Cumulative Risks to Eastern Oysters, Crassostrea virginica in the James River, VA

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CUMULATIVE RISKS TO EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* IN THE JAMES RIVER, VIRGINIA

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL STUDIES AT VIRGINIA COMMONWEALTH UNIVERSITY

BY

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1.0 Abstract

In an effort to apply Cumulative Risk Assessment (CRA) as developed by the U.S. EPA, the present study investigates the cumulative risks to Eastern oysters due to multiple stressors such as salinity, temperature and oxygen and carbon dioxide. I also compared the effectiveness of the Hazard Quotient Method (HQ) in CRA. Ambient conditions in the James River, VA were obtained from the Virginia DEQ database and respiratory responses were estimated using values from the literature. The multiple environmental stresses are evaluated using a probabilistic analysis that combines the environmental conditions. It was concluded that salinity was the most influential stressor in the model. Other risks were identified contributing to the vulnerability of the oysters. Crystal Ball simulations yielded that the oxygen uptake of oysters reduced by more than 29%. The HQ method was found to be inappropriate in analyzing cumulative risks for CRA. Oyster populations are dramatically declining in the James River and the Chesapeake Bay. Hence, effective oyster restoration activities are underway to rebuild oyster populations in the James River and throughout the Bay area.
2.0 INTRODUCTION

The U.S. Environmental Protection Agency (EPA) uses risk assessment as a key method to analyze scientific information for sound decision making to manage and protect human health and the environment. The latest innovative methodology published by EPA is the Cumulative Risk Assessment (CRA) Framework that involves assessment of combined risks from multiple stressors. CRA utilizes a holistic approach with multiple non-chemical or chemical stressors in conjunction and incorporating cumulative risks (U.S. EPA, 2003). CRA has the potential to address complex environmental conditions, including aquatic ecosystems in the Chesapeake Bay area. Traditional risk assessments use the hazard quotient (HQ) method in the characterization of risks. HQ method is a simple and an inexpensive method. However, application of this method in ecological and cumulative risk assessments has limitations (U.S. EPA, 2003). Two important limitations are that the HQ method does not present the probability and the magnitude of the risk because it is essentially the ratio of exposures.

Eastern Oysters (*Crassostrea virginica*) are subjected to variable environmental conditions of salinity, temperature and oxygen. Amongst all the abiotic factors, combined effects of salinity and temperature have the most profound effects on the physiology of the oysters. Other stressors that affect oyster metabolism are hypoxia and hypercapnia (Shumway and Koehn, 1982; Willson and Burnett, 2000). Eastern Oysters play an important role in the economy and ecology of the Chesapeake Bay. The James River remains the only river of note in Virginia that has supported
and continues to support a commercial public oyster fishery. Nevertheless, the population is declining due to several risks (Mann et al., 2009). Other risks that pose stress on oyster survival are diseases like MSX and Dermo. High salinity and temperature waters increase the persistence of the pathogens and in turn increase the susceptibility of oysters to infectious diseases. Dermo was first detected in the Chesapeake Bay in 1949 and MSX first appeared in 1959 and are still persistent (Carnegie and Burreson, 2009).

In the present investigation, elements from the Cumulative Risk Assessment Framework (2003) were used to assess the combined risks due to the multiple stressors salinity, temperature and hypoxia by performing a probabilistic risk analysis. In addition, other risks associated with these multiple stressors are also addressed. Applicability of the hazard quotient method in CRA was also evaluated, i.e. if the hazard quotient method is suitable in assessing cumulative risks in CRA.
3.0 LITERATURE REVIEW/BACKGROUND

3.1 NON-CUMULATIVE ECOLOGICAL RISK ASSESSMENT

The U.S. Environmental Protection Agency (EPA) developed Ecological Risk Assessment (ERA) in 1992 with an objective “to evaluate the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors”. ERA originated from Human Health Risk Assessment (HHRA), hence the approach was similar to traditional risk assessments with ecological endpoints. ERA includes initial planning, problem formulation, analysis of stressors and effects, and risk characterization. However, the current practices have certain limitations in applying ERA. Ecological systems are stochastic and it is important to include and characterize the uncertainties associated with this nature in the assessment. The second limitation is not connecting the relevant spatial and temporal scales to the assessment. Selecting the relevant spatial and temporal scale in order to understand the population dynamics is essential. Third, interactions of multiple and complex stressors in the system are seldom incorporated in ERA. The fourth limitation is that contemporary ERAs focus primarily on chemical effects and exposures. Further, assessments should consider chemical and nonchemical stressors. The most important critique for ERA discussed by Glen Suter is the relative lack of influence in EPA’s decision making. Although ecological receptors are commonly more sensitive and more exposed than humans, human health risks dominate rule
making, remedial actions, and other regulatory decisions (Dale et al., 2000; Kapustka, 2008; deFur, 1997; Suter, 2008).

### 3.2 Cumulative Risk Assessment: A Review

Although the risk assessment approach has been developing over the years at the U.S. EPA, evaluating cumulative risks from multiple stressors has been a rather slow process. After the first breakthrough report published by National Research Council (NRC) in 1983, EPA followed the recommendations and published a series of risk assessment guidelines. Subsequently, in 2003, EPA adopted a holistic approach in developing the “Framework for Cumulative Risk Assessment” (Callahan and Sexton, 2007).

The U.S EPA Framework defines CRA as “an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors”. CRA has been developed using traditional risk assessment methods. Several distinctive and essential key features are derived in CRA. First, combined effects of more than one agent or stressor are studied in concert, i.e. rather than studying effects of a single stressor or a chemical, multiple stressors or chemicals in conjunction are involved. Second, stressors are not limited to chemicals. They could be biological, physical, social, economical, psychological, behavioral stresses, or natural or anthropologic activities that could disturb the equilibrium of any system. The third feature calls for combining the risks from the multiple stressors. It does not necessarily mean “adding” the risks but identifying interactions between the stressors and the related hazards. Fourth, assessments could be quantitative or qualitative in nature.
characterization of risks is not possible, identifying the essential sources, stressors, or other qualitative approaches can provide deeper insight in the situation. Fifth, since CRA involves multiple stressors, they are population-focused assessments (U.S. EPA, 2003; Fox et al., 2002).

CRA is an analytic-deliberative process that involves three main phases: (1) planning, scoping, and problem formulation; (2) analysis; and (3) risk characterization. The first phase includes creating a backbone and defining the risk assessment. Identifying and evaluating stressors, sources, pathways, exposures, routes, population to be evaluated, scope and limitations of assessment, data gaps, analysis methods, uncertainties of the study, stake holders involved and other essential information required for the study. This phase calls for systematic planning and execution. The first phase produces a conceptual model and an analysis plan. The conceptual model describes relationships between all possible sources, stressors, endpoints and interactions between them, while the analysis plan includes the course of action or strategy behind the analysis described in the conceptual model. Information about measurement and assessment endpoints, uncertainties and data gaps, scope of the assessment and other key components of the assessment are discussed explicitly.

For the analysis phase, EPA suggests integrating exposure, hazard, and dose-response information. This process can create complexity in a CRA due to three main factors: 1) time-related aspects, 2) vulnerability, and 3) subpopulations with special or particularly distinctive exposures. By the end of the analysis phase, it is important to come up with estimates of the
combined risks of exposure to multiple stressors for the population(s) of interest, and the associated uncertainty and variability.

Different approaches for predicting risk of multiple stressors could be utilized. These include 1) single stressor information could provide information about the joint impact of multiple stressors; 2) combining effects of stressors that have a common mode of action, not necessarily adding toxicologically similar stressors, but probabilistic approaches predicting number of cases affecting a population due to the multiple stressors could also be applied; 3) utilizing some type of decision index like the hazard index or any other type of a common metric could be useful; or 4) engaging in any qualitative approaches that provide insight about the risks, location of the hazards, exposure pathways or any information pertaining to the study. In addition, quantitative results might also be changed to a qualitative scale (e.g. high, low and medium), to provide better understanding and depth in the study. Any other available scientific methods or approaches could be used to characterize risks, leading to the last phase of CRA. As mentioned in the framework, “Risk Characterization integrates results from the analysis phase and addresses the problem(s) formulated in the planning and scoping phase. It should describe the qualitative and/or quantitative risk assessment results; list the important assumptions, limitations, and uncertainties associated with those results; and discuss the ultimate use of the analytic-deliberative outcomes”. Figure 1 briefly illustrates the details in the three phases of CRA (U.S. EPA, 2003; Callahan and Sexton, 2007; deFur et al., 2007; Fox et al., 2002).
One of the key features and a requirement that makes CRA different is evaluating multiple stressors and their risks. In most ecosystems, single stressors seldom occur; multiple stressors come into action at some level. The stressors could be chemical, physical, or biological and may occur at different temporal and geographical scales. Thus, understanding the synergy between the stressors and their risks is imperative (Suter et al., 1999).
Hence, CRA is an important tool of EPA because the methodology has been refined to reflect the real world. EPA is slowly transitioning from single (primarily chemical) stressors, end points, sources, pathways and routes of exposure to a holistic and an integrated approach. Table 1 points out the differences between the traditional and emerging approaches at the U.S. EPA.

**Table 1: Comparison of Risk Assessment and Management Characteristics for the Traditional Versus Emerging Approaches at the U.S. EPA**

<table>
<thead>
<tr>
<th>Traditional risk assessment and management characteristics</th>
<th>Emerging risk assessment and management characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single end point</td>
<td>Multiple end points</td>
</tr>
<tr>
<td>Single source</td>
<td>Multiple sources</td>
</tr>
<tr>
<td>Single pathway</td>
<td>Multiple pathways</td>
</tr>
<tr>
<td>Single route of exposure</td>
<td>Multiple routes of exposure</td>
</tr>
<tr>
<td>Single-media focus</td>
<td>Multimedia focus</td>
</tr>
<tr>
<td>Single-stressor risk reduction</td>
<td>Multistressor risk reduction</td>
</tr>
<tr>
<td>Centralized decision making</td>
<td>Community-based decision making</td>
</tr>
<tr>
<td>Command-and-control strategies</td>
<td>Flexibility in achieving goals</td>
</tr>
<tr>
<td>One-size-fits-all responses</td>
<td>Case-specific responses</td>
</tr>
</tbody>
</table>

(Source: Callahan and Sexton, 2007)

**3.3 Hazard Quotient Method in Cumulative Risk Assessment**

One of the approaches used by EPA in predicting risk is the Hazard Quotient Method (HQ) or Hazard Index (HI). Typically, HQ is the ratio of site exposure (e.g. predicted exposure concentration or estimate of exposure) divided by the predicted concentration (e.g. reference dose, toxicity reference value or any standard designed to be protective). If the HQ >1, then the stressor is of potential concern. HI is calculated for a group of chemicals often with mode of action the same, simply by adding/combining the respective HQs (U.S. EPA, 2003).
Although, HQ/HI is widely used, due to the fact that the method is easy, inexpensive and risk assessors are familiar with the approach, it has certain limitations in its application in CRA. First, it is the measure of hazard and not risk. A HQ of 0.1 (for human or non-human endpoints) does not mean that there is one-in-ten chance that any adverse effects will occur nor does an HQ of 0.1 mean that the harm will be 10%. In addition, when HQs/HIs are below 1, toxicological effects are unlikely to occur and the chance for an unacceptable risk is present when HQs/HIs are above 1. Second, HQs do not measure the magnitude of the effect. For e.g. HQ of chemical A is 5 and HQ of chemical B is 10, this result does not mean that the level of concern for chemical B is two times more than chemical A. 5 and 10 are merely numbers that each is greater than the ratio of 1.0. Third, HQ does not account for the temporal scale. HQ for a 5 year old contaminated site or 50 years old contaminated site will be the same. The fourth main disadvantage is it does not represent any uncertainties related to the study (US EPA, 2003; Volosin and Cardwell, 2002; Tannenbaum et al., 2003; Tannenbaum, 2005). Thus, it is important to tap into other approaches and methods that are not generalized but are rather case-specific to each assessment.

3.4 HYPOXIA AND HYPERCAPNIA AS MULTIPLE STRESSORS

Estuarine and coastal marine ecosystems around the world show a drastic change in the dissolved oxygen conditions. Hypoxia is a low dissolved oxygen condition. Environmental hypoxia can be designated as moderate (75 to 50 % air saturation) or severe (20 to 30 % air saturation) hypoxia. Scientists have observed physiological changes in organisms below 50% saturation or when partial pressure of oxygen (PO$_2$) < 80 mmHg (at sea level) (Diaz and Rosenberg, 1995; Hypes
Hypoxic zones can sometimes be created due to natural processes; nevertheless, anthropogenic activities have increased the frequency and severity of hypoxic events. In 2008, Diaz and Rosenberg have reported 400 aquatic systems world-wide, affecting a total area of more than 245,000 square kilometers with dead zones; amongst them were the Chesapeake Bay, York (Kuo and Neilson, 1987) and Rappahannock (Kuo and Neilson, 1987; Kuo et al., 1991) Rivers (Diaz and Rosenberg, 2008). Instances of hypoxia in the James River have also been reported by Kuo and Neilson, 1987. Hypoxia is a concern because it not only affects the ability for the organisms to thrive, but also alters the population structure within a species, and decreases the population density of organisms (Burnett and Stickle, 2001).

Other concerns with environmental hypoxia are co-occurrence of hypercapnia (increase in water carbon dioxide) and acidic conditions in water (decrease in water pH) (Burnett, 1997). During the day, algal photosynthesis produces oxygen and removes carbon dioxide, whereas, at night due to absence of photosynthesis, the reverse process occurs. A similar increase in CO$_2$ production results from bacterial activity. This production of carbon dioxide results in hypoxic and hypercapnic water. As the partial pressure of carbon dioxide (PCO$_2$) in water rises, the PCO$_2$ in tissues of aquatic animals also rise. The equation representing this dynamics is:

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+
\]

The decrease in water pH co-occurs with hypoxia and hypercapnia. Christmas and Jordan (1987) demonstrated a positive correlation between the amount of oxygen in water and pH over oyster
bars in the Choptank River oyster bars in Maryland. Thus, “a functional link exists between the consumption of oxygen, reducing oxygen levels, carbon dioxide production, and lowering pH” (Burnett, 1997). In addition, since, organisms facing hypoxic conditions also face hypercapnia and acidosis, hypoxia cannot be considered as a single stressor, but can be viewed as multiple stressors (Hypes and deFur, 1999).

Scientists have documented many morphological, physiological, behavioral and molecular adaptations that organisms exhibit to deal with hypoxia. Few studies describe effects of hypercapnic environments. However, the effects of hypoxia combined with hypercapnia and the responses to organisms have not been studied well. This combination is essential as they co-occur, and the two gases have profound and independent effects on the physiology of estuarine organisms (Burnett, 1997; Willson and Burnett, 2000).

3.5 EASTERN OYSTER RESPONSES TO MULTIPLE STRESSES

The Eastern oyster, *Crassostrea virginica* (Gmelin), is a mollusk that is subjected to extreme environmental conditions of temperature, salinity, pH and oxygen. *C. virginica* plays an important role in the economy and ecology of the Chesapeake Bay. Over the last three decades, MD and VA economies have cumulatively lost more than $4 billion due to the decline of oysters (Chesapeake Bay Foundation, 2010). Oysters are suspension-feeders that enhance water quality, increase light penetration and trap contaminants entering coastal waters. They also create biogenic reef structures that provide refuge to other organisms, increasing the ecosystem

Oysters have been found at low temperature levels of about 1 °C in the northern states of the U.S. to about 36 °C in Texas, Florida and Louisiana (Galtsoff, 1964). At 6 to 7 °C, feeding is ceased and above 32 °C ciliary movement rapidly declines (Galtsoff, 1964; Stanley and Sellers, 1986). Optimum water temperature for growth, reproduction, and survival of Eastern Oysters ranges from about 20 to 30 °C (Stanley and Sellers, 1986).

In case of salinity, two favorable ranges- 18 to 30 parts per thousand (‰) and 5 to 18 ‰ have been suggested by Galtsoff (1964). However, oysters have also been reported at 2 ‰ and 36 ‰. Populations of oysters found beyond this range exhibit inhibited growth, reproduction, discontinued feeding or they either die (Galtsoff, 1964; Stanley and Sellers, 1986). The first symptoms of oysters subjected to lower salinities are partial or complete contraction of the adductor muscle and closing the shells. Nevertheless, one of the major concerns is that unsuitable salinity and temperature ranges make the organism more susceptible to infections of pathogens like *Haplosporidium nelsoni* (MSX) and *Parkinsus marinus* (Dermo) (Galtsoff, 1964). MSX and Dermo have the most oyster mortalities on the James River, VA oyster beds.

Amongst all the abiotic factors, synergistic effects of temperature and salinity probably have the most significant effects on *C. virginica*. Numerous researchers have pointed out that different biological responses are observed in organisms when subjected to two or more environmental factors acting in concert than the same factors acting independently. Temperature or salinity
impacts every function of the oyster’s life cycle. Many researchers have discussed the role of temperature and salinity on the physiological and behavioral mechanisms; however, few data are available on effects of temperature and salinity in concert on oxygen consumption ($\dot{VO}_2$) in whole oysters, though there is considerable data for individual tissues (Shumway and Koehn, 1982; Shumway, 1982).

Oysters cannot avoid hypoxia. In order to withstand hypoxia they engage in anaerobic metabolic pathways for energy production (Willson and Burnett, 2000). Declining oxygen levels affect the embryonic development and growth of larvae which can slowly affect the recruitment of adult oysters (Baker and Mann, 1994). Larvae are able to sustain short-term hypoxia (hours), but it reduces the feeding activity, in turn reducing the growth rate (Widdows et al., 1989). Baker and Mann (1992) have stated that hypoxic and anoxic events (84 hours) have adverse effects on the larval settlement, juvenile growth and survival, which would influence oyster distribution.

Shumway and Koehn (1982) have studied the oxygen consumption ($\dot{VO}_2$) of oysters exposed to combined effects of temperature, salinity and oxygen tensions. They indicated that oysters have remarkable regulating capabilities under declining oxygen tensions. However, high salinity and low temperature conditions decrease the oxygen consumption of oysters, in presence of hypoxia (Shumway and Koehn, 1982, Stanley and Sellers, 1986).

Many scientists have studied the physiological responses of hypoxia in oysters, but responses due to hypercapnia and acidosis, which co-occur with hypoxia, have not been addressed. Few studies have described the effects of hypercapnic environments. In Bivalves, hypercapnia-
induced acidosis involves dissolution of the calcium carbonate shell (Burnett, 1997; Willson and Burnett, 2000). Willson and Burnett’s (2000) study demonstrated that moderate hypercapnia depresses the oxygen uptake of oysters under well aerated condition at water pH down to 6.6. Hypoxic and hypercapnic conditions also reduce the ability of oysters to defend themselves against infection (Boyd and Burnett, 1999).

3.6 James River Watershed and Eastern Oysters

The James River is one of the most historic rivers in North America and the James River Basin occupies about 24 percent of Virginia’s area. It is Virginia’s largest river basin encompassing 10,265 square miles of the total land area. It is subdivided into 4 Sub-basins: Upper, Middle and Lower James Sub-basins and the Appomattox River Sub-basin. The James River is surrounded by six basins, the Potomac-Shenandoah, Rappahannock and York River Basins in the North and the New, Roanoke and the Chowan River Basins in the south. The James originates along the Virginia and West Virginia state line at the Alleghany Mountains, flowing southeast to meet the Chesapeake Bay. It is formed by the confluence of the Jackson and Cowpasture Rivers. The Basin is also divided into four physiographic provinces, each with a different topography and species diversity. The Valley and Ridge province spans from the Appalachian Plateau to the Blue Ridge Province. The fall line at Richmond separates two provinces Piedmont and the Coastal Plain. Major tributaries of the James River are the Jackson River, Cowpasture River, Craig Creek, Maury River, Tye River, Rockfish River, Slate River, Rivanna River, Willis Creek,

Land use types and population are predominantly different above and below the fall-line. The fall-zone is a three-mile stretch of river at Richmond. Above the fall-line, the watershed is essentially rural with 66% forest cover and 16% agricultural land. Below the fall-line, only 42% of the land is forested, 38% is developed and over 15% is characterized by impervious surfaces. It also has the lowest percentage of agricultural land (11%) compared to other river basins (Dauer et al., 2010). One of the reasons for the loss of agricultural land is population growth and urbanization. The total 2006 population for the James River Basin was approximately 2,092,278, concentrated in two major metropolitan areas: Tidewater with over 1 million people, and the Greater Richmond – Petersburg area with over 650,000. The other two population centers are the Lynchburg and Charlottesville areas, each with over 100,000 people. The basin has 38 counties and 17 cities (VA DEQ Impairment report, 2010).

According to the Chesapeake Bay Program (at ODU) the overall water quality of the James River is deteriorating. Limited improvements have been found upstream, with degrading trends in water quality downstream. The main sources of impairment affecting the river and the estuary described by VA DEQ in the Final 2010 305(b)/303(d) Water Quality Assessment Integrated Report are agriculture, atmospheric deposition (acidity, nitrogen), contaminated sediments, discharges from municipal storm sewer systems, livestock grazing, industrial point source discharge, loss of riparian habitat, municipal point sources discharge (high urbanized area), non-
point sources and many other unknown sources. All these sources directly or indirectly contribute in the deterioration of water quality of the river (VA DEQ Impairment report, 2010).

Historically, the James River has served as a microcosm for oyster populations. The river is “self-recruiting” because of the circulation patterns and frontal features of the river. Large populations of eastern oysters once existed in abundance, however, the populations and harvests have declined by more than 90% during the last century (Mann et al., 2009). In 2000, the Chesapeake Bay Agreement had called for at least a 10-fold increase by 2010 in *C. virginica* populations, based on 1994 levels. No significant increase has been noted in oyster levels (Dr. Mann, email communication). Figure 2 represents the oyster spat in the James River from 1947-2007. Spat data indicate that oysters are still able to spawn and have the potential for restoration.

**Figure 2: James River Spat Set (Spat/Bushel)**

3.6.1 Conceptual Model

In a risk assessment the conceptual model serves as a basis for further analysis. The model represents the relationship between all the various aspects of an assessment. The five main aspects of a conceptual model include sources (of the stressors), stressors, exposure pathways/routes, receptors/population and endpoints/measures. In this study, sources include all the sources that deteriorate the water quality like point and nonpoint source discharges, acid rain, atmospheric deposition, agriculture etc. Stressors are all those elements that produce an adverse response like changes in salinity, temperature, hypoxia, susceptibility to infection. Exposure pathways are the routes that the stressor/s follows to reach the receptor population like water quality deterioration, habitat alteration, feeding, and ingestion. An endpoint is an observable biological or chemical event that is used an index of the effect a stressor on an organism like growth, metabolism, and reproduction. (U.S. EPA, 2003). The Conceptual model pertaining to this study is represented in Figure 3.

In a nutshell, the present investigation uses elements from the Cumulative Risk Assessment Framework to assess the combined risks due to multiple stressors salinity, temperature and hypoxia. Water quality monitoring data were collected from Virginia Department of Environmental Quality’s water quality monitoring database. Oxygen consumption of oysters was calculated adjusted for salinity, temperature and oxygen saturation. A probabilistic risk analysis was performed using Monte-Carlo Analysis on Crystal Ball software. Sensitivity Analysis was performed to help in determination of the most influential factor in the model. In addition, effectiveness of the hazard quotient method was evaluated for its application in CRA.
FIGURE 3: CONCEPTUAL MODEL FOR THE STUDY

Sources
- Agricultural/Industrial Runoff
- Point/Non Point Sources
- Eutrophication
- Contaminant sediments
- Anthropogenic activities like overharvesting of natural resources

Stressors
- Changes in Salinity, Temperature
- Hypoxia, Hypercapnia, Acidosis
- Vulnerability to infection

Pathways
- Water Quality deterioration, Habitat alteration
- Physiological functions like reproduction, respiration, etc.

Receptors
- Eastern Oysters
- Invertebrates depending on oyster reefs, fishes etc.
- Sea food consumers, economy

Endpoints
- Oxygen Uptake of Oysters
- Vulnerability to diseases
4.0 METHODS

4.1 STUDY DESIGN

This study is an effort to apply cumulative risk assessment in an ecological situation. It aims to study the cumulative risks to the Eastern Oysters in the James River subjected to multiple stressors of changing salinity, temperature, hypoxia (decreasing oxygen levels) and simultaneous hypercapnia (increasing carbon dioxide levels). A second objective of the study is to calculate the oxygen consumption ($\hat{V}O_2$) when exposed to the same multiple conditions, using real field water monitoring data from VA Department of Environmental Quality. The third objective of the study is to compare the effectiveness of the hazard quotient method in CRA, i.e. to assess the robustness of the method in ecological cumulative risk assessment.

Two main studies (Shumway and Koehn, 1982 and Willson and Burnett, 2000) were utilized here to calculate the oxygen consumption ($\hat{V}O_2$) of the Eastern Oysters. The main features of both studies are that environmental variation is studied in combination and $\hat{V}O_2$ is measured in whole animals (and not individual tissues). Shumway and Koehn (1982) measured the oxygen consumption ($\hat{V}O_2$, ml O$_2$/hr) of $C.\ virginica$ under 9 salinity-temperature combinations and declining oxygen tensions. Three salinity conditions- 7, 14 and 28 parts per thousand (‰) and three temperature conditions- 10, 20, 30 °C were maintained. Measured $\hat{V}O_2$ values were standardized for 0.4 gm dry weight of oysters. Several important conclusions were drawn from
this study. First, with every 10 °C rise, rate of oxygen consumption significantly increases (refer to table 3 in methods for \( Q_{10} \) values). Second, oysters were successful in regulating \( \dot{V}O_2 \) in all 9 experimental conditions, but under declining oxygen conditions (hypoxia), as the salinity decreases and temperature increases, the ability to regulate \( \dot{V}O_2 \) also increases. Third, for all 9 combinations, no significant change in the \( \dot{V}O_2 \) was observed after 60 % saturation. Thus, using this study, \( \dot{V}O_2 \) adjusted for salinity, temperature and declining oxygen tensions (hypoxia) were calculated.

Willson and Burnett (2000) also measured the oxygen uptake (\( \mu \text{mol / hr} \)) under 25 ‰ salinity and 25 °C temperature. Other than hypoxia, organisms were also subjected to hypercapnia, low (< 1 Torr) and high (6-8 Torr) \( CO_2 \) pressures were maintained. \( \dot{V}O_2 \) values were measured in gm wet weight of oysters. This study has pointed out that moderate hypercapnia depresses the oxygen uptake of oysters under well aerated conditions. Second, \( \dot{V}O_2 \) decreases when oxygen pressure is less than 60 Torr (50 % saturation). Thus, using this study, \( \dot{V}O_2 \) adjusted for \( CO_2 \) was calculated. However, \( \dot{V}O_2 \) adjusted for pH was not calculated in the study.

Once relationships between the three multiple stressors were established, the same relationships were used to build a probabilistic calculation with a Crystal Ball (Oracle) Model. Crystal Ball uses Excel Spreadsheet models to quantify risks and aids in understanding the most influential factors in the model. This study used Monte Carlo Analysis which randomly generates values for uncertain variables repeatedly to simulate a model. All the uncertain variables of the model
(here, salinity, temperature, DO) are called assumptions. The probability distribution for each assumption is defined before starting a simulation. After assumption cells are defined forecast cells are defined (here, final $\dot{V}O_2$). During a simulation, Crystal Ball randomly selects values defined by that corresponding distribution and produces a probability distribution for the output variables (forecast) (Crystal Ball Manual, 2009).

### 4.2 Dataset Details

Water quality monitoring data were obtained from Virginia Department of Environmental Quality’s (VA DEQ) water quality monitoring database, dated from 1999 to 2009. This dataset contains a specific suite of measurements including temperature, salinity, dissolved oxygen saturation percentage and details about the monitoring stations (station ID #, date and time of recording, basin and sub-basin name etc.). Data were sorted based on two conditions. Data must fall between the salinity range 7 and 28 parts per thousand ($‰$) and a temperature range between 10 and 30 °C. All the data points that did not fall into these two criteria were excluded from the study. These criteria were selected based on experimental temperatures and salinities maintained in Shumway and Koehn’s and Willson and Burnett’s studies. All the monitoring stations were located below the fall-line. Figure 4 shows a map of all the monitoring stations on the river used in the study.
FIGURE 4: MAP OF MONITORING STATIONS USED IN THE STUDY

(Source: Mr. Roger Stewart, Virginia Department of Environmental Quality)
Green dots indicate monitoring stations with the station ID #s
4.3 Spreadsheet Analysis

All the spreadsheet analyses were performed in MS Excel 2007. A series of calculations were performed in the following sequence, in order to calculate the oxygen consumption (\( \dot{V}O_2 \)) of oysters adjusted for salinity, temperature and dissolved oxygen.

In the first step, using \( \dot{V}O_2 \) values from Shumway and Koehn’s study (Table 2) and the equation for a straight line (\( Y = mx+c \)), \( \dot{V}O_2 \) adjusted for salinity were calculated. \( Y \) is \( \dot{V}O_2 \) adjusted for salinity and \( X \) is the salinity value from the dataset. Slope of the line was -0.0096 (m) and intercept was 0.3913 (c).

**Table 2: Table Used to Calculate Slope (m) and Intercept (c) for a Straight Line**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Salinity (X) (‰)</th>
<th>( \dot{V}O_2 ) (Y) (ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>7</td>
<td>0.1317</td>
</tr>
<tr>
<td>20</td>
<td>7</td>
<td>0.2464</td>
</tr>
<tr>
<td>30</td>
<td>7</td>
<td>0.5031</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>0.0933</td>
</tr>
<tr>
<td>20</td>
<td>14</td>
<td>0.1875</td>
</tr>
<tr>
<td>30</td>
<td>14</td>
<td>0.6216</td>
</tr>
<tr>
<td>10</td>
<td>28</td>
<td>0.0531</td>
</tr>
<tr>
<td>20</td>
<td>28</td>
<td>0.0962</td>
</tr>
<tr>
<td>30</td>
<td>28</td>
<td>0.1783</td>
</tr>
</tbody>
</table>

(Source: Shumway and Koehn, 1982)

The second step was to calculate \( \dot{V}O_2 \) adjusted for temperature. \( Q_{10} \) values were utilized from Shumway and Koehn’s study. For each salinity and temperature range, a \( Q_{10} \) value was assigned (table 3). These corresponding \( Q_{10} \) values were multiplied with salinity adjusted \( \dot{V}O_2 \) values from step one.
The next step was to calculate $\dot{V}O_2$ adjusted for dissolved oxygen (DO). Using Shumway and Koehn’s study, relative changes were calculated for each salinity and temperature range. If DO saturation percentage was less than 60 %, corresponding calculated relative change was assigned (table 4). No relative change was found above 60 % oxygen saturation. Hence, relative change was assumed as 1. This relative change was multiplied with the temperature adjusted $\dot{V}O_2$ values from step two. Only 5 % of the data had oxygen saturation values below 60%.

### Table 3: $Q_{10}$ Values for Salinity and Temperature Ranges to Calculate Temperature Adjusted $\dot{V}O_2$

<table>
<thead>
<tr>
<th>Salinity (%‰)</th>
<th>Temperature (°C)</th>
<th>$Q_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-13</td>
<td>10-20</td>
<td>1.79</td>
</tr>
<tr>
<td>7-13</td>
<td>20-30</td>
<td>2.38</td>
</tr>
<tr>
<td>14-20</td>
<td>10-20</td>
<td>2.05</td>
</tr>
<tr>
<td>14-20</td>
<td>20-30</td>
<td>3.55</td>
</tr>
<tr>
<td>21-28</td>
<td>10-20</td>
<td>1.52</td>
</tr>
<tr>
<td>21-28</td>
<td>20-30</td>
<td>2.18</td>
</tr>
</tbody>
</table>

(Source: Shumway and Koehn, 1982)

### Table 4: Relative Change for Salinity and Temperature Ranges to Calculate DO Adjusted $\dot{V}O_2$

<table>
<thead>
<tr>
<th>Salinity (%‰)</th>
<th>Temperature (°C)</th>
<th>DO Saturation Percentage (%)</th>
<th>Calculated Relative Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-13</td>
<td>10-16</td>
<td>&lt; 60</td>
<td>0.518</td>
</tr>
<tr>
<td>7-13</td>
<td>17-23</td>
<td>&lt; 60</td>
<td>0.6112</td>
</tr>
<tr>
<td>7-13</td>
<td>24-30</td>
<td>&lt; 60</td>
<td>0.562</td>
</tr>
<tr>
<td>14-20</td>
<td>10-16</td>
<td>&lt; 60</td>
<td>0.02</td>
</tr>
<tr>
<td>14-20</td>
<td>17-23</td>
<td>&lt; 60</td>
<td>0.7223</td>
</tr>
<tr>
<td>14-20</td>
<td>24-30</td>
<td>&lt; 60</td>
<td>0.9113</td>
</tr>
<tr>
<td>21-28</td>
<td>10-16</td>
<td>&lt; 60</td>
<td>0.667</td>
</tr>
<tr>
<td>21-28</td>
<td>17-23</td>
<td>&lt; 60</td>
<td>0.813</td>
</tr>
<tr>
<td>21-28</td>
<td>24-30</td>
<td>&lt; 60</td>
<td>0.6277</td>
</tr>
<tr>
<td>7-28</td>
<td>10-30</td>
<td>&gt; 60</td>
<td>1</td>
</tr>
</tbody>
</table>

(Source: Shumway and Koehn, 1982)
The last step was to calculate $\dot{V}O_2$ values adjusted for hypercapnic conditions coexisting with hypoxia. Willson and Burnett’s study was used to find the relative change between the low and high CO$_2$ curve, compensating for CO$_2$ changes in water. No relative change was found below 50 % DO saturation. Hence, the relative change was assumed to be 1. Above 50 % DO saturation, the relative change was 0.158 (table 5). This relative change was multiplied with DO adjusted $\dot{V}O_2$ from step three.

Last step was to adjust the units and mass. Willson and Burnett measured oxygen uptake in $\mu$mol/hr gm$^{-1}$ wet weight (WW) of the organism, while Shumway and Koehn’s study expressed oxygen uptake in ml/hr 0.4 gm dry weight (DW) of the organism. Therefore in the present study, $\mu$mol were converted to ml by multiplying it with 0.0224 (Dejours, 1972). DW was converted to WW by multiplying it with 14.8 (shell-free DW/WW = 2.7%, Ricciardi and Bourget, 1998).

Figure 5 represents the sequence of steps used to calculate the final $\dot{V}O_2$.

**Table 5: Relative Change for Salinity and Temperature Conditions to Calculate CO$_2$ Adjusted $\dot{V}O_2$**

<table>
<thead>
<tr>
<th>Salinity (‰)</th>
<th>Temperature (°C)</th>
<th>DO Saturation Percentage (%)</th>
<th>Calculated Relative Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>25</td>
<td>&lt; 50</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
<td>&gt; 50</td>
<td>0.158</td>
</tr>
</tbody>
</table>

(Source: Willson and Burnett, 1997. Low CO$_2$ pressure was <1 Torr and High CO$_2$ pressure was 6-8 Torr)
**Figure 5: Sequence of Steps Used to Calculate Adjusted \( \dot{VO}_2 \) for Salinity, Temperature, DO and CO\(_2\)**

Calculated slope (m), intercept (c), and substituted salinity values (x) from dataset (y = mx+c)

- **Temperature**
  - Multiplied \( Q_{10} \) values from the study with \( \dot{VO}_2 \) values from step 1

- **DO (hypoxia)**
  - Calculated relative change (refer table 4) and multiplied with \( \dot{VO}_2 \) values from step 2

- **CO\(_2\) (hypercapnia)**
  - Calculated the relative change (refer table 5) and multiplied with \( \dot{VO}_2 \) values from step 3

- Multiplied step 4 \( \dot{VO}_2 \) by 0.0224 to convert µmol/hr to ml/hr and 14.8 to convert dry weight to wet weight

- **Final \( \dot{VO}_2 \) in ml/hr gm\(^{-1}\) wet weight**

**Study:** Shumway and Koehn, 1982
- Salinity Range: 7 to 28 ‰
- Temperature Range: 10 to 30 °C

**Study:** Willson and Burnett, 2000
- Salinity: 25 ‰
- Temperature: 25 °C
4.4 Statistical Analysis

Data were obtained from VA DEQ in MS Excel and imported to SAS 10.1 for all statistical analysis. Descriptive statistics and frequency distribution for all parameters (salinity, temperature, DO saturation %) including final VO$_2$ were calculated.

4.5 Crystal Ball Analysis (Risk Assessment Model)

Crystal Ball 11.1 was used in the study in order to perform a Probabilistic Risk Assessment (PRA). Instead of relying on single point estimates, PRA uses probability distributions for each parameter. The computer simulation runs thousands of trials by randomly selecting values for the variables and producing a probability distribution that can be used to determine the likelihood of exceeding a particular concentration or risk level.

This study evaluated one outcome, calculated final VO$_2$ (called forecast in crystal ball). The study intended to investigate the cumulative effects of changing salinity, temperature, DO and CO$_2$ conditions in water on the oxygen uptake of oysters, in addition, it provides insights into understanding whether the present conditions that are existing in the river are healthy for the oyster population to thrive.

The conditions of salinity, temperature and DO that influence the oxygen uptake (VO$_2$) of oysters were assigned as assumptions (in crystal ball). The model was created in such a way that all the assumption values were randomly generated. Each parameter had 4533 data points to run 10,000 trials/simulations. Since the study is limited to certain salinity and temperature ranges, the
distribution for each parameter was set to triangular distribution. Triangular distributions allowed selecting the minimum, maximum and the likeliest values from the dataset and limit the range of values in the Crystal Ball model. The following equation was used for creating a crystal ball model:

\[
\text{Final } \dot{V}O_2 = (mx+c) \times Q_{10} \times \text{OU}_{\text{(sal)}} \times \text{RC} \times \text{OU}_{\text{(Temp)}} \times \text{RC'} \times W \times G
\]

Where
- \( m = \) slope of the line (= -0.098)
- \( c = \) intercept of the line (=0.3913)
- \( x = \) salinity (‰)
- \( Q_{10} = \) factor by which the reaction rate increases for every 10-degree rise (table 3)
- \( \text{OU}_{\text{(sal)}} = \) oxygen uptake adjusted for salinity in ml/hr 0.4 gm dry weight
- \( \text{RC} = \) calculated relative change for adjusting changing DO (table 4)
- \( \text{OU}_{\text{(Temp)}} = \) oxygen uptake adjusted for temperature in ml/hr gm wet weight
- \( \text{RC'} = \) calculated relative change for adjusting changing CO\(_2\) levels (table 5)
- \( W = \) weight constant for converting dry weight into wet weight of the organism
- \( G = \) gas constant for converting µmole/hr into ml/hr
- \( \text{Final } \dot{V}O_2 = \) oxygen consumption of oysters in ml/hr gm\(^{-1}\) wet weight

Sensitivity analysis was also performed on the model to understand the influence of each parameter (assumption). Sensitivity analysis was performed in order to understand the variance each parameter contributes on the forecast (\(\dot{V}O_2\)) and uncertainty of the assumptions.

Assumption with the strongest correlation coefficient has the highest sensitivity and typically has a dominant effect on the uncertainty of the forecast. Figure 6 shows a screen shot of the Crystal Ball model.
FIGURE 6: SCREENSHOT OF CRYSTAL BALL MODEL IN EXCEL
5.0 RESULTS

5.1 STATISTICAL ANALYSIS

The data set contained 4533 samples from 72 monitoring stations (refer appendix 1 for map). Descriptive statistics and frequency distributions for each parameter and final \( \dot{V}O_2 \) are presented below (table 6, figure 7, 8, 9 and 10). Frequency distribution of salinity was negatively skewed. Data for temperature had a uniform distribution. Percent air saturation had a bell-shaped curve. In addition, majority of the air saturation levels were between 80 to 100 %, which suggests that most of the James River (only the area included in this study between the 72 monitoring stations) does not suffer from severe hypoxia. Data for final \( \dot{V}O_2 \) were skewed to the right.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Median</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (‰)</td>
<td>20.24</td>
<td>21.59</td>
<td>5.67</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20.56</td>
<td>21.38</td>
<td>5.62</td>
</tr>
<tr>
<td>Dissolved Oxygen (% Saturation)</td>
<td>85.28</td>
<td>86.95</td>
<td>13.93</td>
</tr>
<tr>
<td>Final ( \dot{V}O_2 ) (ml/hr)</td>
<td>0.0241</td>
<td>0.0203</td>
<td>0.0189</td>
</tr>
</tbody>
</table>
FIGURE 7: FREQUENCY DISTRIBUTION OF SALINITY (‰)
FIGURE 8: FREQUENCY DISTRIBUTION OF TEMPERATURE (°C)
Figure 9: Frequency Distribution of Dissolved Oxygen (% saturation)
Figure 10: Frequency Distribution of Final $\dot{V}O_2$ (ML/HR)
5.2 CRYSTAL BALL SIMULATIONS

A cumulative probability distribution of final $\dot{V}O_2$ generated by Crystal Ball is shown in Figure 11. This forecast was achieved in 10,000 trials/simulations. The expected final $\dot{V}O_2$ values are plotted on the horizontal axis. The vertical axis displays the cumulative probability (and cumulative frequency) with which a certain final $\dot{V}O_2$ is expected. For example there is a 29% chance that the expected final $\dot{V}O_2$ would fall below 0.1052 ml/hr or there is a 71% chance that the expected final $\dot{V}O_2$ would fall between 0.1052 ml/hr and 0.1972 ml/hr. This percentile information, in 10% increments is displayed in table 7. A percentile is the percent chance, or probability, of a forecast value (final $\dot{V}O_2$) being less than or equal to the value that corresponds to the percentile. Probabilistic analysis yields a range and probability associated with $\dot{V}O_2$ values, rather than single estimates.

5.2.1 SENSITIVITY ANALYSIS

Sensitivity chart generated by Crystal Ball is shown in Figure 12. Sensitivity is calculated by computing rank correlation coefficients between every assumption and every forecast while the simulation is running. In a sensitivity chart, assumptions that are most important and influencing (to the forecast) are ranked and displayed first. Assumptions with a negative relationship have bars on the left size of the zero line. Salinity contributed most to variability and uncertainty of the model and the forecast, followed by temperature.
Figure 11: Cumulative Probability Distribution of Final $\tilde{V}_O$ Using Crystal Ball

Table 7: Percentile Calculation of Final $\tilde{V}_O$ (Forecast) Generated by Crystal Ball

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Forecast values (final $\tilde{V}_O$, ml/hr gm WW of oysters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 %</td>
<td>0.0013</td>
</tr>
<tr>
<td>10 %</td>
<td>0.0407</td>
</tr>
<tr>
<td>20 %</td>
<td>0.0481</td>
</tr>
<tr>
<td>30 %</td>
<td>0.0567</td>
</tr>
<tr>
<td>40 %</td>
<td>0.0639</td>
</tr>
<tr>
<td>50 %</td>
<td>0.0703</td>
</tr>
<tr>
<td>60 %</td>
<td>0.0823</td>
</tr>
<tr>
<td>70 %</td>
<td>0.1063</td>
</tr>
<tr>
<td>80 %</td>
<td>0.1221</td>
</tr>
<tr>
<td>90 %</td>
<td>0.1384</td>
</tr>
<tr>
<td>100 %</td>
<td>0.2753</td>
</tr>
</tbody>
</table>
Figure 12: Sensitivity Analysis Chart Generated by Crystal Ball
6.0 **Discussion**

6.1 **Cumulative Risks on Eastern Oysters**

The calculated final $\dot{V}O_2$ values in this study demonstrated a similar trend found in Shumway and Koehn’s study (table 2) i.e. as salinity decreases and temperature increases, the oxygen consumption ($\dot{V}O_2$) of oysters also increases. This pattern was observed based on the sensitivity analysis generated by Crystal Ball. Sensitivity analysis calculated that salinity was the most influential assumption in the model (-79.3%) followed by temperature (20.7%). The negative sign indicates the direction of the relationship of the assumption, which confirms the trend observed in Shumway and Koehn’s study.

Second, it is essential to note that temperature and salinity are the two important environmental factors influencing Dermo (*Perkinsus marinus*) and MSX (*Haplosporidium nelsoni*) diseases that pose serious risks on survival of oysters. Willson and Burnett’s (2000) study demonstrated that light to moderate infections of *P. marinus* present in oysters did not affect the oxygen uptake of the oysters. *P. marinus* only require 0.3% of the total oxygen uptake, suggesting it does not appear to compete with the infected oyster for the available oxygen. Responses due to heavy infections of *P. marinus* were not stated in their study (Willson and Burnett, 2000). Elevated water temperatures and salinities provide favorable conditions for the two pathogens to grow and proliferate. The prevalence of these pathogens makes oyster survival difficult. Dermo was first
detected in the Chesapeake Bay in 1949 and MSX first appeared in 1959 and are still persistent (Carnegie and Burreson, 2009).

Below 15-20 °C, Dermo infections decline. Nevertheless, above a threshold of 20 °C, infections intensify and the parasite proliferates. Above 25 °C, the parasite rapidly multiplies and kills the oysters. Elevated salinity levels provide favorable conditions for the parasite to multiply. Salinity areas above 12-15 ppt have high oyster infections and mortalities. Below 9 ppt, infection intensities remain low (Burreson and Calvo, 1996). MSX infections are acquired at temperatures above 20°C. H. Nelsoni usually occurs at salinity conditions above 10 ppt. Below 10 ppt the parasite gets eliminated. The parasite starts proliferating at temperatures of about 10°C. H. nelsoni infects and kills sooner than P. marinus (National Research Council, 2004). Hence, combinations of salinity and temperature ranges can affect the growth of the pathogens and the susceptibility of the oysters towards these diseases.

Many other factors that increase the susceptibility of oysters towards Dermo infections were proposed by Lenihan et al. (1999) like flow speed, position of oyster on the reef, and food quality and availability. Low flow speed can decrease the physiological activity of the oysters and hence increase the susceptibility. Oysters located at the base of the reefs have a greater susceptibility than those located at the crest. The proposed reason for increased susceptibility was that oysters at the base of the reefs are subjected to low flow speed, reduced and poor food quality, and sedimentation rates are highest. These conditions depress the physiological activity and in turn increase the vulnerability of oysters (Lenihan et al, 1999). Hypoxia could also be a responsible
factor because oysters infected with *Perkinsus* die faster than uninfected oysters (Dwyer and Burnett, 1996).

Other than risks of infectious diseases, several other multiple risks stress the oyster population. Habitat degradation/loss, eutrophication, sedimentation, unsuitable oyster-substrate for the spats to latch on, tidal erosion etc. are few of them.

Third, when multiple risks of two or more independent stressors act simultaneously and are presented as a probability, the risk is the product of individual risks, not the sum (S. Ferenc et al., 1999). In this study, in order to calculate the oxygen uptake of oysters, cumulative risks (salinity, temperature, hypoxia and hypercapnia) were sequentially multiplied to understand if there was “synergy” between the risks. Therefore, cumulative risks may need to be multiplied and not added. Ferenc et al. (1999) also note that combinations of stress conditions can provoke responses that are not observed with single stresses, because of either the accumulation of responses, or the manifestation of unique responses.

### 6.2 Hazard Quotient Method and Cumulative Risk Assessment

The hazard quotient method has been a traditional risk estimation method for single-stressor assessments. Nevertheless, the method may be inappropriate for a risk assessment that involves multiple stresses and conditions like CRA. The first reason for its inappropriateness is that the estimates used in the method are calculated for single-stressor, single-response situations. Also, the final HQ is a mere number. Hence, when hazard index is calculated by adding/combining all
the HQs for a group of chemicals with the same mode of action, the number is not a real number. HQs cannot be added, multiplied or divided. Second limitation is that HQ does not represent any probability of the risk. Thus, risk assessors would not be able to predict the likelihood of the risks. Third, numerators and denominators reflect different estimates (i.e. effect and exposure estimates) that represent different uncertainties (S. Ferenc et al., 1999). Fourth, particularly with reference to CRA, HQ method cannot be used since it does provide any insight about the vulnerability of populations or organisms to the exposed risks. It is an important feature of CRA that HQ cannot address. Fifth, CRA involves assessing non-chemical stresses like social, psychologic, economical, geographic etc. Risks associated due to these suite of stressors cannot be captured by the HQ method (deFur et al., 2007).

For the present study, HQ was calculated for DO percent saturation. Usually, in order to calculate the HQ, mean of DO (85% saturation) would be divided by the EPA standard for hypoxia, 2 mg/L ≈ 30% saturation. The ratio is greater than one, indicating potential adverse effects would occur in organisms exposed to 85% DO saturation. In a situation like this, when a stressor like oxygen is involved, one would have to invert the ratio to yield the correct result, i.e.

\[
\text{HQ of % Saturation} = \frac{0.3}{0.85} = 0.36 < 1; \text{No adverse effects would occur}
\]

However, it is important to note that 0.85 is a single estimate for the dissolved oxygen concentration, it does not account for any seasons, particular location on the river, nor does it represent any probability. Since 0.85 is the mean of DO concentration, probability distribution is also lost. Probabilistic methods represent the likelihood of the risk and characterize the
uncertainties in the assessment. It provides a more comprehensive characterization of variability in risk estimates. In addition, sensitivity analysis aids in identifying the most influential parameters in risk estimates that would help in reducing the uncertainties associated with the assessment and other data gaps in the study. Probabilistic methods provide deeper insight than point estimate approach (U.S. EPA, 2001).

6.3 LIMITATIONS OF THE PRESENT STUDY

Although this study aimed at studying the cumulative effects of salinity, temperature, hypoxia and hypercapnia, it could not study effects due to acidosis (decrease in pH) that coexists with hypoxia and hypercapnia (Burnett, 1997). The study has not modeled $\dot{V}O_2$ adjusted for pH. Willson and Burnett (2000) demonstrated low oxygen uptake in high CO$_2$ and low pH conditions in gill tissues. It would be interesting to study the oxygen uptake responses of whole oysters subjected to multiple stressors (hypoxia related or any other).

The dataset used in the study was sorted based on experimental conditions found in the two studies (discussed in literature review). Therefore, $\dot{V}O_2$ below 7 ‰ and 10 °C and above 28 ‰ and 30 °C could not be calculated. Other differences in the two studies might have also contributed towards errors in the model, and eventually uncertainties in the model. Both studies have analyzed the data under different experimental conditions. Shumway and Koehn’s study had measured oxygen consumption in varying salinity and temperature conditions, while the other study measured oxygen consumption at constant salinity and temperature. Therefore the second study has not taken into consideration changing salinity and temperature while
calculating the oxygen uptake. Also, it is important to note that Shumway and Koehn had not accounted for varying CO$_2$ levels that Willson and Burnett’s study did. This study modeled oxygen consumption of oysters for varying salinity, temperature, DO and CO$_2$ conditions cumulatively, using real water monitoring data.

Other uncertainties include any other unnoticed data gaps in the water monitoring data obtained from VA DEQ.

**6.4 RECOMMENDATIONS**

It would be worthwhile to investigate models generated for other river systems, especially highly stressed water bodies. Further, studying other combinations of stressors in the ecosystem affecting the living resources would also be interesting. For example in the existing study, cumulative effects of other stressors like heavy metals or pesticides could be added to examine the responses on oysters. Furthermore, vulnerability of organisms or populations is also an important aspect in risk characterization. For instance, oysters have the ability to cope with fluctuating salinity and temperature ranges. These changing conditions provide a favorable environment for the pathogens to grow, proliferate, and hence (with the combination of other stressors) make the oysters more vulnerable to infectious diseases. Therefore, it is necessary to explore this aspect in a cumulative risk assessment.
7.0 BIBLIOGRAPHY


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