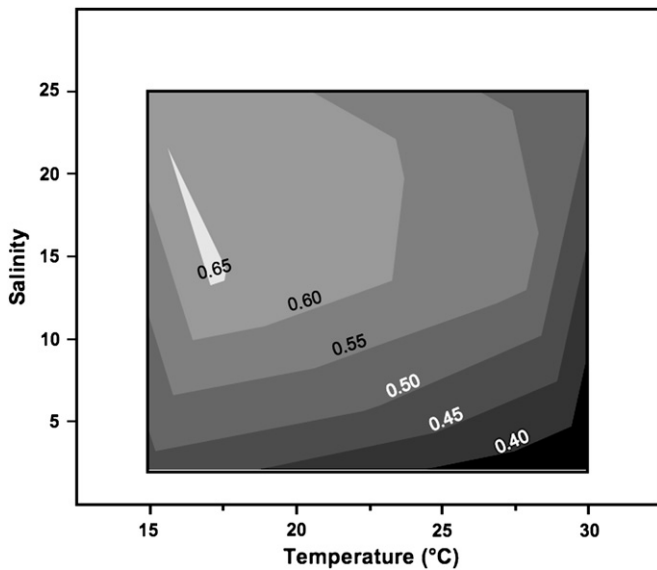


Fig. 4. Response surface plot showing RNA/DNA ratios of *Crassostrea virginica* maintained at combinations of temperature and salinity for 2 weeks. Isobars show the RNA/DNA ratio determined from $\mu\text{g RNA}/\mu\text{g DNA}$ (interval size is 0.05 units). The model is best described by the equation given in Section 3.2.

Bataller et al., 1999), two or more environmental variables working in concert have more profound biological consequences than any one of those factors acting independently. Accordingly, in our study, the effect of salinity could not be isolated, but, instead, were considered together with temperature. Adult oysters can tolerate salinities from 0 to 42, but the optimum range is 14–28 (Shumway, 1996). A minimum salinity of 10 is required for growth, with little growth occurring at salinities less than 5 (Shumway, 1996). Butler (1952) reported self-sustaining populations in Florida in areas, where salinities were as low as 0.2 for five consecutive months annually. Loosanoff (1953) and Wells (1961) reported minimum values for normal survival at salinity of 7 and 7.5, respectively, for the species. However, our results clearly showed that oyster fitness (measured as BCI) in individuals from the St. Lucie Estuary can remain relatively high at salinities below 7.5, provided the corresponding temperature is sufficiently low, i.e. below about 20 °C. These would represent temperatures corresponding to cooler seasons in this habitat. Our results are in agreement with those of Andrews et al. (1959) and Kennedy (1991), who determined adult oysters could survive periods of low salinities by inducing a state of metabolic depression through prolonged valve closure, providing corresponding low ambient temperatures. In contrast, throughout the summer/wet season when temperatures reach levels approaching 30 °C and higher, the detrimental effects of low salinity are exacerbated. While higher temperatures can increase the assimilation efficiency of the organism, subsequently leading to an increase in somatic growth (Nair and Appukuttan, 2003), the present study suggests reduced somatic growth when coupled with low salinity levels. In the present study, lowest BCI values occurred at combinations of salinities less than 5 with temperature above 25 °C. Throughout the experiment, oysters subjected to salinity treatments below 5 ceased feeding, produced no feces or pseudofeces and maintained their valves in a closed position. During the close-valve stage an increase of anaerobic metabolism ensures the energy supply for the organism (Michaelidis et al., 2005). The drawbacks of this behaviour are accumulation of anaerobic end products (i.e. succinate) and a nearly complete cessation of gas exchange between the oyster and the external medium. This causes the buildup of carbon dioxide in tissues, i.e. respiratory acidosis,

which is discussed to be one of the main reasons for summer mortalities in oyster populations (Michaelidis et al., 2005; Lannig et al., 2008). These findings are in agreement with previous work (Loosanoff, 1953; Shumway, 1996) that long-term valve closure will result in mortality particularly when coupled with high temperatures. Even with valves completely closed oyster gill cilia continue to beat, with the pumping rate being a direct function of the valve aperture (Jorgensen, 1990), potentially inducing anaerobiosis (Lannig et al., 2008). Higher temperatures increase metabolic activities such as pumping rate, heart rate and respiration, while low salinities (e.g. <5) likely interfere with the osmotic balance of somatic tissues (Anderson and Anderson, 1975; Shumway, 1996), disrupting whole system functioning. Oysters are generally thought to be osmotic conformers, which will put a burden on cells with regard to maintain their cell volume, i.e. cell function and constituents (Shumway, 1996). However, osmotic concentration was not measured during the cause of this experiment.

The response surface analysis of oyster RNA/DNA ratio did not yield a statistically significant model, explaining only 30% of the observed variance. However, the RNA/DNA ratio contour plot from the model described a similar physiological response to the effects of temperature and salinity to that of the plot generated for BCI (Fig. 4). It follows that the overall somatic condition of the oyster mirrors the metabolic status of the organism. The lack of significance for the RNA/DNA ratio model is attributable to the high variability associated with the treatment means. Careful examination of the controls from each fluorometer measurement did not link this variability to procedural errors. Although RNA/DNA ratios are a highly sensitive measure of the metabolic status of an organism (Wright and Hetzel, 1985; Chicharo et al., 2001; Dahlhoff, 2004), Okumura et al. (2002) recommended that other indices of metabolic health be taken in conjunction to RNA/DNA ratios due to its large variability. Furthermore, RNA/DNA ratios reflect short term responses in gene expression that exaggerate intra-individual differences of the oysters (Dahlhoff, 2004), unlike that seen with the BCI, which reflects changes in biomass resulting from the integration of bioenergetic fluxes over time.

4.2. Relevance to the SLE

The hydrology of the SLE is characterized by mean water temperatures ranging from 17.1 to 38.8 °C and bottom salinity values from 0 to 39. While temperature changes seasonally, salinity depressions occur suddenly due to the canal infrastructure and watershed development (Chamberlain and Hayward, 1996; Millie et al., 2004; Sime, 2005). This study aimed to determine site-specific responses of a *Crassostrea virginica* population that is challenged by anthropogenic freshwater discharges from the Lake Okeechobee in addition to natural freshwater inflows. The existence of physiological races that are geographically separated has been demonstrated quite clearly, and differences can exist between populations located in close geographic proximity (Newkirk et al., 1977; Shumway, 1996). Gaffney (1996) postulated that differences between Gulf and Atlantic oyster populations are more likely due to local environmental conditions rather than genetic differences. Hence, we hypothesize that the specific conditions of the SLE sustain an oyster population by removing weaker individuals and selecting those that are more tolerant to such extreme conditions.

While oysters are capable of surviving in a wide range of habitat conditions, the preferred habitat (general range) conditions of eastern oysters are considered to be (Shumway, 1996 and references therein): salinity – larvae (10–27.5; Davis and Calabrese, 1964), adults (normally ~5–40; Loosanoff, 1953); temperature – larvae (optimum ~20–32 °C) (Davis and Calabrese, 1964); adults optimum temperatures range from 20 to 30 °C (Brown and

Hartwick, 1988; Fisher et al., 1996). When compared with results of previous studies, we did not detect any phenotypic differences characteristic of the SLE oyster, i.e. no indication for formation of a physiological race. This is also seen with other marine animals with a large geographic range (e.g. Bullock, 1955; Levinton and Monahan, 1983; Conover and Schultz, 1995; Pörtner et al., 2005). Additionally, the temperature and salinity ranges of the present experiment replicated that of the subtropical St. Lucie Estuary and did not include extreme combinations of conditions found throughout the range of *Crassostrea virginica* (Chamberlain and Hayward, 1996).

However, our study is the first to determine the baseline for future research attempts in the SLE. We could show that controlled laboratory experiments are an effective way to determine the combined effects of environmental conditions on the biology of adult oysters. Future research should include conditions such as ambient hypoxia and hypercapnia caused by low water oxygen (Millie et al., 2004). Additionally diseases affect oyster fitness. As shown by an environmental survey many oysters are infected with the protozoan parasite *Perkinsus marinus* (Craig et al., 1989). Infected oysters might be more vulnerable to extreme temperature and salinity situation than healthy ones due to the demands of combating infection since survival rate depends on previous oyster conditions (Davis and Calabrese, 1964; La Peyre et al., 2003; Soniat et al., 2006).

Unlike larvae or juveniles, adult oysters can tolerate a wide range of environmental extremes for prolonged periods (Loosanoff, 1953; Robert et al., 1988; Shumway, 1996). In the broader context of recruitment in dynamic environments, tolerance of larvae and juveniles may be more significant than that of adults and should be considered in future studies.

4.3. Conclusions and perspectives

Oysters have a greater ability to survive extreme salinity conditions at lower temperatures and their capability to withstand temperature extremes is greater at near optimum salinities (Davis and Calabrese, 1964). This is consistent with the temperature–salinity response surface for BCI and RNA/DNA ratios in 2-year old oysters described in the present study. Stress due to salinity is intricately tied to interactions with temperature and must be evaluated as a function of interactive effects. The findings from this study can serve as a building block towards the development of a robust predictive tool for modeling the health of oyster populations in the St. Lucie Estuary, especially including further parameters such as prevalence (*Perkinsus marinus*), hypoxia and different ontogenic stages.

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