1990

Effects of low dissolved oxygen on the macrobenthos of the lower Rappahannock River, Chesapeake Bay

Roberto Javier Llanso

*College of William and Mary - Virginia Institute of Marine Science*

Follow this and additional works at: [https://scholarworks.wm.edu/etd](https://scholarworks.wm.edu/etd)

Part of the Environmental Sciences Commons, and the Fresh Water Studies Commons

**Recommended Citation**


[https://dx.doi.org/doi:10.25773/v5-vqfd-7f64](https://dx.doi.org/doi:10.25773/v5-vqfd-7f64)

This Dissertation is brought to you for free and open access by the Theses, Dissertations, & Master Projects at W&M ScholarWorks. It has been accepted for inclusion in Dissertations, Theses, and Masters Projects by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.
INFORMATION TO USERS

The most advanced technology has been used to photograph and reproduce this manuscript from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
Effects of low dissolved oxygen on the macrobenthos of the lower Rappahannock River, Chesapeake Bay

Llansó, Roberto Javier, Ph.D.
The College of William and Mary, 1990
EFFECTS OF LOW DISSOLVED OXYGEN ON THE MACROBENTHOS
OF THE LOWER RAPPAHANNOCK RIVER, CHESAPEAKE BAY

A Dissertation
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

by
Roberto J. Llansó
1990
APPROVAL SHEET

This dissertation is submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Roberto J. Llansó

Approved, December 1990

Robert J. Diaz, Ph.D.
Committee Chairman

Roger L. Mann, Ph.D.

Mark W. Luckenbach, Ph.D.

Bruce Neilson, Ph.D.

Charlotte P. Mangum, Ph.D.
This dissertation is dedicated to my mother, Esperanza, and to the memory of my father, Luis.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ix</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>2</td>
</tr>
<tr>
<td>CHAPTER 1. MACROBENTHIC POPULATION DYNAMICS DURING SEASONAL HYPOXIA IN THE LOWER RAPPAHANNOCK RIVER (CHESAPEAKE BAY).</td>
<td>5</td>
</tr>
<tr>
<td>Abstract</td>
<td>6</td>
</tr>
<tr>
<td>Introduction</td>
<td>7</td>
</tr>
<tr>
<td>Methods</td>
<td>12</td>
</tr>
<tr>
<td>Results</td>
<td>17</td>
</tr>
<tr>
<td>Discussion</td>
<td>60</td>
</tr>
<tr>
<td>CHAPTER 2. TOLERANCE OF LOW DISSOLVED OXYGEN BY THE POLYCHAETE STREBLOSPIO BENEDICTI (WEBSTER)</td>
<td>69</td>
</tr>
<tr>
<td>Abstract</td>
<td>70</td>
</tr>
<tr>
<td>Introduction</td>
<td>71</td>
</tr>
<tr>
<td>Methods</td>
<td>73</td>
</tr>
<tr>
<td>Results</td>
<td>78</td>
</tr>
<tr>
<td>Discussion</td>
<td>85</td>
</tr>
<tr>
<td>CHAPTER 3. TOLERANCE OF LOW DISSOLVED OXYGEN BY THE TUBICULOUS POLYCHAETE LOIMIA MEDUSA (SAVIGNY)</td>
<td>91</td>
</tr>
<tr>
<td>Abstract</td>
<td>92</td>
</tr>
<tr>
<td>Introduction</td>
<td>93</td>
</tr>
<tr>
<td>Methods</td>
<td>95</td>
</tr>
<tr>
<td>Results</td>
<td>98</td>
</tr>
<tr>
<td>Discussion</td>
<td>108</td>
</tr>
<tr>
<td>CHAPTER 4. EFFECTS OF HYDROGEN SULFIDE ON SURVIVAL AND BEHAVIOR OF THE POLYCHAETES STREBLOSPIO BENEDICTI (WEBSTER) AND LOIMIA MEDUSA (SAVIGNY)</td>
<td>112</td>
</tr>
<tr>
<td>Abstract</td>
<td>113</td>
</tr>
<tr>
<td>Introduction</td>
<td>114</td>
</tr>
<tr>
<td>Methods</td>
<td>118</td>
</tr>
<tr>
<td>Results</td>
<td>124</td>
</tr>
<tr>
<td>Discussion</td>
<td>133</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>137</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>139</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>142</td>
</tr>
<tr>
<td>VITA</td>
<td>160</td>
</tr>
</tbody>
</table>

iv
ACKNOWLEDGEMENTS

I gratefully acknowledge the support and guidance provided by my major professor, Dr. Robert Diaz, throughout the course of this research. Financial support, laboratory space and all needed equipment were always provided with generosity. I thank my Advisory Committee members for their time and constructive review of endless chapters of the manuscript. I especially thank Dr. Mark Luckenbach for field assistance, stimulating conversations and generous advise that helped to clarify important ecological concepts.

Successful completion of this study would not have been possible without the assistance of a number of people. David Stilwell, Maryann Wohlgemuth, Beverly Baker, Jerome La Peyre, Jia Chen, Berch Smithson, Eric Zobrist, Cindy Hutton, Lino Gallo and Gustavo Calvo spent long days assisting in the field. Dr. Charlotte Mangum, Dr. Fu-lin Chu, Steve Snyder, Dan Sved and Mary Ann Vogelbein let me borrow equipment. Nancy Wilson provided some of the water quality data presented in this study. Betty Salley and Grace Battisto provided prompt and generous help with water sample analyses. Dr. John Greaves was always ready to listen to questions and find better ways to build artificial worm tubes. Dr. Mike Ewing (ODU) provided some of the polychaetes used in experimental work. Sharon Miller generally had available and ready that much needed boat I reserved at the last minute. Diane Walker was always willing to help in library matters. To all of them, thank you. Finally, special thanks to my wife, Rita, who endured my grouchy mood and the many hours of work I spent away from home; her love never failed.
LIST OF TABLES

Chapter 1.
1. Percent total organic carbon content of sediments ............... 27
2. Thirteen most abundant species of macrobenthos .................. 29
3. Station locations versus species abundances .................... 33
4. Species richness and evenness .................................. 35
5. Abundances versus depth in sediment ............................ 53

Chapter 2.
1. Treatment conditions of Streblospio in low dissolved oxygen .... 74
2. Tolerance of experimental anoxia by polychaetes ................. 86

Chapter 3.
1. Treatment conditions of tests of Loimia medusa .................. 96
2. Ammonium concentrations and pH ................................ 99
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Chapter 1.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Map of the study area and station locations</td>
<td>13</td>
</tr>
<tr>
<td>2. Dissolved oxygen concentrations of water column</td>
<td>18</td>
</tr>
<tr>
<td>3. Salinity and temperature</td>
<td>20</td>
</tr>
<tr>
<td>4. Sediment composition at the mouth and C stations</td>
<td>23</td>
</tr>
<tr>
<td>5. Sediment composition at the upriver and E stations</td>
<td>23</td>
</tr>
<tr>
<td>6. Sediment composition at station F</td>
<td>23</td>
</tr>
<tr>
<td>7. Temporal changes of abundance and species number</td>
<td>31</td>
</tr>
<tr>
<td>8. Temporal changes of abundance of <em>S. benedicti</em></td>
<td>36</td>
</tr>
<tr>
<td>9. Temporal changes of abundance of <em>H. ambiseta</em></td>
<td>38</td>
</tr>
<tr>
<td>10. Temporal changes of abundance of <em>P. pinnata</em></td>
<td>41</td>
</tr>
<tr>
<td>11. Temporal changes of abundance of <em>P. levifuscina</em></td>
<td>43</td>
</tr>
<tr>
<td>12. Temporal changes of abundance of <em>L. medusa</em></td>
<td>45</td>
</tr>
<tr>
<td>13. Total number of individuals collected on a 250-um sieve</td>
<td>48</td>
</tr>
<tr>
<td>14. Vertical distribution of macro-infauna in sediments</td>
<td>51</td>
</tr>
<tr>
<td>15. Vertical distribution of <em>P. levifuscina</em> and <em>N. succinea</em> in sediments</td>
<td>55</td>
</tr>
<tr>
<td>16. Vertical distribution of <em>S. tentaculata</em> and <em>L. medusa</em> in sediments</td>
<td>57</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 2.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Survival of Streblospio adults after exposure to hypoxia and air-saturated seawater</td>
<td>79</td>
</tr>
<tr>
<td>2. Number of Streblospio adults surviving as a function of time during exposure to anoxic seawater</td>
<td>83</td>
</tr>
</tbody>
</table>
Chapter 3.
1. Survival of *Loimia* adults after exposure to hypoxia and air saturated seawater ......................................................... 101

2. Number of *Loimia* adults surviving as a function of time during exposure to anoxic seawater ............................................. 104

3. Cumulative number of *Loimia* adults dead on surface versus dead in tube as a function of percent air saturation .......................... 106

Chapter 4.
1. Number of *Streblospio* adults surviving as a function of time during exposure to anoxia with sulfide, anoxia, and air-saturated seawater ......................................................... 125

2. Number of *Streblospio* adults surviving as a function of time during exposure to low dissolved oxygen with sulfide, low dissolved oxygen, and air-saturated seawater ................................. 127

3. Number of *Loimia* adults surviving as a function of time during exposure to anoxia with sulfide, anoxia, and air-saturated seawater ................................................................. 131
ABSTRACT

The lower Rappahannock River, a subestuary of the Chesapeake Bay, is affected by seasonal low dissolved oxygen events that are intermittent and vary in intensity. The effects of these seasonal hypoxic events on macrobenthic population dynamics were studied in a meso-polyhaline, deep-water sandy-mud habitat, with the objectives of documenting the patterns of spatial and temporal variation of abundance and species composition with the onset of hypoxic conditions, vertical distribution of species in sediment during hypoxia, and patterns of recovery from hypoxia.

Overall, macrobenthic abundance and species number were low, decreasing towards the channel and upriver, where dissolved oxygen concentrations were lowest. Polychaetes comprised the largest percentage of the population, with dominance of "opportunists". Other taxa were present, but in very low numbers. Mortality of macrobenthos in early summer was attributed to hypoxia. Species abundances and number declined with the onset of hypoxia, and did not recover in the deep channel until the fall. Temporal changes of abundance in the vertical distribution in sediment were observed, with lower overall abundance at depth in sediment during hypoxia than in normoxia. When analyzed by species, trends in the vertical distribution were not clearly related to hypoxia for most species.

Tolerance of macrobenthos to hypoxia appeared to be species-specific. Recruitment of hesionid polychaetes occurred in summer during normoxia, but mortality followed during hypoxia, suggesting that macrobenthic assemblages in affected areas may be largely structured by the relationship between the timing of low dissolved oxygen events and species life histories. Dominant species with apparent differences in response to hypoxia in the field, were investigated further in laboratory experiments to determine their tolerance and behavior to hypoxia, anoxia, and hydrogen sulfide. Two polychaetes were selected, the small "opportunist" spionid Streblospio benedicti, and the larger terebellid Loimia medusa.

Under severe hypoxia, S. benedicti survived for two weeks without significant mortality at two experimental dissolved oxygen concentrations (14.5 and 7% air saturation). In anoxia, all worms died within 55 hrs. Larvae survived severe hypoxia without displaying behavioral ill effects for at least 4 days. The behavior of S. benedicti was modified; feeding and burrowing activities ceased, and worms came out of their tubes onto the sediment surface. L. medusa showed higher tolerance of anoxia, surviving up to 5 days. Survival under prolonged hypoxia was significantly reduced. The behavior of L. medusa was also modified by hypoxia and anoxia. Worms ceased feeding and protruded head and abdominal tip out of their tubes. Worm occurrences on the sediment surface at time of death were treatment-dependent.

Survival of both species was not significantly reduced when worms were exposed to anoxia with moderate concentrations of hydrogen sulfide. These results are discussed in terms of species distributions, presence of hydrogen sulfide in sediment, and mechanisms of resistance to sulfide toxicity.
EFFECTS OF LOW DISSOLVED OXYGEN ON THE MACROBENTHOS
OF THE LOWER RAPPAHANNOCK RIVER, CHESAPEAKE BAY
INTRODUCTION

Considerable scientific and public attention has recently focused on the deterioration of the marine milieu. Specifically, there is an increasing concern about water quality alterations in estuarine and coastal areas resulting from pollution and eutrophication. Proximity to urban centers and agricultural fields, makes estuaries and coastal areas susceptible to nutrient accumulation. Eutrophication has been implicated in changing estuarine ecosystem processes, resulting in habitat loss and decreased productivity of species of commercial and recreational importance (Flemmer et al. 1983).

One of the effects attributed to eutrophication is the stimulation of phytoplankton growth, with a concomitant increase in sedimentation of organic matter, elevation of chemical and biological oxygen uptake, and bottom water deoxygenation (Rainer and Griffiths 1980). While there is increasing evidence of a relationship between eutrophication-induced organic enrichment and the development of low dissolved oxygen in bottom waters (Rosenberg and Loo 1988, Baden et al. 1990), the occurrence of hypoxia is often a natural phenomenon determined primarily by physical factors, such as water mass movements, temperature, and salinity gradients. How much of the long-term variation in the development, extent, and intensity of hypoxic events is related to nutrient loading, is one of the central interests of current scientific and management programs (Mackiernan 1987).

In order to evaluate the relative importance of hypoxia disturbance on estuarine processes, the response of both species and community must be assessed. Estuarine benthos are particularly important to study because of
their value as a resource for higher trophic levels. Most previous studies of biological effects of hypoxia on benthos have focussed either on the tolerance and physiological adaptations of species, or on changes in community structure alone. A dual approach, however, permits the identification of species-specific responses to environmental hypoxia (or anoxia), and provides insight on how these responses may affect population dynamics and community structure in oxygen-stressed environments.

The Chesapeake Bay and its subestuaries constitute an excellent system for the study of benthic responses to hypoxia because of the occurrence of seasonal hypoxic and anoxic events. In the lower Rappahannock River, low dissolved oxygen events are intermittent and vary in extent and intensity (Brooks 1983). Therefore, their effect on benthos may be subtle and vary with species tolerance and behavior.

The first chapter of this dissertation is a study of the effects of environmental hypoxia on estuarine macrobenthos. It focusses on population dynamics, community structure, and species responses during seasonal hypoxia (Spring to Fall). Hypotheses concerning potentially different effects of hypoxia on the distribution of macrobenthos were posed and tested. The results from this investigation are subsequently used to identify benthic species of numerical or biomass importance exhibiting specific responses to low dissolved oxygen. Tolerance and behavior of these species to hypoxia, anoxia, and hydrogen sulfide are further evaluated in laboratory experiments.

The tolerance and behavior of the spionid polychaete *Streblospio benedicti* to experimental hypoxia and anoxia are studied in chapter 2, while in chapter 3 the terebellid polychaete *Loimia medusa* is the object of study. The ecology, distribution within the sediment, and body size of these two
species differ, providing an opportunity for between species comparisons of low dissolved oxygen effects. Chapter 4 is a study of the behavioral responses and tolerance of both species to hydrogen sulfide.

No work on the effects of experimental hypoxia, anoxia, and hydrogen sulfide, or on the respiratory physiology of these two species has been published to date. With the exception of Polydora ligni (Mangum and Van Winkle 1973), no information on the responses of spionid polychaetes to low dissolved oxygen is available, despite the fact that members of this family are common in a variety of estuarine habitats. Research on the responses of these and other benthic species to reduced dissolved oxygen concentrations is needed before inferences can be extended to populations and communities. The present study links field observations with laboratory experiments to evaluate more clearly the importance of oxygen-related disturbance on estuarine macrobenthos.
CHAPTER 1.

MACROBENTHIC POPULATION DYNAMICS DURING SEASONAL HYPOXIA

IN THE LOWER RAPPAHANNOCK RIVER (CHESAPEAKE BAY)
ABSTRACT

The effects of hypoxia on population dynamics of macrobenthic organisms were studied at a meso-polyhaline, deep sandy-mud habitat in the lower Rappahannock River, a tributary of Chesapeake Bay. Water quality was monitored on a monthly to weekly basis, Spring to Fall, to determine the extent, intensity and duration of hypoxic events. Core samples collected along a five-station transect were used to identify species, discern patterns of spatial and temporal variation, and determine vertical distribution patterns in sediment in relation to dissolved oxygen fluctuations.

A significant decrease in macrobenthic abundance followed by defaunation at some stations was attributed to intermittent hypoxia. Abundance and species number were low during summer, decreasing with increasing water depth and upriver direction in relation to lowest dissolved oxygen concentrations. Species composition appeared to be influenced by hypoxia. The magnitude of the responses to hypoxia appeared to be species-specific. Overall, vertical distribution of macrobenthos in sediment changed with season and water-column oxygen concentrations. When data were analyzed for individuals species, no clear trends of temporal change in vertical distributions were discerned. Other factors, such as recruitment pulses, that may account for such patterns are discussed.

The intensity of hypoxia was found to change benthic community structure and distribution. Results suggest that the periodicity of hypoxic events may lead to long-term alterations of local benthic populations.
INTRODUCTION

Hypoxia, defined here as the occurrence in bottom waters of dissolved oxygen (DO) concentrations below 2 mg/l (about 30% air saturation at 26°C), has been frequently observed in estuaries and coastal areas (May 1973; Christensen and Packard 1976; Taft et al. 1980; Harper et al. 1981; Stuntz et al. 1982), and anoxia, the absence of oxygen, is a common feature of deep basins (Richards 1965). The identification of hypoxia as an indicator of reduced environmental quality has brought public and scientific attention to a problem that is often considered a consequence of pollution and eutrophication (Rosenberg and Loo 1988; Baden et al. 1990). Recently, this concern has also made hypoxia the focus of intensive scientific and management programs in the Chesapeake Bay (Virginia, USA) (Mackiernan 1987). Seasonal hypoxia appears to have increased both in intensity and extent in the Chesapeake Bay during the last decade (Flemor et al. 1983; Officer et al. 1984; but see Seliger and Boggs 1988), although earlier reports (Newcombe et al. 1939) had already emphasized the importance of this phenomenon and its ecological significance for the bay ecosystem.

Low-oxygen induced stress may have the most severe ecological consequences when its effects are manifested over large areas through a major reduction of prey for higher trophic levels. Wherever hypoxic events are frequent, benthic communities may become dominated by short-lived, low biomass species (Mountford et al. 1977). Under severe hypoxia or persistent anoxia, benthic population abundances decrease and defaunation may follow. Anoxia-induced faunal mortalities have been documented for some areas of the

Summer hypoxia in the Chesapeake Bay is estimated to cover large areas from Baltimore to the Rappahannock Shoals (Taft et al. 1980), encompassing some 603 million m² (Flemer et al. 1983), mainly along the deep main stem of the bay and at the mouth of the major tributaries: Patuxent, Potomac, Rappahannock and York Rivers (Haas, 1977; Officer et al. 1984; Kuo and Neilson 1987). The volume of water in Chesapeake Bay with DO concentrations equal or less than 0.7 mg/l was estimated at 4.3 billion m³ in 1980, an increase of 4 billion m³ since 1950 (Flemer et al. 1983).

Factors likely to contribute to the development and spatial variation of hypoxia in the Chesapeake Bay are salinity, temperature, wind stress, and tidal circulation (Kuo and Neilson 1987; Tuttle et al. 1987). The development of vertical salinity gradients during the spring freshwater run off leads to water column density stratification. The establishment of a pycnocline,
association with periods of calm and warm weather, restricts water exchange between the surface and the bottom layers of the estuary, where oxygen consumption is large. The formation or the disruption of the pycnocline is probably the most important process determining the intensity and extent of hypoxia (Seliger et al. 1985; Turner et al. 1987), albeit not the only one. Biological processes have been suggested to contribute to deep water oxygen depletion (Taft et al. 1980; Officer et al. 1984). Benthic metabolic rates increase during spring and early summer, leading to an increase of the rate of oxygen consumption in bottom waters. This depends in part on the amount of organic carbon available for the benthos, which appears to be derived from seasonal phytoplankton blooms of the previous fall (Taft et al. 1980; Officer et al. 1984). Phytoplankton growth may further be stimulated by benthic remineralization of nutrients, which may be transported through the lateral boundaries of anoxic water, or advected into the euphotic zone during periods of destratification (Webb and D'Elia 1980; Gavis and Grant 1986).

Anthropogenic nutrient inputs to the Chesapeake Bay ecosystem may also influence the development and intensity of hypoxia (Mearns and Word 1982; Malone 1987).

The Rappahannock River is one of the tributaries to the lower Chesapeake Bay (Fig. 1). It is affected by summer hypoxic events that are more severe and of longer duration than those in the other Virginian tributaries, the York and the James Rivers (Kuo and Neilson 1987). Available data (Brooks 1983) indicate that, while hypoxia is common upriver during summer months, the mouth of the Rappahannock River experiences intermittent hypoxia, and anoxia may become established during short periods of time. These conditions render the lower Rappahannock River an area of special interest for the study of benthic
population responses during hypoxia.

The study of the effects of intermittent hypoxia or anoxia on estuarine benthos is particularly interesting because the frequency of low DO events probably sets up the conditions for which species populations fluctuate. For instance, recruitment levels are of critical importance for the establishment of annual patterns in species composition (Holland et al. 1987). Therefore, the coincidence of hypoxic events with larval availability and settlement may have severe consequences for the maintenance of benthic populations. The intensity and duration of hypoxia or anoxia may further change patterns of species composition as a result of varying low DO tolerances of benthic organisms. Additionally, moderate hypoxia may affect the availability of infaunal benthic organisms to fishes and crabs through a shift in their vertical distribution in sediment. We know that widespread anoxia leads to defaunation of large coastal areas (Swanson and Sindermann 1979; Rossignol-Strick 1985); however, less information is available on how hypoxia may lead to more subtle changes in benthic community structure, such as transitory and long-term alterations in taxonomic composition, diversity and biomass. On a local scale, comparison of sites affected differently by hypoxia may also reveal unequal responses of species among sites.

In an attempt to establish the relationships between seasonal hypoxia and macrobenthic population dynamics, a meso-polyhaline, deep sandy-mud habitat of the lower Rappahannock River was sampled from spring to fall 1987 for a study designed to (1) document changes in species abundances and composition of the macrobenthos with the onset of hypoxia, (2) compare population changes in species abundances between sites affected by different intensity of hypoxia and (3) determine whether there is any shift in the
burrowing depth of macroinfauna during hypoxia.
METHODS

Sampling Design

Macrobenthos (invertebrates collected on a 0.5-mm screen), sediments and water quality were sampled monthly from April to October 1987 at four deep stations, and from May to September 1987 at two shallower stations in the lower Rappahannock River. In addition, water quality was also sampled fortnightly in June and September and weekly in July and August at three of the deep stations (data provided by A. Kuo, VIMS). The stations were located along two transects (Fig. 1). One transect was established from the mouth of the river to about 20 km upstream, and consisted of four stations (A, B, C and E) of water depths of 12.8 to 18.6 m MLW. A second transect, perpendicular to the first, consisted of two stations (F and G) with water depths of 10.6 m and 7.6 m MLW.

Sampling and Laboratory Procedures

Three Smith-McIntyre grabs (0.1 m²) were collected at each station, May through October. In April, three grabs were collected at stations A and B, and one grab was collected at stations C and E. Three replicates were collected because between-replicate variability was low during preliminary sampling. Upon retrieval, the sediment surface was examined carefully for the presence of organisms and their structures. Temperature, color and depth of the Redox Potential Discontinuity (RPD) layer of surface sediments were noted.

Sub-samples were taken from each grab by inserting vertically into the sediment two acrylic cores (56.7 cm² surface area per core) to a depth of 15 cm. Each core was sectioned into layers by horizontally inserting acrylic
Fig. 1. Map of the study area and station locations
cutting plates into the sediment at depths of 2 and 5 cm. Three sediment layers were obtained in this way: 0 to 2 cm, 2 to 5 cm and >5 cm. Two additional replicate sediment cores (3.5 cm²) were removed at each sampling site and date for analyses of particle size distribution and organic carbon content. Two sediment layers (2 to 5 and >5 cm) were sieved through a 0.5-mm mesh screen using seawater. Organisms were fixed in 10% buffered formalin in seawater to which Rose Bengal had been added. The top sediment layer (0 to 2 cm) was fixed in formalin, and later sieved through nested 0.25 and 0.5-mm mesh screens. In the laboratory, samples were washed in fresh water and the organisms sorted, identified to the lowest possible taxon and stored in 70% ethanol.

Temperature, salinity and DO of bottom (0.5 to 1 m above bottom), mid-column and surface water (3 m) were obtained in situ using a Conductivity-Temperature-Depth (CTD) sensing instrument to which a calibrated oxygen probe (YSI Model 54) was attached. Salinity of some samples was also determined with a salinometer (Beckman Model RS-10). Sediment particle size analyses were conducted as described by Folk (1980). Total organic carbon was measured by high temperature combustion using a carbon-nitrogen analyzer (Carlo Erba CNA 500). Inorganic carbon was first digested with 5% HCl, and the samples air dried and weighed.

Data Analyses

Species richness (SR) and evenness (J') were computed using the formulas given by Margalef (1958) and Pielou (1966). Mean faunal abundances were compared using one-factor analyses of variance (ANOVA) on transformed data after assuring that the assumptions of the test were met as indicated in
Underwood (1981). The null hypothesis tested was of no difference in total species abundances between sampling locations differently affected by hypoxia. Transformation of counts to $\log_{10}(X+1)$ was necessary to remove heterogeneity of variances and normalize the data. The logarithmic transformation was used because of the binomial character of the original count distribution.

Independence and normality were checked by plotting the data. Homogeneity of variances before and after transformation was tested using Cochran’s test (Zar 1984). The Student-Newman-Keuls procedure (Zar 1984) was used for aposteriori comparisons of means. Changes in the distribution of macrobenthic organisms with depth in sediment was tested using G-test (Sokal and Rohlf 1981) under the null hypothesis of independence between depth of burrowing and sampling data. The 5% level of probability was selected for all tests.
RESULTS

Hypoxia and the Environment

For convenience, I will refer to station A as the "mouth" station, and to station B as the "upriver" station, identifying in this way the two end points of the transect.

DO concentrations varied extensively from week to week, exhibiting similar trends among sampling locations (Fig. 2). The short-term variation in DO was missed when measurements were taken monthly (e.g., at stations C and F). Bottom DO concentrations first decreased to hypoxic levels in June at the upriver, F and E stations (Fig. 2). Intermittent periods of hypoxia followed at all stations throughout summer. Minimum DO concentrations were recorded upriver in August at a water depth of 19 to 20 m. Hypoxia appeared to be of greater intensity at the upriver station than at the mouth of the river. Average DO concentrations of bottom water for the summer (June through September) were 2.1 mg/l at the upriver station, and 3.6 mg/l at the mouth station. Hypoxia also may have been of longer duration upriver than at the mouth, as suggested by the low DO concentration values recorded at the upriver station consecutively on July 28 and August 4.

Salinity varied spatially and temporally, as is characteristic of a partially-mixed estuary (Fig. 3). It ranged from 12.7-21.7%o at the surface, and from 14.4-26.0%o at the bottom. The upriver station exhibited the largest variation in bottom water salinity.

Water temperature (Fig. 3) increased uniformly at all depths, reaching a maximum at the surface in July (average =-27.5°C), and at the bottom in August (average =-27.1°C).
Fig. 2. Dissolved oxygen concentrations in bottom [o—o], mid-column [o—o] and surface waters [o...o] at five stations of the Rappahannock River from April 14 to October 23 1987.
Fig. 3. Salinity (open symbols) and temperature (dark symbols) of bottom 
[●...●], mid-column [▲...▲], and surface [○...○] waters at five stations 
of the Rappahannock River from April 14 to October 23 1987.
Salinity data reveal some periods of water column density stratification followed by events of density destratification. The latter coincided with an increase of bottom DO concentrations, as indicated on July 21 and August 17 upriver and at station E, and on July 16 at the mouth of the river (Fig. 3). The July 21 cruise was preceded by a week of north-westerly winds and a summer storm, which may explain the increase in deep-water DO by surface mixing.

Deep-water sediments in the lower Rappahannock River were muds comprised of >95% silts and clays upriver, and 80 to 90% silts and clays at the mouth (Figs. 4-5). Sediments of the shallow station F contained a higher proportion of sands (Fig. 6). The proportion of sand increased markedly at the mouth of the river and station C in October. Station G was located on a shallow area (7 m) characterized by sands and numerous oyster shells. The collection of benthic grabs at this station proved difficult, with several samples lost as shells prevented the sampler from sediment penetration and grab closure. Since data for station G are incomplete, they were not included in this study.

Total organic carbon in sediments was slightly higher upriver than at the mouth of the river (Table 1). It was generally lowest at station F. The monthly variation of total organic carbon in sediments was small, except for a substantial decrease at the mouth station in October.

The surface of the sediment appeared to be well oxygenated at all stations in May, exhibiting a light-brown color and a RPD layer of about 10 to 15 mm. Numerous small polychaete tubes protruded from the sediment surface of grabs collected at the mouth station. In June, the color of surface sediments had turned gray and, judging by the change in color with depth, the RPD layer was shallow (1 to 2 mm) at the mouth, C and E stations, and absent upriver.
Figs. 4-6. Sediment composition by month at five stations of the Rappahannock River, April through October 1987.
CUMULATIVE PERCENTAGE

STATION E

UPRIVER

MONTH OF SAMPLING

MONTH OF SAMPLING

Sediment type
- Sand
- Silt
- Clay
Table 1. Percent (by weight) of total organic carbon content of sediments at stations in the lower Rappahannock River, April through October 1987. Station designation as in Fig. 1.

<table>
<thead>
<tr>
<th>Month</th>
<th>A</th>
<th>C</th>
<th>E</th>
<th>F</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>2.04</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>2.90</td>
</tr>
<tr>
<td>May</td>
<td>1.97</td>
<td>2.40</td>
<td>2.56</td>
<td>1.99</td>
<td>2.86</td>
</tr>
<tr>
<td>June</td>
<td>2.22</td>
<td>2.54</td>
<td>2.75</td>
<td>1.78</td>
<td>2.94</td>
</tr>
<tr>
<td>July</td>
<td>2.22</td>
<td>1.85</td>
<td>2.61</td>
<td>1.27</td>
<td>2.87</td>
</tr>
<tr>
<td>August</td>
<td>2.11</td>
<td>2.36</td>
<td>nd</td>
<td>nd</td>
<td>2.95</td>
</tr>
<tr>
<td>September</td>
<td>1.99</td>
<td>2.24</td>
<td>2.63</td>
<td>1.51</td>
<td>2.86</td>
</tr>
<tr>
<td>October</td>
<td>0.32</td>
<td>nd</td>
<td>2.72</td>
<td>nd</td>
<td>2.59</td>
</tr>
</tbody>
</table>

nd — no data.
Similar observations were noted in July and August. At station F, the sediment surface had a brown color in June and July, with an estimated RPD depth of 5 to 10 mm; no observations were made in August. By September, an RPD depth of about 2 mm was estimated from sediments of all deep stations.

The Macrobenthos: Species Composition

A total of 63 species were collected and identified at the lower Rappahannock River during this study. An additional 7 taxa were identified to the family or higher-order levels (See Appendix). Annelids were the dominant components (Table 2) with 32 species, followed by crustaceans (12 species) and molluscs (11 species). Most of the individuals collected were annelids, accounting for 94% of the total abundance. Molluscs and crustaceans were sparse with only the gastropod *Acteocina canaliculata* being common, accounting for 25% of all non-annelid taxa.

Infauna was mostly restricted to the top 5 cm of the sediment, and was comprised of small annelid polychaetes and their juveniles (e.g., *Streblospio benedicti, Mediomastus ambiseta*). Large invertebrates were not collected at deep stations, with the exception of the tubiculous polychaete *Loimia medusa*. Large epifauna was only observed at the shallow station G, and included oysters (*Crassostrea virginica*), tunicates (*Molgula manhattensis*), gastropods (*Nassarius* spp.), sea-anemones (*Diadumene leucolena*?) and black-fingered crabs (*Xanthidae*).

Some species were only present, as small recruits, in October. Among these species, the presence of hemichordates, turbellarians, the polychaetes *Spiochaetopterus costarum* and *Phylloodoce arenae*, and the bivalve *Lucina multilineata* at the mouth and C stations, may have been related to the higher
Table 2. Thirteen most abundant species of macrobenthos in the lower Rappahannock River from April to October 1987, and their relative percent abundance.

<table>
<thead>
<tr>
<th>Species</th>
<th>Percent of Total Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Streblospio benedicti</em> Webster</td>
<td>37.0</td>
</tr>
<tr>
<td>2. <em>Mediomastus ambiseta</em> Hartman</td>
<td>26.0</td>
</tr>
<tr>
<td>3. <em>Paraprionospio pinnata</em> (Ehlers)</td>
<td>7.0</td>
</tr>
<tr>
<td>4. <em>Sigambra tentaculata</em> (Treadwell)</td>
<td>2.8</td>
</tr>
<tr>
<td>5. <em>Podarkeopsis levifuscina</em> Perkins</td>
<td>2.7</td>
</tr>
<tr>
<td>6. <em>Pectinaria gouldii</em> (Verrill)</td>
<td>2.3</td>
</tr>
<tr>
<td>7. <em>Eteone heteropoda</em> Hartman</td>
<td>2.3</td>
</tr>
<tr>
<td>8. <em>Polydora cornuta</em> Bosc</td>
<td>2.2</td>
</tr>
<tr>
<td>9. <em>Loimia medusa</em> (Savigny)</td>
<td>2.2</td>
</tr>
<tr>
<td>10. <em>Nereis succinea</em> (Frey and Leuckart)</td>
<td>1.8</td>
</tr>
<tr>
<td>11. <em>Pseudeurythoe paucibranchiata</em> Fauvel</td>
<td>1.7</td>
</tr>
<tr>
<td>12. <em>Acteocina canaliculata</em> (Say)</td>
<td>1.5</td>
</tr>
<tr>
<td>13. <em>Glycinde solitaria</em> Webster</td>
<td>1.3</td>
</tr>
</tbody>
</table>
proportion of the sand fraction of sediments in October. Similarly, the gastropods Acteocina canaliculata and Odostomia sp. had increased abundances at the mouth in October, which may have been related to a shift in sediment composition.

Patterns of Spatial and Temporal Variation

Total abundance showed a recruitment peak in May followed by a sharp decline during June and July, reached very low numbers in August and September, and recovered in October (Fig. 7). Species number also declined after May, but an increase occurred in July at stations C and F (Fig. 7). It was highest at the mouth and at station F, and lowest at the upriver station. During the recruitment peak, species abundance decreased along the transect from the mouth to the upriver station. The latter station exhibited the lowest abundance of the study period, with defaunation occurring in July. The recovery peak in October did not reach the abundance values of the spring, but was pronounced both upriver and at the mouth.

In order to assess the relative station-to-station variation in macrobenthic abundance, data were analyzed with ANOVA for each of the sampling dates (Table 3). Differences in mean abundances among stations are significant for each of the months tested (overall p < 0.05). The month of April was not included in the analysis because of inequality of sample size. The null hypothesis of no difference in total species abundance among station locations was rejected. Comparison of means (Table 3) reveals that the upriver station had abundances that were significantly lower (p < 0.05) than those of all other stations in May and July. In June, the upriver and C stations were significantly different from all others. Station F had
Fig. 7. Changes of mean abundance (total number ± 1 Standard Error per core) (solid line) and total species number (broken line) of macrobenthos at five stations of the Rappahannock River from April to October 1987.
Table 3. One-way ANOVA results for station location effects upon macrobenthic species abundances during May to October 1987. * 0.05>p>0.01, ** 0.01>p>0.001, *** p<0.001. Underlined stations are not statistically different in Student-Newman-Keuls aposteriori comparisons (p> 0.05). Station designation as in Fig. 1.

<table>
<thead>
<tr>
<th>Month</th>
<th>Station mean</th>
<th>F</th>
<th>df</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>A 2.03</td>
<td>C 2.02</td>
<td>E 1.93</td>
<td>F 2.21</td>
</tr>
<tr>
<td>June</td>
<td>A 1.73</td>
<td>E 1.68</td>
<td>F 1.61</td>
<td>C 0.49</td>
</tr>
<tr>
<td>July</td>
<td>C 0.75</td>
<td>A 0.88</td>
<td>E 1.01</td>
<td>F 1.48</td>
</tr>
<tr>
<td>August</td>
<td>C 0.59</td>
<td>A 0.61</td>
<td>B 0.06</td>
<td>E 0.06</td>
</tr>
<tr>
<td>September</td>
<td>C 0.38</td>
<td>B 0.16</td>
<td>A 0.62</td>
<td>E 1.07</td>
</tr>
<tr>
<td>October</td>
<td>A 1.62</td>
<td>C 1.67</td>
<td>E 1.15</td>
<td>B 1.29</td>
</tr>
</tbody>
</table>
significantly higher abundances than the upriver and C stations in July and September, and than all other stations in August. In October, the station at the mouth and its closest station C differed from the upriver and E stations in having higher mean abundances.

Species richness was highest at the mouth in April and October (Table 4). Richness was highest at the shallow station F, May through September, and lowest at the upriver station. All other stations had low richness during summer. Evenness (Table 4) was low in May and June, higher in April and highest July through October at all stations. Low evenness values at the mouth, C and E stations reflect the numerical dominance of the annelid polychaetes *Streblospio benedicti* and *Mediomastus ambiseta* during spring. High evenness values during summer reflect a decrease of species and their abundances as hypoxia developed, with no strong dominance of any particular species.

Most species exhibited a trend of abundance decrease from May to July at deep stations, often with local extinction in August and September. Detailed trends of abundance of five of the most abundant species of annelid polychaetes are presented below. The species examined were the spionids *Streblospio benedicti* and *Paraprionospio pinnata*, the capitellid *Mediomastus ambiseta*, the hesionid *Podarkeopsis levifuscina* and the terebellid *Loimia medusa*.

*Streblospio benedicti* exhibited an abundance peak in May, with decrease in June and local extinction in July (Fig. 8). Abundance was highest at the mouth and station F, and decreased along the deep transect upriver. Likewise, *Mediomastus ambiseta* had high abundance in May, followed by a decrease in June and local extinction in July or August (Fig. 9). Both species recruited in
Table 4. Species richness (SR) and evenness ($J'$) of samples of macrobenthos collected at stations in the lower Rappahannock River, April through October 1987. Station designation as in Fig. 1.

<table>
<thead>
<tr>
<th>Station</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>SR</td>
<td>J'</td>
<td>SR</td>
<td>J'</td>
<td>SR</td>
<td>J'</td>
<td>SR</td>
</tr>
<tr>
<td></td>
<td>4.89</td>
<td>0.53</td>
<td>1.25</td>
<td>0.84</td>
<td>2.23</td>
<td>0.70</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>3.45</td>
<td>0.40</td>
<td>2.02</td>
<td>0.39</td>
<td>2.53</td>
<td>0.40</td>
<td>4.03</td>
</tr>
<tr>
<td></td>
<td>2.08</td>
<td>0.42</td>
<td>1.80</td>
<td>0.70</td>
<td>1.22</td>
<td>0.36</td>
<td>3.16</td>
</tr>
<tr>
<td></td>
<td>2.64</td>
<td>0.78</td>
<td>3.09</td>
<td>0.88</td>
<td>2.25</td>
<td>0.66</td>
<td>6.43</td>
</tr>
<tr>
<td></td>
<td>1.57</td>
<td>0.77</td>
<td>2.23</td>
<td>0.84</td>
<td>nc</td>
<td>3.97</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>2.44</td>
<td>0.75</td>
<td>2.49</td>
<td>0.90</td>
<td>2.73</td>
<td>0.85</td>
<td>5.78</td>
</tr>
<tr>
<td></td>
<td>5.43</td>
<td>0.83</td>
<td>4.28</td>
<td>0.80</td>
<td>2.50</td>
<td>0.75</td>
<td>nd</td>
</tr>
</tbody>
</table>

nd - no data.
nc - not calculated, near zero abundance.
Fig. 8. Change in mean abundance (number ± 1 Standard Error per core) of *Streblospio benedicti* at five stations of the Rappahannock River, April through October 1987.
Fig. 9. Change in mean abundance (number ± 1 Standard Error per core) of
*Mediomastus ambiseta* at five stations of the Rappahannock River, April
through October 1987.
low numbers in October, but were collected only in the 250-um sieve fraction.

Population dynamics of *Paraprionospio pinnata* were different. This polychaete had low abundance in the lower Rappahannock River, but was collected continuously from April to October at the mouth and F stations (Fig. 10). Its abundance declined from May to July at the mouth of the river, but increased through the next three months. This recovery started earlier at station F, and reached a maximum in August. At other stations, *P. pinnata* abundances were low during summer, with local extinction in July and August upriver, and recovery in October. The species exhibited highest abundance at station F.

Several species of the family Hesionidae had a recruitment peak in July, and, except for a few individuals collected at the mouth, they were absent from previous months. These are small, motile, subsurface polychaetes comprising at least four species, all found in the lower Rappahannock River. Juveniles of *Podarkeopsis levifuscina* (formerly *Gyptis brevipalpa*) were sampled July through October. They exhibited a peak of abundance in July, a decrease in August and a small increase in September and October (Fig. 11). *P. levifuscina* had highest abundance at station F in August, and lowest abundance upriver, where only a few individuals were collected in October.

*Loimia medusa* showed various patterns of abundance (Fig. 12). It was present during spring (April-May) and summer (July-August) in samples collected at the mouth, was absent in June and September, and increased in abundance in October. No individuals were found in June at station C, but were collected during the rest of summer and fall. At station F, this polychaete was collected throughout the sampling period, showing highest abundance in August. *L. medusa* was absent during summer upriver and at
Fig. 10. Change in mean abundance (number ± 1 Standard Error per core) of *Paraprionospio pinnata* at five stations of the Rappahannock River, April through October 1987.
Fig. 11. Change in mean abundance (number ± 1 Standard Error per core) of *Podarkeopsis levifuscina* at five stations of the Rappahannock River, April through October 1987.
Fig. 12. Change in mean abundance (number ± 1 Standard Error per core) of *Loimia medusa* at five stations of the Rappahannock River, April through October 1987.
station E, but present in samples collected at these two stations in September and October.

In summary, the general trend of species abundances is one of decrease in June, after the onset of hypoxia in the river. This decline occurred at all stations, but was largest upriver, where presumably few or no organisms survived DO concentrations below 1 mg/l (<13% air saturation). A second and more intense hypoxic event on July 8 is probably related to the population crash of some species such as *Mediomastus ambiseta* and *Streblospio benedicti*. On the other hand, two species, *Paraprionospio pinnata* and *Loimia medusa*, maintained or increased their abundance near the mouth of the river during subsequent hypoxic events (Figs. 10 and 12). Likewise, these species maintained their abundances at station F, which may be related to the occurrence of higher bottom DO concentrations in this shallow area than in deeper ones.

Examination of the fraction of organisms retained on a 250-μm sieve revealed recruitment of a few polychaete species during summer, and a proportionally larger increase of juvenile molluscs, turbellarians and nemerteans in fall (Fig. 13). The amphinomid polychaete *Pseudeurythoe paucibranchiata* exhibited a recruitment peak in July primarily at the mouth of the river, and hesionid polychaetes recruited in July and August at the mouth and C stations. No macrobenthic organisms were collected in the 250-μm sieve fraction at the upriver station June through September.

**Vertical Distribution in Sediment**

For all locations and months, most organisms (75.2%) occurred in the upper 2 cm of sediment, a smaller fraction (16.7%) occurred from 2 to 5 cm,
Fig. 13. Total number of individuals of macrobenthic species retained on a 250-μm sieve at four stations of the Rappahannock River, June through October 1987. ND= no data.
and few organisms (8.1%) were found deeper than 5 cm. Temporal patterns of variation in vertical distribution for the mouth, C and F stations are presented below. For statistical analyses, data of summer months from the mouth and C stations were combined into one season because of the occurrence of some zero values.

Species at the mouth exhibited changes in abundance with depth in the sediment that were related to the overall increase or decrease of total abundance with time (Fig. 14). A larger proportion of organisms occurred in the upper 2 cm of sediment during spring recruitment than in previous or following months (Table 5). The proportion of organisms below 5 cm was higher in early spring (5.2%) and fall (15.1%) than in summer (3.9%). In fall, a large shift of distribution took place, with proportionally more organisms occurring in deeper layers than in the upper 2 cm of sediment. These changes in the vertical distribution of macrobenthos were statistically significant (p <0.001). Therefore, the null hypothesis of independence between depth of burrowing and sampling date was rejected.

Species at station C also exhibited changes in abundance with depth in sediment related to the overall temporal change of abundance (Fig. 14). The change in the proportion of organisms between sediment layers was not significantly different from spring to summer (p >0.05) (Table 5). In fall, however, there was a significant increase in the proportion of organisms occurring from 2 to 5 cm of sediment and deeper than 5 cm (p <0.001).

Species at station F exhibited higher abundance below the top 2 cm of sediment than at other stations, reflecting an overall increase of abundance (Fig. 14). The proportion of organisms occurring deeper than 5 cm decreased from May (8.9%) to June (5.7%), and increased in July (9.1%) (Table 5). This
Fig. 14. Mean abundance (number per core) of macro-infauna in three layers of sediment at three stations of the Rappahannock River, April through October 1987. ☐ 0-2 cm., ☑ 2-5 cm, □ >5 cm.
Table 5. Temporal changes in total abundance of macrobenthos with depth in sediment from April to October 1987. The percent contribution of each sediment layer to the abundance of all three layers combined is indicated in parenthesis. Temporal changes in the distribution of organisms between layers indicated by G-tests. * 0.05>p>0.01, *** p<0.001, ns =not significant.

<table>
<thead>
<tr>
<th>Station</th>
<th>Month</th>
<th>0-2</th>
<th>2-5</th>
<th>&gt;5</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouth</td>
<td>April</td>
<td>365 (78.6%)</td>
<td>75 (16.2%)</td>
<td>24 (5.2%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>688 (87.3%)</td>
<td>69 (8.8%)</td>
<td>31 (3.9%)</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>236 (73.8%)</td>
<td>70 (21.8%)</td>
<td>14 (4.4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July-Sep</td>
<td>110 (85.3%)</td>
<td>14 (10.8%)</td>
<td>5 (3.9%)</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>158 (62.9%)</td>
<td>55 (21.9%)</td>
<td>38 (15.1%)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>April-May</td>
<td>583 (91.8%)</td>
<td>39 (6.1%)</td>
<td>13 (2.1%)</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>June-Sep</td>
<td>94 (95.0%)</td>
<td>3 (3.0%)</td>
<td>2 (2.0%)</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>161 (58.7%)</td>
<td>72 (26.3%)</td>
<td>41 (15.0%)</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>May</td>
<td>686 (66.1%)</td>
<td>260 (25.0%)</td>
<td>92 (8.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>211 (70.8%)</td>
<td>70 (23.5%)</td>
<td>17 (5.7%)</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>132 (66.7%)</td>
<td>48 (24.2%)</td>
<td>18 (9.1%)</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>110 (55.3%)</td>
<td>47 (23.6%)</td>
<td>42 (21.1%)</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>85 (47.2%)</td>
<td>35 (19.5%)</td>
<td>60 (33.3%)</td>
<td></td>
</tr>
</tbody>
</table>
change, matched by an increase of organisms occurring in the upper 2 cm of sediment in June, was not significant (p >0.05). In August and September, a significant shift in the proportion of organisms occurred from shallow to deeper layers of sediment (p <0.001).

Vertical distributions of the most abundant species were examined in detail. Two dominant species, the polychaetes *Mediomastus ambiseta* and *Streblospio benedicti* occurred primarily in the upper 2 cm of sediment. The former species is a burrowing sub-surface deposit-feeder, and the latter is a tube builder which feeds at the sediment-water interface. No trend of temporal change in their vertical distributions was observed, mainly because of the local extinction of both species in early summer.

*Podarkeopsis levifuscina* occurred in the surface layer of sediment in July, during a peak of recruitment, but it was also found below 2 cm at station F in July and August (Fig. 15a). No clear trend of temporal change was observed. *Nereis succinea* occurred primarily in the upper 2 cm of sediment, although a trend of abundance increase below 5 cm was apparent in spring and fall at station E, and in spring and late summer at station F (Fig. 15b). This polychaete is a mobile carnivore and omnivore which lives in burrows generally occurring deeper than 5 cm in sediment. A similar species in trophic mode and mobility, the pilargid polychaete *Sigambra tentaculata*, occurred in all three layers of sediment, with no clear trend of temporal shift (Fig. 16a). At station F, however, a higher proportion of individuals occurred deeper than 5 cm, with increased abundance in the 2-5 cm sediment layer in June.

*Loimia medusa* exhibited low abundance in summer, but these individuals were found in the upper 2 cm of sediment (Fig. 16b). In spring and fall, this
Fig. 15. Changes of abundance (mean number per core) in the vertical distribution of *Podarkeopsis levifuscina* (A) at three stations, and of *Nereis succinea* (B) at four stations of the Rappahannock River, April through October 1987. □□ 0-2 cm, □□□ 2-5 cm, □□□□ □□□ □□□□ □□□□ >5 cm.
Fig. 16. Changes of abundance (mean number per core) in the vertical distribution of Sigambra tentaculata (A) and Loimia medusa (B) at four stations of the Rappahannock River, April through October 1987.

- 0-2 cm, ■■ 2-5 cm, □□ >5 cm.
surface deposit-feeding polychaete, was found primarily below 2 cm, with an increased proportion of individuals occurring deeper than 5 cm. Other species examined (i.e., the polychaetes *Eteone heteropoda*, *Glycine solitaria* and *Paraprionospio pinnata*) occurred primarily in the surface layer of sediment, with no trends of temporal change.
DISCUSSION

Hypoxia was observed intermittently in the lower 20 km of the Rappahannock River during summer of 1987. Although anoxia was not recorded in this area, average DO concentrations of 2.1 mg/l (about 30% air saturation) prevailed at the upper-most station (Fig. 2). Hypoxia has been observed repeatedly in deep waters of the Rappahannock River (Kuo and Neilson 1987), and anoxia or near anoxic conditions have been reported further upriver (Brooks 1983). During 1987 anoxia occurred in bottom waters covering extensive areas 20 to 50 km upriver in June, July and August (Kuo, unpublished data). In June, about 30% of the water column from the mouth to 60 km upriver had DO concentrations below 30% air saturation. Anoxia occurred again 20 to 39 km upriver in July and August 1989 (Kuo, unpublished data). These conditions undoubtedly exert an intense stress on deep-water macrobenthic populations, as well as on demersal fish and crustacean populations. In the lower Rappahannock, defaunation occurred 20 km upriver in summer 1987, and a dramatic decrease of macrobenthic abundances took place at the mouth of the river (Fig. 7). Although the actual cause of the defaunation is not known, hypoxia is presumed to be the main factor. The effect of hypoxia was most evident upriver, where intense hypoxic events were significantly related to low macrobenthic abundance. At the mouth of the river and at a shallow station, a decrease in the severity of hypoxia resulted in higher abundances.

In addition to hypoxia, other factors may have contributed to macrobenthic mortality. Among these, predation and food limitation have been cited as potential causes for macrobenthic reductions during summer in the
Chesapeake Bay (Holland et al. 1987). The importance of predation by bottom-feeding fish and crabs on controlling the abundance and distribution of infaunal benthos in the Chesapeake Bay has been manifested through predator-exclusion experiments (Virstein 1977; Holland et al. 1980). These type of experiments show a marked increase in the abundances of several common infaunal species inside cages that exclude large predators. In the mesohaline region of the upper Chesapeake Bay, species living near the sediment surface exhibited the largest increase in the absence of predators (Holland et al. 1980). Shallow-dwelling species, such as _Streblospio benedicti_ and _Mediomastus ambiseta_, may have been adversely affected by predation during spring in the lower Rappahannock River. This predation effect, however, is probably manifested only through a reduction in the amplitude of the recruitment peak (Holland et al. 1987). Large numbers of bottom-feeding fish were present in spring and summer 1987 in the lower Rappahannock River (Bonzek, unpublished data). Therefore, predation on macrobenthos would have been expected to occur with relatively similar intensity at same water depths throughout the river. Thus, predation alone does not explain the observed differences in macrobenthic abundances between stations. Moreover, fish such as the spot, _Leiostomus xanthurus_, and the blue crab, _Callinectes sapidus_, were absent from trawls in June, at the time of major benthic faunal reductions which coincided with the development of hypoxia in the river (Bonzek, unpublished data).

Mortality of dominant species following the spring recruitment peak could be partially attributed to food limitation. Spring planktonic blooms provide increased suspended food for invertebrate larvae. In addition, they are related to an increase in the load of organic matter reaching the bottom.
This supply of food is readily available to the benthos, explaining the rapid growth, reproduction and recruitment of benthic populations during spring and summer. Tenore and Chesney (1985) and Chesney and Tenore (1985) suggest that opportunistic species respond to increased food supply by increasing the rate of population growth, leading to a rapid change in population density. This explosive increase of population abundance may exceed the carrying capacity of the environment. As a consequence, food becomes limited and a precipitous population decline follows.

*Streblospio benedicti*, one of the dominant species in the lower Rappahannock River, responds to enhanced food supply by accelerating growth and increasing reproductive output (Levin 1986). In addition to food supply, spring and fall temperature regimes further stimulate growth and reproduction in this polychaete (Levin and Creed 1986). Therefore, the large recruitment peak of *S. benedicti* observed in May 1987 was probably related to warming water temperatures and organic enrichment. The rapid population decline can be partially explained by subsequent food limitation; however, mean densities of *S. benedicti* in deep (11,000 individuals/m²) and shallow (15,000 individuals/m²) areas of the lower Rappahannock River were much lower than densities attained in other estuaries (Diaz 1984; Levin 1984) or in field manipulations where there was organic enrichment (346,359 individuals/m², Levin 1986). Food availability, therefore, may not have been of critical importance for the demise of *S. benedicti* populations in the lower Rappahannock River.

Although laboratory experiments have demonstrated that *Streblospio benedicti* is tolerant of low DO concentrations (Chapter 2), the stress of hypoxia is increased by the diffusion of hydrogen sulfide from the sediments
in natural situations (Jørgensen 1980). The synergistic effects of hypoxia, high temperature and elevated concentrations of hydrogen sulfide may have been related to the extensive mortalities affecting populations of opportunist polychaetes in the lower Rappahannock River (but see Chapter 4). Population oscillations of *S. benedicti* have been reported from other estuaries and embayments (Boesch 1973; Watling 1975; Whitlatch 1977; Dauer et al. 1979; Santos and Simon 1980b; Diaz 1984; Levin 1984). Patterns of population increase are largely reflections of the species seasonal spawning cycle, but summer abundance declines have been attributed to unknown causes (Boesch 1973), temperature stress (Watling 1975) and low oxygen stress (Santos and Simon 1980b).

The lower Rappahannock River can be regarded as an area where physical rather than biological factors have an overriding influence on structuring community. Summer hypoxia, in combination with high temperature and salinity fluctuations, seems to control macrobenthic population dynamics. Abundance and species number were low during summer of 1987, decreasing with increasing water depth and distance from the mouth of the river. These parameters were also low for the entire year when compared to those of benthic populations in the York River, a similar mesohaline subestuary of the lower Chesapeake Bay (Dauer et al. 1989). Biomass at one station of the lower Rappahannock River was 2.4 g/m² ash free dry wt. in 1987 (Dauer et al. 1989), and the overall biomass for the period 1985-1988 was low (2.3 g/m²) when compared to the York River (5.4 g/m²). Hypoxia occurs in the York River, but is more attenuated than in the Rappahannock River (Kuo and Neilson 1987); its effects on macrobenthic populations in 1987 (Neubauer and Luckenbach, pers. comm.) were not as devastating as in the Rappahannock River. Macrobenthic productivity in
the York River exhibits patterns that seem to be unrelated to low DO stress
(Diaz et al., in press).

Species composition in the lower Rappahannock River seemed to be
influenced by seasonal hypoxia. Polychaetes were the dominant taxa, with few
crustaceans and molluscs collected. This is in general agreement with the
findings of other benthic studies, suggesting that polychaetes are more
tolerant of hypoxia than other taxa such as crustaceans, gastropods and
Bivalves appear to be variously affected (Theede et al. 1969). Dominance of
opportunistic species, such as the polychaetes *Streblospio benedicti*,
*Mediomastus ambiseta* and *Paraprionospio pinnata*, also occurs in other oxygen-
stressed environments (Mountford et al. 1977; Dauer et al. 1979; Gaston 1985).
With an increase in the frequency of hypoxic and anoxic events, benthic
populations become transitory, dominated by few short-lived species, and their
overall productivity is decreased (Mountford et al. 1977; Rainer and

The magnitude of the apparent responses of annelids to hypoxic events in
the lower Rappahannock River varied with species. Like other species,
*Paraprionospio pinnata* and *Loimia medusa* had reduced abundance during low DO
events. In contrast to the large mortalities of *Streblospio benedicti* and
*Mediomastus ambiseta*, however, *P. pinnata* and *L. medusa* were present during
most of summer and fall. By maintaining their presence near the mouth of the
river and at the shallow station, *P. pinnata* and *L. medusa* appear to survive
moderate hypoxia. Laboratory experiments have indicated that *L. medusa*
survives short periods of hypoxia and anoxia (Chapter 3). Observations of *P.*
pinnata population responses to hypoxia vary. While Gaston (1985) reported
increasing abundances from July to September 1981 in the Gulf of Mexico as DO concentrations declined below 1 mg/l, Harper et al. (1981) showed a large decrease of *P. pinnata* populations in a nearby area during hypoxia in June and July 1979. The specific low DO tolerance of this species is unknown.

Species of the polychaete family Hesionidae, mainly *Podarkeopsis levifuscina*, exhibited a peak of recruitment in mid-summer. Juvenile abundances declined in August with the development of an hypoxic event, suggesting that the species is not particularly resistant to low DO. Larval availability and settlement probably occurred in mid-July, during a period of water column destratification that brought oxygenated waters into the area. This provides an indication that the timing of low DO events can be of critical importance to species colonization and survivorship.

After disturbance, first colonists are generally species with dispersal abilities (Simon and Dauer 1977). For example, the success of *Streblospio benedicti* in recolonizing marine soft-bottom environments after disturbance has been attributed to its opportunistic life history (Santos and Simon 1980b). High fecundity, short generation times (Levin et al. 1987) and rapid dispersal ability (McCall 1977; Levin 1984) of planktotrophic populations are responsible for its success. In the lower Rappahannock River, a recolonization pulse of *S. benedicti* was indicated by the presence of juveniles in the 250-um sediment fraction of fall samples. Populations of *S. benedicti* may have recolonized deep-water areas from shallow-water colonizing stocks. In fall, *S. benedicti* does not typically attain high abundances, and maintains low over-wintering stocks (Santos and Simon 1980a; Levin 1984).

*Mediomastus ambiseta* also exhibited a small pulse of larval recolonization in fall. The biology of this infaunal polychaete is not well known, but brooding
has been observed in a sibling species (Santos and Simon 1980a). The benefits from brooding are rapid population increase and larval survivorship (Levin et al. 1987), a successful strategy in environments subjected to periodic disturbance. Given that the present study ended in October, temporal patterns of recovery after summer hypoxia are largely unknown.

During hypoxia, species behavior is often modified, and may result in a shift in the burrowing depth of infaunal organisms. Some species come to the sediment surface to avoid low oxygen and increasing levels of hydrogen sulfide in sediment (Swanson and Sindermann 1979; Jørgensen 1980; Stachowitsch 1984). In the lower Rappahannock River, patterns of temporal change in the vertical distribution of macrobenthos showed a trend of decrease in abundance below 2 cm of sediment during summer. The overall small proportion of organisms occurring below 5 cm of sediment and the insufficient frequency in the measurements of vertical distributions during DO fluctuations, make the interpretation of these trends difficult. It is tempting to relate the paucity of organisms at depth in sediment with the development of hypoxia in summer; however, other interpretations are possible. The predominance of small organisms in the upper 2 cm of sediment reflects pulses of recruitment in spring and fall. The two dominant species of spring and early summer live typically near the sediment surface. Large mortalities coinciding with the development of hypoxia in June prevented the observation of patterns of temporal shift in several species. One exception is perhaps *Loimia medusa*. The occurrence of this species in shallow layers of sediment during summer but in deeper ones during spring and fall, may be a response to low DO concentrations in bottom waters. During experimental hypoxia (Chapter 3), *L. medusa* was often observed protruding from its tube above the sediment surface.
A significant increase of organisms occurring deeper than 2 cm of sediment in fall may be indicative of high DO concentrations, but may also reflect the recruitment of species of different burrowing behavior as a result of a shift in sediment composition. The alteration of the substrate with the advection of sandy sediment into the area, may have been favorable for the establishment of species that were absent from previous months, such as some gastropods and bivalves. Thus, high species richness in fall samples could be partially attributed to a shift in sediment composition in addition to relief from hypoxia. In the upriver station, however, sediments remained unchanged; therefore, the increase in species richness in that station seems to be directly related to oxygenation.

The consequences of low DO stress upon the dynamics of benthic populations have been discussed. Additionally, the effects of hypoxia are also manifested at higher trophic levels, providing an indication of the overall health of the system. The lower Rappahannock River provides habitat for numerous bottom-feeding fish and crustaceans. During periods of hypoxia, few fish and no crabs were collected from monthly trawls (Bonzek, unpublished data). Whether there were fish mortalities or fish were able to avoid the affected area, is unknown. Most fishes are generally able to avoid hypoxia by migrating to shallow areas (Loesch 1960; May 1973; Garlo et al. 1979; Levings 1980a; Arntz 1981). This may have been the case in the lower Rappahannock River, since high abundances of demersal fish were observed as soon as oxygen conditions improved in deep water (Bonzek, unpublished data). Pihl et al. (in preparation) have documented migration of demersal fish to shallow areas in the York River during hypoxia, and a rapid return to deep-water as DO concentrations increase. The return of fish and crabs to deep-water habitats
after hypoxia is probably affected by the loss of food resources from the benthos. If hypoxia intensifies, the seasonal reduction in abundance of benthic organisms may show an additive effect, resulting in a long-term impoverishment of benthic resources and a concomitant local decline of demersal fish stocks. Further, a long-term change in benthic habitat and water quality could lower the value of estuaries as nursery areas for bottom-feeding fish.
CHAPTER 2.

TOLERANCE OF LOW DISSOLVED OXYGEN BY THE POLYCHAETE

STREBLOSPIO BENEDICTI (WEBSTER)
ABSTRACT

The spionid polychaete *Streblospio benedicti* (Webster) is common in surface sediments of estuarine intertidal and subtidal habitats. Populations of *S. benedicti* may be exposed to hypoxia or anoxia during the summer. Laboratory experiments were designed to document the behavior and determine the tolerances of adults and larvae in 14.5% and 7% air-saturated seawater, and anoxic seawater. Exposures to 14.5% air saturation for at least 14 days had no significant effect on adult survivorship. Larvae tolerated similar conditions for at least 4 days. Mortality of less than 50% of the adults occurred in 7% air saturation. Survival under hypoxia was partially achieved by reducing activity. Under anoxic conditions, all worms died within 55 h. These results suggest that field populations of *S. benedicti* may survive intermittent periods of hypoxia, but that the intensity and duration of low oxygen events are critical to survivorship and larval recolonization.
INTRODUCTION

Estuarine invertebrates vary in their tolerance of hypoxia depending on factors such as temperature, salinity, activity and metabolism, but they are not likely to survive under persistent anoxia or prolonged exposures to hypoxia in combination with high summer temperatures (e.g., Santos and Simon 1980b). Under such conditions complete defaunation may occur, resulting in a reduction of food resources for higher trophic levels (Rosenberg 1980, Rainer and Fitzhardinge 1981). Estuaries with periodic deoxygenation often show a decrease in bottom oxygen concentration in early summer, with oxygen levels remaining below 2 mg/l throughout most of the summer, and occasionally reaching anoxia. As a consequence, macrobenthic species abundances decline, defaunation may follow, and assemblages do not recover until the fall (e.g., Holland et al. 1977, 1987).

The recovery of macrobenthic assemblages from hypoxia should be largely structured by the relationship between the timing of the return to normoxic conditions and species life histories. Opportunistic species often colonize benthic habitats rapidly after defaunation as a result of physical disturbance or pollution abatement (McCall 1977, Pearson and Rosenberg 1978, Santos and Simon 1980). Therefore, euryhaline opportunists that have competent larvae in the vicinity are expected to colonize the defaunated habitats when oxygen levels rise. Likewise, mobile species in surrounding normoxic areas may migrate into the affected area when oxygen conditions improve. In this scheme, faunal colonization depends on a combination of water exchange, proximity of reproducing organisms and larval availability. An important, but
often overlooked factor is the oxygen tolerance of larvae, new recruits and adults, which may determine patterns of reestablishment of the community. For instance, survival of larvae under moderate hypoxia may partially determine the recolonization success of the species in habitats affected by hypoxia.

The spionid polychaete *Streblospio benedicti* (Webster) is a common surface deposit feeder in muddy intertidal and subtidal estuarine habitats (Light 1978). Populations of this species are likely to encounter summer hypoxia because of their wide distribution in estuaries. The species can promptly colonize an area following disturbance, maintaining high population abundance (Levin 1984). High concentrations of this polychaete were present in bottom samples collected at water depths of 13 to 19 m during spring 1987 in the lower Rappahannock River (Chesapeake Bay, Virginia, USA) during a study designed to document macrobenthic species population responses to hypoxia (see Chapter 1). The Rappahannock River subestuary experiences severe hypoxia every summer (Kuo and Neilson 1987). The presence and dominance of *S. benedicti* in some estuaries with periodic deoxygenation may be a consequence of its life history traits (i.e., small adult size, brood protection, rapid development, short generation time) or a combination of these with behavioral and physiological adaptations to seasonal hypoxia or anoxia. Since its specific tolerance of low dissolved oxygen is unknown, laboratory experiments using *S. benedicti* were conducted to (1) determine hypoxia and anoxia tolerance of adults, (2) hypoxia tolerance of larvae, and (3) document behavioral responses of larvae and adults to reduced dissolved oxygen concentrations.
METHODS

Test of adults in low dissolved oxygen

Adult *Streblospio benedicti* were obtained from laboratory cultures started in November 1987. Worms were of similar size, although age and sex were not determined. The contents of several culture dishes were sieved through a 250-μm mesh and tubes with worms were obtained. Worms were forced out of their tubes by gently probing the lower end of the tube, and transferred to experimental dishes.

Ten small dishes (5.5 cm diameter) each containing 6 ml of defaunated sediment were set up in each of six 19-l aquaria. The dishes were placed near the top of the aquaria to facilitate observation. Each dish received one worm, and all dishes were covered with a 125-μm mesh screen to prevent worms from swimming away. Aquaria were filled with 1-μm filtered seawater and tightly covered with a glass top perforated in two of the corners to allow a water-heater cable, tubing and oxygen probe to pass through. Worms were held in the aquaria with aeration at 26° C for 7 days prior to the initiation of the experiment. During this acclimation period, fecal pellets were removed from the dishes with a pipette and the worms were fed with a slurry of fine sediment and filtered seawater every other day. Unhealthy worms (i.e., non-burrowers and non-feeders) were replaced by additional worms kept in similar aquaria.

Worms were randomly assigned to the six aquaria before the initiation of the experiment. Two aquaria were randomly selected for each of the following three oxygen treatments: 100% (controls), 14.5%, and 7.0% air saturation (Table 1). Temperature and salinity (Table 1) were maintained within the
Table 1. Treatment conditions during a test of adults and larvae of *Streblospio benedicti* in low dissolved oxygen.

<table>
<thead>
<tr>
<th>% Air Saturation</th>
<th>mg/l</th>
<th>PO$_2$(Torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.0</td>
<td>7.2</td>
<td>153.9</td>
</tr>
<tr>
<td>14.5</td>
<td>1.0</td>
<td>22.3</td>
</tr>
<tr>
<td>7.0</td>
<td>0.5</td>
<td>10.8</td>
</tr>
</tbody>
</table>

T = 26±0.5° C
S = 19-21%
range usually encountered by the worms during summer hypoxia. Dissolved oxygen concentrations were maintained by continuously bubbling air (20.9% O₂) or a gas mixture of either 3% or 1.5% O₂ and the balance nitrogen. Gas mixtures were provided in gas cylinders, and the rate of gas bubbled was adjusted using flow meters. Oxygen was monitored daily with a microelectrode (Microelectrodes, Inc.), calibrated in moist air at the temperature of measurement. Calibration was checked daily.

The experiment was initiated on September 15, 1989, and lasted for 330 h (13.7 days). Worms were fed and observed throughout the length of the experiment with the unaided eye or by using a dissecting microscope mounted on an extension arm. A preliminary experiment was conducted in order to establish the appropriate experimental procedures.

Test of larvae in low dissolved oxygen

Larvae of *Streblospio benedicti* were obtained from laboratory cultures and were less than 24 h old at the initiation of the experiment. Larvae are planktotrophic for 7-10 days and are capable of growing in filtered seawater without the addition of algae (pers. obs.). They reached a size of 125-200 μm with 3-4 setigers one day after release.

Larvae were kept in 25-ml test tubes with 1-μm filtered seawater of 20% salinity and dissolved oxygen reduced to either 14.5% or 7.0% air saturation. Penicillin G (10,000 units/l) and Streptomycin (12.5 mg/l) were added to the seawater in order to reduce bacterial growth. Oxygen concentrations were attained by bubbling a gas mixture of either 3% or 1.5% oxygen and the balance nitrogen in a 2.5-l beaker with filtered seawater for about 15 min. The water was then siphoned into the experimental tubes and larvae added with a pipette.
Tubes were closed with screw caps and teflon tape, leaving no gas space, sealed with wax, and immersed in an aquarium maintained at constant temperature of 26° C. Controls consisted of tubes with filtered seawater and antibiotic at 100% air saturation. Oxygen measurements were taken using a calibrated microelectrode at the beginning and at the end of the experiment.

Two trials were conducted using 10 and 6 replicate tubes per treatment, each containing 5 (first trial) or 10-12 larvae (second trial). Few larvae were used in order to determine individual larval behavior and survival. Tubes were kept in the dark except when observed for 1-2 min under a dissecting microscope. Larvae were observed hourly, and identified as swimming or dead. In the latter case, dead larvae were counted by looking at the bottom surface of the tube. Time to mortality was recorded.

Test of adults in anoxic water

Adult *Streblospio benedicti* of uniform size were obtained from laboratory cultures, following the procedure described for the hypoxia experiment. Worms were assigned to jars (4.5 cm diameter x 4.5 cm high) and held in aquaria with aeration at 26°C for 7 days prior to the experiment. During this period, worms were observed and fed as described above.

Ten jars, each containing 6 ml of defaunated sediment, were set up in each of four 19-1 aquaria. Each jar received one worm. Two aquaria were randomly selected for a treatment consisting of 1-um filtered seawater deoxygenated by bubbling nitrogen for 2.5 h. Jars were sealed with a plastic screw cap and teflon tape as soon as deoxygenation was reached. The aquaria then functioned as water baths. Temperature was maintained at 26±0.5° C, and salinity at 18%. Seawater in control aquaria was withdrawn to the upper rim
level of the jars, leaving the jars open to atmospheric air. The centers of the jar caps were cut out and replaced by round plexiglass sheets firmly glued to the rim of the cap. The sediment surface could then be easily seen by placing each jar under a dissecting microscope. Each worm was observed for 1-3 min every hour throughout the length of the experiment. Worms were determined dead when no response was observed after tapping the side of the jar. Control jars were handled in a similar manner. Time to mortality was recorded, the jar opened and the oxygen concentration measured.

All oxygen measurements were made using a microelectrode calibrated in moist air at the temperature of measurement. A preliminary experiment was conducted in order to set the appropriate experimental procedures.
RESULTS

Tolerance of hypoxia by adults

Dissolved oxygen concentrations generally fluctuated between 5.3 (0.4 mg/l) and 7% air saturation (0.5 mg/l) in the 7% air saturation treatment, and between 11.5 (0.8 mg/l) and 14.5% air saturation (1.0 mg/l) in the 14.5% air saturation treatment. Measured concentrations increased only on one occasion to 10.6% air saturation (0.8 mg/l) in the 7% air saturation treatment.

Worms were active during the acclimation period, burrowing throughout the dish and feeding at multiple tube openings. Most of the worms in the low oxygen treatments were observed at the opening of their tubes, without feeding, at the beginning of the experiment. They remained inactive except for occasional undulatory body movements. Seven worms out of forty were observed half way out of their tubes, or lying on the sediment surface, obviously stressed. Five of these worms died within the first 66 h in low dissolved oxygen (7% air saturation treatment). After 3.5 days, most of the worms in the 14.5% air saturation aquaria resumed feeding. Feeding was observed in the 7% air saturation aquaria after 4.5 days. By the end of the experiment, 86% of all the dishes with live worms contained fresh fecal pellets, an indication of feeding activity.

There was no significant difference in survival among the three oxygen treatments (G-test of independence, G = 4.71, p >0.05). Seventeen out of 19 worms were recovered live in the 14.5% air saturation treatment at the conclusion of the experiment (Fig. 1). Fourteen out of 20 worms were recovered live in the 7% air saturation treatment. One death occurred in control aquaria. Mortality in the 7% air saturation treatment was observed in
Fig. 1. Survival of *Streblospio* adults after 14 days exposure to hypoxia and air-saturated seawater.
both replicate aquaria. No aquarium effects were detected after plotting the
data and testing for homogeneity of replicates within each treatment (G-test,
p >0.05).

Tolerance of hypoxia by larvae

Treatment oxygen concentrations were difficult to maintain, presumably
because of bacterial growth in the test water. Similar experiments with 0.2-
mum filtered seawater, autoclaved seawater, and no-larvae controls suggested
that bacterial oxygen consumption was the cause for the observed oxygen
decrease. The addition of antibiotic in the present two trials only delayed
the time at which seawater became anoxic.

Although the experiment could not be conducted beyond 92 h, it provided
valuable information. Larvae remained swimming and exhibited phototactic
behavior until dissolved oxygen concentrations decreased below 7% air
saturation. All larvae of two replicate tubes in the 7% air saturation
treatment survived this oxygen concentration for 48 h. No mortality was
observed in controls. All larvae of one replicate tube in the 14.5% air
saturation treatment survived for 92 h. The dissolved oxygen concentration at
the end of this 92 h period was 3% air saturation (0.2 mg/l).

Tolerance of anoxia by adults

Complete deoxygenation was not reached in all treatment jars, although
the oxygen concentrations (≤2% air saturation) were within the limits of
instrumental error. Two jars had slightly higher oxygen concentrations (3% air saturation, 0.2 mg/l). Control jars had oxygen concentrations in the
range 79-88% air saturation (5.7-6.4 mg/l).
Worms remained inactive at the opening of their tubes, with branchiae and palps extended. Some worms stretched half way out into the water column, displaying sporadic ventilatory movements. With time, they vacated their tubes and laid on the sediment surface.

The first death occurred 24 h after the initiation of the experiment (Fig. 2). Time to mortality of half of the experimental population ($LT_{50}$, obtained from Fig. 2) was 43 h. All control worms were alive at the end of the experiment (55 h). No significant difference in time to mortality was observed among replicate aquaria (Student's t-test, $p > 0.05$). Three worms showed some response after opening the jar. They may have been temporarily narcotized after prolonged exposure to anoxic seawater. Their jars were immediately immersed into deoxygenated seawater, lids reset, and the experiment continued.
Fig. 2. Number of *Streblospio* adults surviving as a function of time during exposure to anoxic seawater.
Number of worms surviving

![Graph showing the number of worms surviving over time.](image-url)
The polychaete *Streblospio benedicti* tolerated oxygen concentrations of 14.5% and 7% air saturation for 14 days under experimental laboratory conditions, although it showed little tolerance of anoxia. There was some evidence suggesting that larvae survive without behavioral ill effects in seawater of 14.5% air saturation for at least 4 days.

The anoxia tolerance of *Streblospio benedicti* contrasts with the higher tolerances of other polychaetes under experimental conditions (Table 2). The experimental population of *S. benedicti* reached 100% mortality in 2.3 days, and had a LT₅₀ value of 1.8 days at a temperature of 26°C. Given the temperature difference between the experiments of this study and those of previously published work, the tolerance of anoxia by *S. benedicti* should not be regarded as atypical among polychaetes. Lower temperatures probably increase survival time; however, some estuarine populations are exposed to high temperatures during summer hypoxia (Chapter 1).

Physiological and biochemical adaptations largely account for the observed differences in survival ability of polychaetes under hypoxia and anoxia (Warren 1984). For instance, most polychaetes are able to switch from aerobic to anaerobic metabolism at low oxygen tensions and during anoxia (Mangum and Van Winkle 1973). Anaerobiosis partially supplies the energy requirements of the animal through the activation of metabolic pathways in which glycolysis is linked to non-oxidative electron transfer systems (Newell 1970). The type of pathway seems to be related to the interspecific differences in anoxia tolerance of many polychaetes and other invertebrates (Schöttler 1979, Warren 1984).
Table 2. Tolerance of experimental anoxia by polychaetes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Survival time</th>
<th>Experimental temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arenicola marina</strong></td>
<td>6</td>
<td>15-18</td>
<td>Hecht 1932.</td>
</tr>
<tr>
<td></td>
<td>up to 5</td>
<td>12</td>
<td>Schöttler &amp; Grieshaber 1988.</td>
</tr>
<tr>
<td></td>
<td>3.8 to 16.6</td>
<td>10</td>
<td>Groenendaal 1980.</td>
</tr>
<tr>
<td><strong>Nereis diversicolor</strong></td>
<td>LT&lt;sub&gt;50&lt;/sub&gt; = 5</td>
<td>10</td>
<td>Theede et al. 1969.</td>
</tr>
<tr>
<td></td>
<td>LT&lt;sub&gt;50&lt;/sub&gt; = 8</td>
<td>4</td>
<td>Henriksson 1969.</td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>12</td>
<td>Schöttler &amp; Grieshaber 1988.</td>
</tr>
<tr>
<td><strong>Nereis virens</strong></td>
<td>&gt;5</td>
<td>12</td>
<td>Schöttler &amp; Grieshaber 1988.</td>
</tr>
<tr>
<td><strong>Scoloplos armiger</strong></td>
<td>LT&lt;sub&gt;50&lt;/sub&gt; = 5</td>
<td>4</td>
<td>Henriksson 1969.</td>
</tr>
<tr>
<td><strong>Capitella capitata</strong></td>
<td>LT&lt;sub&gt;100&lt;/sub&gt; = 7</td>
<td>4</td>
<td>Henriksson 1969.</td>
</tr>
<tr>
<td></td>
<td>LT&lt;sub&gt;50&lt;/sub&gt; = 7</td>
<td>15</td>
<td>Warren 1977.</td>
</tr>
<tr>
<td></td>
<td>LT&lt;sub&gt;100&lt;/sub&gt; = 30</td>
<td>15</td>
<td>Warren 1977.</td>
</tr>
<tr>
<td><strong>Streblospio benedicti</strong></td>
<td>LT&lt;sub&gt;50&lt;/sub&gt; = 1.8</td>
<td>26</td>
<td>This study.</td>
</tr>
<tr>
<td><strong>Ampharete grubei</strong></td>
<td>LT&lt;sub&gt;50&lt;/sub&gt; = 3</td>
<td>4</td>
<td>Henriksson 1969.</td>
</tr>
<tr>
<td><strong>Terebellides stroemi</strong></td>
<td>LT&lt;sub&gt;100&lt;/sub&gt; = 5</td>
<td>4</td>
<td>Henriksson 1969.</td>
</tr>
<tr>
<td><strong>Cirriformia tentaculata</strong></td>
<td>3</td>
<td>15</td>
<td>Dales &amp; Warren 1980.</td>
</tr>
<tr>
<td></td>
<td>LT&lt;sub&gt;50&lt;/sub&gt; = 10</td>
<td>12</td>
<td>Bestwick et al. 1989.</td>
</tr>
<tr>
<td><strong>Euzonus mucronatus</strong></td>
<td>18-20</td>
<td>22</td>
<td>Ruby &amp; Fox 1976.</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>(?)</td>
<td>Brand 1927.</td>
</tr>
</tbody>
</table>
The variation in the resistance of polychaetes to severe hypoxia or anoxia is sometimes reflected in the ecological differences between their habitats (Theede et al. 1973). This relationship, however, is not always clear (Warren 1984). Among the polychaetes cited in table 2, Capitella capitata, like S. benedicti, is an opportunist (Grassle and Grassle 1974), able to exploit disturbed environments through rapid colonization. It is abundant in organically enriched environments, where other species are excluded, to the point that it has often been used as an indicator of polluted conditions (Wass 1967). Organically enriched environments are prone to develop hypoxia and anoxia because of their high chemical and biological oxygen demand. Therefore, C. capitata would be expected to survive prolonged exposures to hypoxia or anoxia. The polychaete is tolerant of hypoxia; however, its ability to tolerate anoxia is not greater than that of several other polychaetes not associated with organically polluted habitats (Warren 1977).

The resistance of Streblospio benedicti to anoxia is lower than the resistance of Capitella capitata (Table 2). Gray (1979) has suggested that the presence of opportunists, such as C. capitata, in polluted environments may be a consequence of their life history strategies, rather than resistance to disturbance. As results from the present study suggest, the presence of S. benedicti in estuaries with periodic anoxia can also be attributed to resilience from physical disturbance. Short periods of anoxia may eliminate entire populations of S. benedicti. For instance, local populations of S. benedicti became extinct at four stations monitored in a subestuary of the Chesapeake Bay, just after severe hypoxia had become established in early summer (see Chapter 1). Anoxia was not observed in water samples taken at 0.5
to 1 m above the bottom, but oxygen depletion might have occurred at the water-sediment boundary or between sampling intervals. Mortalities of *S. benedicti* during low dissolved oxygen events have been documented for other estuaries (Santos and Simon 1980).

Stress from low oxygen may also be complicated by the diffusion of hydrogen sulfide from the sediment into the water column. Increased mortality at low oxygen in the presence of hydrogen sulfide has been demonstrated for some polychaetes such as *Arenicola marina* (Groenendaal 1980), *Nereis diversicolor* (Theede et al. 1969, Vismann 1990), *Nereis virens* (Vismann 1990), and *Cirriformia tentaculata* (Bestwick et al. 1989). The fact that *Streblospio benedicti* inhabits tubes just below or at the sediment surface, where a thin normoxic layer prevails over the reducing conditions of the sediment below, may result in a poor adaptation to hydrogen sulfide levels that may not be regularly encountered under normoxia by the worm. Alternatively, physiological protection against hydrogen sulfide occurs in some marine invertebrates that live deeper in the sediment and are exposed to sulfide (Groenendaal 1981, Doeller et al. 1988).

While the development of anoxia leads to the death of *S. benedicti* populations, the prevalence of low oxygen conditions above 7% air saturation should not severely affect population survival. *S. benedicti* confronts hypoxia by modifying its behavior. Feeding activity ceases at least during initial exposures to low oxygen, and the worm spends a good portion of the time inactive at the tube opening, or stretches into the overlying water. Although infrequent undulatory body movements were observed when the animal extended into the water, tube irrigation is not known to occur in this polychaete (Dauer 1984). In the absence of ventilation, gas exchange
presumably takes place only across the branchiae, which are situated near the prostomium and project anteriorly (Dauer 1985).

Inactivity at low oxygen tensions is a behavioral response observed in most polychaetes (Warren 1984), and reflects a decrease in the rate of aerobic metabolism and oxygen consumption. Alternatively, inactivity contributes to maximizing survival during anaerobiosis because of the slower depletion of metabolic reserves (Storey and Storey 1990). As a consequence, feeding and reproduction cease in many polychaetes, generally above their oxygen tolerance limits (Reish and Barnard 1960, Reish 1966). Reproductive activity under hypoxia was not addressed in this study; however, larvae were present in control aquaria, but were absent from low oxygen treatments with some ovigerous females.

The information obtained from these laboratory experiments can be used, with caution, to draw inferences about *S. benedicti* in the field. In oxygenated waters *S. benedicti* is a very rapid colonizer (McCall 1977, Levin 1984). It is highly motile (Dauer et al. 1981, Levin 1981), and will vacate its tube if disturbed (Foster 1971), migrating to nearby areas. Migration of adults, including brooding females, has been commonly observed in recolonization experiments (Dauer and Simon 1976, Levin 1984, Holland et al. 1987). During hypoxia, *S. benedicti* may survive, but activity is reduced, as discussed above. Therefore, it is unlikely that migration of adult worms into or from affected areas occurs until hypoxia ends. Larvae, on the other hand, may be advected to low oxygen waters, their survivorship unaffected during short term hypoxia. Whether they settle and metamorphose under low oxygen conditions is not known, but recruitment may be achieved once oxygenated waters return. Successful colonization will then be dependent upon the timing
of the low dissolved oxygen disturbance, its intensity and duration.
CHAPTER 3.

TOLERANCE OF LOW DISSOLVED OXYGEN BY THE TUBICULOUS

POLYCHAETE LOHIA MEDUSA (SAVIGNY)
ABSTRACT

The polychaete *Loimia medusa* occurs in a broad range of sediments, from intertidal sands to deep-water muds. It is common in deep-water estuarine habitats where summer hypoxic events often occur. Given the importance of *L. medusa* as a major component species in some benthic communities, both in abundance and biomass, its tolerance and behavior to hypoxia and anoxia were examined in the laboratory. The worm is capable of surviving under severe hypoxia (7% air saturation) or anoxia for 3 to 5 days. Under low dissolved oxygen, its behavior is modified. Feeding stops, but activity (body movement and tube irrigation) continues. Most worms come out to the sediment surface, but the proportion of worms dead on the surface is treatment-dependent. Possible physiological mechanisms of tolerance are discussed in relation to available information about other terebellid species.
INTRODUCTION

The terebellid polychaete Loimia medusa is a sedentary, surface deposit-feeding worm common in estuaries and warm coastal areas of North America (Hartman 1951, Kritzler 1984). Predominantly a subtidal polychaete, L. medusa ranges from intertidal and shallow subtidal sands to deep (<100 m) mud habitats. In the intertidal zone, L. medusa regularly experiences hypoxia at low tide. Polychaetes confront these periods of oxygen shortage by developing behavioral and physiological compensations that enable them to survive until the next high tide (Herreid 1980). Different strategies have evolved among the species of the family Terebellidae. These include the development of a vascular system adapted to oxygen transfer at low external oxygen tensions (Terwilliger 1974), oxygen storage by a coelomic cell hemoglobin of high oxygen affinity (Mangum et al. 1975, Wells and Dales 1975), oxyregulation in declining oxygen tensions (Wells et al. 1980) and maintenance of high irrigation rates during oxygen depletion (Coyer and Mangum 1973). The respiratory physiology of L. medusa has not been studied, but oxygen requirements at low tide are probably met by these or similar adaptations.

Unlike intertidal populations, deep-water populations may encounter periods of hypoxia or anoxia of longer duration in areas where summer hypoxic events are common. Under such conditions, the degree of tolerance of hypoxia and anoxia by the species is of critical importance for the maintenance of local deep-water populations. Moreover, L. medusa is a large, apparently long-lived polychaete, and it is unlikely that short-term, rapid population growth occurs after defaunation. With these considerations, the present study
examines *L. medusa* under controlled experimental conditions with the objective of determining its tolerance to and behavior during prolonged periods of hypoxia and anoxia.

*Loimia medusa* inhabits a U-shaped tube formed of small sediment particles cemented by mucus, similar to that of the terebellid *Amphitrite ornata* (Aller and Yingst 1978). The tube is about 0.5-1.0 cm inner diameter and 30-40 cm in length, reaching a depth of 15-20 cm in sediment (pers. obs.). The animal feeds at the sediment surface on silt-size sediment particles in a similar manner to other terebellids (Dales 1955). Numerous bucal tentacles stretch radially on the sediment surface to a distance of tens of centimeters from the tube opening, trapping particles that are brought to the mouth simultaneously when the tentacles retract into the tube. Unconsolidated fecal material is periodically moved out of the tube with a burst of water originating at the same tube-end where feeding occurs.
Hypoxia Experiment

Adult *Loimia medusa* were collected in the intertidal zone of the lower York River, Virginia, on January 11, 1990. They were immediately transferred to seawater tables in the laboratory and gradually acclimated to room temperature.

Thirty-six worms were randomly assigned to individual PVC cores (15 cm diameter x 15 cm deep) containing natural, defaunated sediment, and allowed to burrow. Cores and worms were kept in aerated, filtered seawater for 2 weeks prior to the experiment. Water was changed twice a week and worms fed daily with a slurry of fine sediment. Cores were set up in nine 19-1 aquaria tightly covered by glass sheets. There were two cores per aquarium and two worms in each core. Aquaria were blocked in groups of three, and randomly assigned within a block to each of the following dissolved oxygen treatments: 100% (controls), 14.3% and 7.2% air saturation (Table 1). The combination of aquaria (blocks) was aimed to reduce variability that may arise from the location of the experimental units. Temperature and salinity were maintained within the range to which worms are usually exposed during summer (Table 1). Salinity and pH were measured at the beginning and at the end of the experiment using a refractometer and an Orion pH meter model 601 A, respectively. Ammonium concentrations in the test water were measured at the end of the experiment following procedures described in Grasshoff et al. (1983). Dissolved oxygen concentrations were maintained by continuously bubbling air or a mixture of either 3% or 1.5% oxygen and the balance nitrogen. The rate of gas bubbled was adjusted using flow meters. Oxygen was
Table 1. Experimental conditions of tests of *Loimia medusa* in hypoxic and anoxic seawater.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>DISSOLVED OXYGEN</th>
<th>TEMPERATURE</th>
<th>SALINITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Air Saturation</td>
<td>mg l(^{-1})</td>
<td>°C</td>
</tr>
<tr>
<td>EXPERIMENT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HYPOXIA</td>
<td>100.0</td>
<td>7.3</td>
<td>26±0.5</td>
</tr>
<tr>
<td></td>
<td>14.3</td>
<td>1.0</td>
<td>26±0.5</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>0.5</td>
<td>26±0.5</td>
</tr>
<tr>
<td>ANOXIA</td>
<td>100.0</td>
<td>7.2</td>
<td>26±0.5</td>
</tr>
<tr>
<td></td>
<td>≤1.5</td>
<td>≤0.1</td>
<td>26±0.5</td>
</tr>
</tbody>
</table>
monitored daily with a microelectrode (Microelectrodes Inc.). Calibration was checked often using nitrogen and a 6% oxygen mixture as end points.

The experiment was initiated on January 25, 1990, and lasted for 348 h (14.5 days). Worms were observed three or four times a day and fed daily. Death was determined when worms failed to respond to touch or immersion in oxygenated water for about 15 min. Tube tops were carefully checked for any signs of water flow that might indicate worm activity. Worms remaining in the tube were determined to be dead when no response was observed after repeatedly squeezing the tube top with forceps.

Anoxia Experiment

Adult *Loimia medusa* were collected at the same site on February 11-12, 1990. They were maintained in aquaria for 3 weeks prior to the experiment under similar conditions to those described above. Worm and core assignments to eight 19-l aquaria were as in the hypoxia experiment. Four randomly determined aquaria received a treatment consisting of filtered seawater deoxygenated by a continuous supply of nitrogen. Control aquaria were maintained at saturation with aeration (Table 1). Temperature was maintained at 26±0.5° C. Dissolved oxygen, salinity, pH and ammonium measurements were taken as in the hypoxia experiment.

The experiment was initiated on March 6, 1990, and lasted for 134 h (5.6 days). Worms were checked 8-14 times a day and determined to be dead following the same procedures described above. On six occasions, deaths occurred within a range of 3-6 h, and time to mortality had to be estimated based on observations from this and a preliminary experiment.
RESULTS

Hypoxia Experiment

Dissolved oxygen concentrations were maintained generally at the treatment levels. Measured concentrations decreased only twice to 5.3% air saturation (0.4 mg l\(^{-1}\)) in the 7.2% air saturation treatment, and to 11.9% air saturation (0.9 mg l\(^{-1}\)) in the 14.3% air saturation treatment. pH increased slightly in low oxygen aquaria as compared to control aquaria (Table 2). Ammonium concentrations were lower in low oxygen aquaria than in control aquaria by the end of the experiment (Table 2).

Worms were active during the acclimation period, burrowing as soon as they were transferred to the cores. They reversed frequently within their tube, feeding at both ends. Some worms left the tube and swam for a brief period of time (<1 min). They reburrowed immediately upon settlement on sediment cores.

All worms stopped feeding within 20 h in seawater of low dissolved oxygen, but tube irrigation continued. Two of the worms in one 14.3% air saturation treatment aquarium were observed feeding 42 h after the initiation of the experiment. Hours later (113 and 54 h, respectively), all the worms in the same aquarium, and one worm in a second replicate aquarium (14.3% air saturation treatment) started and continued feeding for the rest of the experiment. Three of these worms came out of the tube and settled on the bottom of the aquaria, reburrowing as soon as they were replaced on the sediment surface. Worms were generally active during the experiment, but activity declined to very low levels before reaching death. Activity consisted of tube irrigation and frequent occurrences of the head out of the
Table 2. Ammonium concentrations (mean of two samples) and pH of treatment aquaria during tests of *Loimia medusa* in hypoxic and anoxic seawater. Ammonium concentrations are those at the end of the experiment. pH$_1$ = at the beginning of the experiment, and pH$_2$ = at the end of the experiment. Treatments are identified as per cent air saturation. Replicate aquaria are indicated in parenthesis.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>HYPOXIA</th>
<th>ANOXIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH$_4$ ($\mu$M)</td>
<td>pH$_1$</td>
</tr>
<tr>
<td>7.2 (1)</td>
<td>3.8</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>4.1</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>8.0</td>
</tr>
<tr>
<td>14.3 (1)</td>
<td>4.7</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>3.8</td>
<td>8.0</td>
</tr>
<tr>
<td>100.0 (1)</td>
<td>9.7</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>8.8</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>5.1</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1.5 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>York River Water</td>
<td>1.6$^{(1)}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) Sample from 2/8/90
(2) Sample from 3/10/90
tube with tentacles and branchiae in constant movement. Epitomization of abdominal fragments occurred in several worms.

Survival was significantly different among the three oxygen treatments (G-test of independence, G with William's correction = 24.88, p < 0.001). After 14.5 days in low oxygen, one worm was recovered live in the 7.2% air saturation treatment, and seven worms were alive in the 14.3% air saturation treatment (Fig. 1). All worms in control aquaria were alive. Mortality of half of the experimental population in the 7.2% air saturation treatment occurred within 5 days. All deaths but one in the 14.3% air saturation treatment occurred within one aquarium. When data within blocks were plotted and tested, there was indication that aquaria in the 14.3% air saturation treatment were not all homogeneous (G-test, p < 0.05).

Anoxia Experiment

Anoxia was reached 5 h after the nitrogen treatment was initiated. Dissolved oxygen concentrations were generally maintained below 1.5% air saturation (about 0.1 mg l⁻¹). There was a small pH increase in anoxic aquaria as compared to control aquaria (Table 2). Ammonium concentrations were higher in anoxic aquaria than in control aquaria by the end of the experiment (Table 2).

As in the hypoxia experiment, worms were active during the acclimation period, feeding frequently at both ends of the tube. Many worms (19 observations in all) vacated their tubes at least once, swimming typically for less than 1 min and burrowing upon settlement. Tube evacuation occurred during periods when worms were not recently fed. Under anoxia, worms stopped
Fig. 1. Survival of *Loimia* adults after 14.5 days exposure to hypoxia and
air-saturated seawater.
Number of worms surviving

Time (Days)
feeding and, except for sporadic excursions half way out of the tube, their activity decreased. Tube irrigation decreased rapidly, reaching a point where it was no longer perceived from observations of tube tops. Epitomization of abdominal fragments was also observed under anoxia.

The first death occurred after 24 h of anoxia. All worms were dead at 5.5 days (Fig. 2). Time to mortality seemed to be independent of aquarium after plotting the data. Worms in control aquaria remained alive except for one death towards the end of the experiment. Mortality of half of the experimental population occurred within 3.3 days of anoxia. All worms in the anoxia treatment but one came out of their tubes and died on the sediment surface. By comparison, only four (7.2% air saturation treatment) and two (14.3% air saturation treatment) of the deaths in the hypoxia experiment occurred on the sediment surface (Fig. 3). This difference is statistically significant (G-test of independence, $G = 12.55$, $p < 0.01$).
Fig. 2. Number of *Loimia* adults surviving as a function of time during exposure to anoxic seawater.
Number of worms surviving

Time (Hours)

- Treatment
- Control
Fig. 3. Cumulative number of Loimia adults dead on surface versus dead in tube as a function of percent air saturation.
DISCUSSION

Under experimental laboratory conditions, *Loimia medusa* is tolerant of severe hypoxia (7% air saturation) or anoxia for short periods of time (3-5 days). This tolerance suggests that intertidal populations can withstand periods of anoxia well in excess to those occurring at low tide. Similarly, subtidal populations of deep estuarine habitats may survive prolonged hypoxia if moderate (>14% air saturation), and severe hypoxia or anoxia if of short duration. These conditions are likely to occur in some estuaries where summer hypoxia develops in bottom waters during water-column density stratification. Dissolved oxygen may decrease to low concentrations, occasionally reaching anoxia, before the situation is relieved by wind mixing, thermal destratification or lateral advection of oxygenated bottom water. Such conditions are known to occur within the Chesapeake Bay, Virginia (Haas 1977, Hayward et al. 1986, Kuo and Neilson 1987).

In the lower Rappahannock River, for instance, *Loimia medusa* is one of the dominant species in biomass, and maintains an average abundance of 32 individuals m$^{-2}$ (Dauer et al. 1989, Chapter 1). During summer of 1987, the species appeared to survive hypoxic events at some stations near the mouth of the river (see Chapter 1). In the present study, worms held in 14% air saturation for two weeks had low mortality. Deaths at this saturation occurred mainly within one aquarium. This raises the possibility that such mortalities were related to aquarium effects, although experimental factors apparently did not differ from similar treatment aquaria.

Tolerance of anoxia by *Loimia medusa* is similar to that of two other terebellids, *Terebella lapidaria* (6 days, Warren 1984) and *Terebellides*
stroemi (5 days, Henriksson 1969). It is also higher than the tolerance of some smaller polychaetes such as Scoloplos armiger (<2 days, Schöttler and Grieshaber 1988) and Streblospio benedicti (2 days, see Chapter 2). Moreover, the capacity of L. medusa to tolerate anoxia may be higher than the experiment indicates because of additional stress from experimental holding conditions. For instance, food shortage may have been related to tube evacuation of worms during acclimation. As a consequence, reburrowing may have inflicted some stress on the animal. It is also possible that increased ammonium concentrations and pH may have further contributed to worm mortality in anoxic aquaria; however, ammonium concentrations in nature are typically higher in polychaete burrows than in the overlaying water (Kristensen 1984). High concentrations of up to 96 µM NH₄⁺ are normal in burrows of the terebellid polychaete Amphitrite ornata, a species morphologically similar to L. medusa, in the absence of irrigation at low tide (Aller and Yingst 1978).

During hypoxia, Loimia medusa probably relies on the contribution of vascular and coelomic cell hemoglobins in a way similar to other terebellids (Wells and Warren 1982, Warren 1984), at least initially while oxygen supply is maintained by irrigation. Oxygen uptake in other terebellids (e.g., Amphitrite ornata, Terebella haplochaeta) occurs mainly across the branchiae. Gas transport also benefits from a well developed vascular system and the presence of a high-molecular weight vascular hemoglobin with positive oxygen-binding properties such as co-operativity (Mangum et al. 1975, Wells et al. 1980). With a fall in ambient oxygen tension, the abdominal body surface and the contribution of high oxygen affinity cell hemoglobins become increasingly important in gas exchange, as it has been suggested to occur in Terebella lapidaria (Warren 1984). In addition, the oxygen storage function of coelomic
cell hemoglobins seems to be of primary importance in the respiratory physiology of the mud-dwelling terebellid *Enoplobranchus sanguineus* (Mangum et al. 1975). On the other hand, oxygen storage in most terebellids, although adequate for short-term hypoxia at low tide, would be insufficient during longer periods of hypoxia affecting subtidal populations (Wells and Warren 1982). In *Amphitrite ornata*, with decreasing ambient oxygen tensions, oxygen consumption continues down to almost anoxia, suggesting that the animal is continuously able to extract oxygen from the ventilatory current with a net oxygen gain (Mangum and Burnett 1975).

Under anoxia, *Loimia medusa* is probably adapted for reliance on anaerobic metabolism. All polychaetes have at least some ability for facultative anaerobiosis (C.P. Mangum, pers. comm.). *Terebella lapidaria* can survive up to six days of anoxia by utilizing a large pool of free amino acids (aspartate and glutamate) to produce succinate and volatile fatty acids which participate in the electron transfer system in the absence of oxygen (Warren 1984). In the anoxia experiment, *L. medusa* reduced irrigation and feeding activities, and metabolism was probably sharply reduced. For instance, the metabolic rate of *Amphitrite ornata* is reduced at rest by about 85% as compared to the active rate at 20° C, although this difference becomes smaller at low oxygen tensions (Mangum and Burnett 1975). During anaerobiosis, metabolic rate depression prevents fast depletion of energy reserves (Storey and Storey 1990).

In field situations, anoxia generally occurs in association with hydrogen sulfide in sediment, which may shorten survival times of polychaetes (e.g., Vismann 1990). Burrowing invertebrates often come to the sediment surface as hydrogen sulfide increases during anoxia (Jørgensen 1980). In the
present study, the smell of hydrogen sulfide was detected in anoxic aquaria towards the end of the experiment, but was not perceived in the hypoxia experiment. This observation suggests that diffusion of hydrogen sulfide into worm tubes may have forced the worms to come out onto the sediment surface. During low oxygen experiments, with hydrogen sulfide presumably absent or in low concentrations, *Loimia medusa* deaths were significantly higher in tubes than on the sediment surface. This effect of hydrogen sulfide has been demonstrated for the burrowing polychaetes *Nereis diversicolor* and *Nereis virens* in experiments where the percentage of worms found on the sediment surface increased in the presence of sulfide (Vismann 1990).

Tolerance to environmental factors has ecological implications for the distribution and coexistence of species in changing estuarine environments. The distribution of *Loimia medusa* in the Chesapeake Bay is known to be limited by salinity (Holland et al. 1988). In addition, if a trend of increase in the extent and intensity of hypoxia is actually occurring (Flemer et al. 1983, Officer et al. 1984), the distribution of *L. medusa* in subtidal habitats may be further limited. A long-term, general trend of decline in abundance of large, long-lived benthos occurring in the upper mesohaline reaches of the Chesapeake Bay, has been related to hypoxia or anoxia effects (Holland et al. 1987, 1988). Such decline in populations of large biomass species may give access to communities dominated by short-lived species with opportunistic life histories. Such a shift in community structure may alter benthic resources because of the reduction of food (i.e., biomass) available for higher trophic levels.
CHAPTER 4.

EFFECTS OF HYDROGEN SULFIDE ON SURVIVAL AND BEHAVIOR OF THE POLYCHAETES

_STREBLOSPIO BENEDICTI_ (WEBSTER) AND _LOIMIA MEDUSA_ (SAVIGNY)
The broad distribution of the polychaetes *Streblospio benedicti* and *Loimia medusa* makes them susceptible to hydrogen sulfide exposure in environments of unpredictable dissolved oxygen concentrations. Tolerance and behavior of these two species were studied in laboratory experiments where worms were exposed to either anoxia or anoxia and sulfide. In addition, the response of *S. benedicti* to low dissolved oxygen in combination with sulfide was examined.

Survival of both species under moderate concentrations of hydrogen sulfide was not decreased in comparison to reference individuals in anoxia alone. Results from a low dissolved oxygen-sulfide treatment were inconclusive since periods of anoxia were present because of the chemical consumption of oxygen by sulfide. The behavior of *S. benedicti* exposed to anoxia plus sulfide was not different from the behavior of worms in anoxia. The behavior of *L. medusa* was changed. The number of worms leaving the tube to die on the sediment surface was proportionally higher in the presence of sulfide.
INTRODUCTION

Sulfide production is common in marine sediments world-wide (Fenchel and Riedl, 1970). Most coastal sediments present a thin, oxidized surface layer below which reducing conditions persist, allowing the generation of sulfur-rich compounds (Fenchel and Riedl 1970, Jørgensen 1977). In habitats where bottom-water stagnation occurs or pore water exchange is poor, the oxidized surface layer of sediment becomes exceedingly thin or disappears, resulting in anoxic sediment columns of strong reducing conditions. These environments, such as mud flats and salt marshes, have high production rates of sulfide (Howarth and Teal 1979), and are prone to develop high concentrations of hydrogen sulfide (about 6 mM at depth), which is often toxic for organisms (Groenendaal 1979; Howes et al. 1985).

A fraction of the sulfide in marine sediments derives from the decomposition of organic matter by heterotrophic microorganisms; but the dominant process leading to the production of sulfide is the anaerobic reduction of sulfate by chemosynthetic bacteria (Jørgensen 1977, 1982, Hansen et al. 1978). Most sulfide in anoxic sediments accumulates by precipitation with ferrous ions, forming insoluble ferrous sulfide (FeS) and pyrite (FeS$_2$), which give a dark color to the bulk of the sediment (Jørgensen 1977). Some sulfide remains dissolved as hydrogen sulfide (H$_2$S) and its ionized form (SH$^-$) in pore water. This pool of hydrogen sulfide may diffuse upwards in the sediment column reaching the oxidized layer or seeping into the water column (Hansen et al. 1978). Upon contact with oxygen, sulfide is chemically oxidized. Much of the sulfide oxidation, however, is mediated by
chemosynthetic, colorless bacteria using oxygen as electron acceptor, or by purple and green sulfur bacteria through photosynthesis under anaerobic conditions (Jørgensen 1977, 1982).

It seems then that the concentration of hydrogen sulfide in sediment varies with oxygen availability and amount of organic matter (Howarth and Teal 1979, Jørgensen 1982); it also varies with seasonality (Jørgensen 1977). This balance between oxic and sulfide-producing anoxic sediments affects the distribution and survival of infaunal benthic organisms (Fenchel 1969). For instance, Fenchel and Riedl (1970) postulated the existence of a variety of meiofauna and microfauna associated with the sulfidic anaerobic environment of sediments. Although the existence of this community has been questioned on grounds that these taxa are merely associated with the oxic layer surrounding macrofaunal burrows (Reise and Ax 1979), more recent experimental work has corroborated the presence of a distinct group of meiofaunal organisms that occupy anoxic microhabitats (Meyers et al. 1987, 1988).

The distribution of macrofauna also is modified by the presence of sulfide in aquatic environments. The existence of a large community of macrobenthos associated with sulfide-emitting hydrothermal vents at oceanic spreading centers is now well established (Lonsdale 1977, Childress 1988). In coastal and estuarine sediments, the distribution of some macrobenthic species seems to be correlated with the presence or absence of sulfide in anoxic sediments, and depends on the organism's tolerance to hydrogen sulfide (Shick 1976, Groenendaal 1979, Vismann 1990). Infaunal burrowing and tube building activities may, in turn, modify the distribution and availability of sulfide in sediments. Bioturbation changes the redox profile of sediments (Rhoads 1974), and may cause hydrogen sulfide to be transported towards the surface,
where it is oxidized (Jørgensen 1982).

Survival of benthic macrofauna is affected by the availability of oxygen. Species tolerances to low dissolved oxygen concentrations vary, but survival is limited in most cases by anoxia or severe hypoxia. The resistance of a species to low dissolved oxygen is of critical importance for the maintenance of local populations in estuaries affected by intermittent periods of seasonal hypoxia or anoxia. Additionally, species survival during hypoxia or anoxia may further be decreased by the presence of unusually high concentrations of hydrogen sulfide or by the diffusion of this to the sediment surface. Although studies of the effects of hydrogen sulfide on estuarine macrobenthos indicate that survival during anoxia is significantly reduced in the presence of sulfide (Groenendaal 1980, Shumway et al. 1983, Bestwick et al. 1989, Vismann 1990), they do not generally take into account the spatial distribution and life habits of macroinfauna as factors that may mitigate exposure to hydrogen sulfide in natural situations.

The present study describes a series of experiments designed to test whether the presence of hydrogen sulfide affects survival of two common estuarine polychaetes under anoxia. It compares two species with different life styles and distributions in the sediment: the spionid *Streblospio benedicti* and the terebellid *Loimia medusa*. The small opportunist *S. benedicti* inhabits tubes at the sediment subsurface. This worm is not known to ventilate its tube; it is very active, able to move to a new feeding area or vacate the tube, migrating to adjacent bottom sediments (Foster 1971). In contrast, *L. medusa* inhabits large U-shaped tubes reaching 15-20 centimeters into the sediment. Water in the tube is renovated by active irrigation (pers. obs.). Although the worm may occasionally vacate its tube (pers. obs.), there
is no evidence to suggest that frequent migrations occur.

Given the rapid oxidation of hydrogen sulfide in the presence of oxygen, oxygen and sulfide probably do not coexist but for short periods of time. Rather, benthic invertebrates are likely to encounter a combination of anoxia and sulfide. A gradient from low dissolved oxygen to anoxia to hydrogen sulfide, however, may be possible. As this gradient migrates within the sediment column or at the sediment-water interface, infauna may be intermittently exposed to hypoxia and hydrogen sulfide. In an attempt to maintain hypoxia and hydrogen sulfide in static seawater, *Streblospio benedicti* was further used to test whether survival in low dissolved oxygen is modified by periods of anoxia and hydrogen sulfide.
Streblospio benedicti

Adult *S. benedicti* were collected from a subtidal mud habitat (Lafayette River, Virginia) between October 8 and 10, 1990. In the laboratory, worms were gently forced out of their tubes and transferred to experimental dishes.

Ten small dishes (5.5 cm diameter) containing a 2-3 mm layer of defaunated sediment were set up in each of six 19-l aquaria. The dishes were placed near the top of the aquaria to facilitate observation, and covered by 125-μm mesh nylon screens to prevent worms from swimming away. Aquaria were filled with 1-μm filtered seawater and tightly covered with glass sheets.

Worms were randomly assigned to dishes, each dish receiving one worm. They were maintained in the aquaria with aeration at room temperature (24° C) for 5 days, and at 26° C for 2 additional days prior to the initiation of the experiment. During this period, fecal pellets were removed from the dishes and worms fed with fine sediment once a day. Non-feeders were replaced by additional worms kept in similar aquaria.

Two aquaria were randomly selected for each of three treatments consisting of (1) deoxygenated seawater with hydrogen sulfide (anoxia-sulfide treatment), (2) deoxygenated seawater without hydrogen sulfide (anoxia treatment), and (3) air-saturated seawater (controls). Temperature and salinity (26±0.5° C; salinity= 20%) were maintained within the range usually encountered by the worms during summer hypoxia.

Anoxia was achieved by continuously bubbling nitrogen gas. The rate of gas introduced in the aquaria was adjusted with flow meters. Hydrogen sulfide was maintained by occasionally adding small amounts (typically <300 ml) of a
stock solution with the aid of a peristaltic pump. Average sulfide
cconcentration in test aquaria was 20 μM (SD=14 μM; n=26), with a maximum
concentration of 66 μM. The stock solution was prepared by dissolving 7.8 g
of Na₂S·9H₂O crystals in 19-l filtered, deoxygenated seawater. Deoxygenation
was maintained through nitrogen bubbling.

Dissolved oxygen was measured with a microelectrode (Microelectrodes,
Inc.) calibrated at the temperature of measurement. Dissolved oxygen
concentrations of samples from the anoxia-sulfide treatment were checked
following the Winkler technique (Grasshoff et al. 1983). pH was measured at
the beginning and at the end of the experiment using a pH-meter (Orion, model
601A). Sulfide measurements were made with a silver-sulfide electrode (Orion,
model 94-16) and a double junction reference electrode (Orion, model 90-02)
connected to a pH-meter with direct concentration display capability (Beckman,
model Phi 12). Calibration was attained by serial dilutions of a commercially
available sulfide standard (100 ppm S²⁻). Standard sulfide dilutions were
initially checked by titration with 0.1 M lead perchlorate, using the
electrode pair as end point indicator of mV change. All standards and samples
were brought to the same temperature, and diluted in proportion 1:1 with a
sulfide anti-oxidant buffer (Orion SA0B II). This buffer consists of a 2 M
sodium hydroxide solution containing ascorbic acid and 0.2 M EDTA.

Sodium hydroxide adjusts the ionic strength of standards and samples to
pH 13, releasing the sulfide ion from hydrogen ions and maintaining its level
fixed. S²⁻ is the chemical species measured by the electrode. Sulfide forms
complexes with hydrogen ion (HS⁻ and H₂S); their relative presence is pH
dependent. At pH of 6.0, 95% of the sulfide is in the form H₂S. Above pH 11,
S²⁻ occurs in significant proportions. Ascorbic acid prevents oxidation, and
the component EDTA avoids precipitation of metals in the form of metal-sulfide complexes. The electrode measuring error was ±4%, and both accuracy and precision of measurements from samples of known concentrations were good during testing sessions.

The experiment was initiated on October 23, 1990, and lasted 48 h. Dissolved oxygen concentrations were first reduced to treatment levels. Sulfide additions were started 3 h later. Five-ml water samples were taken from anoxia-sulfide treatment aquaria for hydrogen sulfide measurements at intervals of 3-6 h. Worms were observed every 2 h except for two intervals of 3 and 4 h, fed daily, and their condition and behavior noted. Worms were determined dead when failing to respond to touch.

In a separate experiment, adult *S. benedicti* obtained from laboratory cultures and from field collections (intertidal marsh, York River, Virginia) were randomly assigned to dishes as described above. The worms were maintained in aquaria with aeration at 26° C for 10 days prior to the initiation of the experiment. Two aquaria were randomly selected for each of the following three treatments: (1) seawater of dissolved oxygen concentration reduced to 7.0% air saturation with the addition of hydrogen sulfide (low DO-sulfide treatment), (2) seawater of dissolved oxygen concentration reduced to 7.0% air saturation without hydrogen sulfide (low DO treatment), and (3) air-saturated seawater (controls).

Desired dissolved oxygen concentrations were achieved by continuously bubbling air, or a gas mixture of air and nitrogen mixed and adjusted with flow meters. Hydrogen sulfide was introduced by adding small amounts (1-2 ml/min) of a stock solution. Equal amounts of untreated seawater and seawater of reduced oxygen concentration were added daily to the control and low DO
aquaria, respectively. The average sulfide concentration in low DO-sulfide treatment aquaria was 67 μM (n=39), but concentrations increased initially up to 330 μM. Both hydrogen sulfide and dissolved oxygen concentrations fluctuated because of the rapid depletion of oxygen in the oxidation of sulfide under static experimental conditions.

The experiment, initiated July 25, 1990, lasted 74 h. Dissolved oxygen concentrations were first reduced to treatment levels, and sulfide flow started 2 h later. The low DO treatment was continued for an additional 17-h period after the low DO-sulfide treatment was discontinued. Ten-ml water samples were analyzed for hydrogen sulfide at intervals of 1-5 h. At equal intervals, worms were observed and determined dead or alive as described above.

Loimia medusa

Adult L. medusa were collected in the shallow subtidal zone of the lower York River, Virginia, October 6-8, 1990. They were immediately brought to the laboratory and transferred to aquaria with circulating seawater at ambient temperature.

Forty-eight hours after the first collection, worms were randomly assigned to plastic containers (10x10x10 cm) filled with natural, defaunated (by microwaving) sediment, and allowed to burrow. Each container received one worm. The volume of sediment per container was smaller than the volume usually utilized for burrowing by average-size adult worms in their natural habitat. Loimia medusa builds a U-shaped tube down to 15-20 cm in sediment, with a distance from head to tail shaft of up to 20 cm (pers. obs.). Reduced sediment volume was used in order to avoid natural build up of hydrogen
sulfide in anoxia treatments. Worm behavior seemed unaffected by container size.

Containers with worms were placed in groups of four in each of nine 19-l aquaria. Aquaria were tightly covered by glass sheets. Worms were maintained in aerated, 10-μm filtered seawater at room temperature (24°C) for 4 days, and at the experimental temperature for 6 additional days prior to the initiation of the experiment. Water was changed once a week and worms fed twice a day with a slurry of fine sediment to which Gerber's mixed cereal was added.

Three aquaria were randomly selected for each of three treatments consisting of (1) deoxygenated seawater with hydrogen sulfide (anoxia-sulfide treatment), (2) deoxygenated seawater without hydrogen sulfide (anoxia treatment), and (3) air-saturated seawater (controls). Temperature and salinity (26±0.5°C; salinity= 20%) were maintained within the range encountered by the worms during summer hypoxia.

Dissolved oxygen and hydrogen sulfide concentrations were achieved as described above. Average sulfide concentration in anoxia-sulfide treatment aquaria was 23 μM (SD=12 μM; n=70), with a maximum concentration of 54 μM. Dissolved oxygen, sulfide and pH measurements were conducted as described above.

The experiment was initiated on October 17, 1990, and lasted for 102 h. Seawater was deoxygenated first. Sulfide additions were started 2.5 h later. Worms were fed twice a day and observed every 3 h throughout the length of the experiment, except for five periods of 4-6 h. Ten-ml water samples were taken from each anoxia-sulfide treatment aquaria for hydrogen sulfide measurements at irregular intervals of 1-12 h, and after sulfide additions. Occasionally,
anoxia treatment aquaria were sampled to confirm the absence of hydrogen sulfide. Death was determined when worms failed to respond to touch after immersion in oxygenated water for 15 min.

Data Analysis

To test the null hypothesis of no hydrogen sulfide effects on time to mortality of worms in anoxia, a nested analysis of variance was applied to the data. A nested ANOVA tested for aquaria effects that may introduce variation. Hydrogen Sulfide was the main factor, with two levels, presence or absence. Aquaria constituted the nested factor. Homogeneity of variances was tested using Cochran's C test.
RESULTS

*Streblospio benedicti*

Worms exposed to anoxia and anoxia with hydrogen sulfide stretched further out of their tubes as time progressed. Feeding was halted and activity reduced. One worm in the anoxia treatment was unaccounted for, presumably dead within the sediment. All other worms in both anoxia and anoxia plus sulfide died on the sediment surface.

Deaths first occurred 18 and 19 h after the initiation of the experiment in anoxia and anoxia-sulfide treatments, respectively (Fig. 1). Time to mortality of half of the experimental population was 27 h for worms in anoxia, and 25 h for worms in anoxia with hydrogen sulfide. No significant difference in time to mortality was observed between the two treatments ($p > 0.05$, 1 and 2 d.f., $F = 0.01$), and there was no significant difference between aquaria within treatments ($p > 0.05$, 2 and 36 d.f., $F = 1.51$). A missing value in the anoxia treatment was estimated as indicated in Zar (1984, p. 216). All worms in the control treatment were alive at the end of the experiment (48 h).

Microelectrode-measured dissolved oxygen concentrations were zero or near zero (<2% of air saturation). Winkler-analyzed samples from anoxia-sulfide aquaria yielded zero oxygen values. pH was slightly higher in anoxia and anoxia-sulfide aquaria (8.1) than in control aquaria (7.7, 7.9) by the end of the experiment as compared to initial values (7.8).

*Streblospio benedicti* exposed to low DO and hydrogen sulfide died within 56 h (Fig. 2). One worm in the low DO-sulfide treatment remained alive at the end of the experiment. Time to mortality of half of the experimental population was estimated at 41 h. Worm burrowing activity decreased and
Fig. 1. Survival of *Streblospio* adults as a function of time during exposure to anoxia with hydrogen sulfide, anoxia, and air-saturated seawater. 

$T = 26 \pm 0.5^\circ C; S = 20\%$. 
Number of worms surviving

Time (Hours)

TREATMENT

- ANOXIA AND SULFIDE
- ANOXIA
- 100% AIR SATURATION
Fig. 2. Number of *Streblospio* adults surviving as a function of time during exposure to low dissolved oxygen (7% air saturation) with hydrogen sulfide, low dissolved oxygen, and air-saturated seawater. T= 26° C; S= 20%.
Number of worms surviving

Time (Hours)

TREATMENT

- LOW DO AND SULFIDE
- LOW DO
- 100% AIR SATURATION
feeding ceased in low DO-sulfide and low DO treatment aquaria within a few hours after initiation of the experiment, as observed before. All worms in low DO and control treatments survived the length of the experiment (74 h).

All worms previously exposed to low DO had recovered and exhibited normal burrowing and feeding behavior in normoxic seawater twenty hours after the conclusion of the experiment.

Dissolved oxygen concentrations in low DO-sulfide treatment aquaria fluctuated between 0 and 7% air saturation as a consequence of the oxidation of hydrogen sulfide. Although the duration of anoxic periods was not measured, data from experiments to determine interaction of oxygen and sulfide suggest that worms may have been exposed to prolonged periods of anoxia. At the reduced experimental concentrations, dissolved oxygen was depleted by the addition of hydrogen sulfide. Sulfide concentrations varied because of the difficulty of maintaining hydrogen sulfide in the presence of oxygen, but initially increased to a maximum of 190 μM and 330 μM in the two low DO-sulfide aquaria, respectively. In low DO-sulfide treatment aquaria pH decreased from 7.6 and 7.8 to 6.7 and 6.9, respectively, by the end of the experiment. pH decrease reflects the release of hydrogen protons from the chemical oxidation of hydrogen sulfide.

Loimia medusa

Feeding ceased in anoxia and anoxia plus sulfide treatments. Head, anterior region, and posterior abdominal segments of worms were occasionally observed protruding out of tubes. Epitomization of abdominal fragments occurred in some worms. Tube ventilation continued, but its amplitude appeared to decrease in sulfide-treatments.
The first death occurred after 15 h of anoxia and sulfide (Fig. 3). Time to mortality of half of the experimental population was 41 h for worms in anoxia, and 45 h for worms in anoxia with sulfide. Time to mortality between anoxia and anoxia-sulfide treatments was not significantly different ($p > 0.05$, 1 and 4 d.f., $F = 0.00$). No significant differences were observed between aquaria within treatments ($p > 0.05$, 4 and 18 d.f., $F = 0.60$).

One worm in each anoxia and anoxia-sulfide treatment survived for the length of the experiment. The anoxia-exposed worm recovered in normoxic seawater and resumed feeding. All control worms were alive and feeding at the end of the experiment. In the presence of sulfide, 10 worms came out of their tubes and died on the sediment surface; one worm died in its tube. In the absence of sulfide, 5 deaths occurred on the sediment surface, and 6 deaths in the tubes. This difference is statistically significant ($G$ test of independence, $G$ with William's correction $= 5.233$, $p < 0.05$), suggesting that worm location at death depends on the presence of hydrogen sulfide.

Both, dissolved oxygen concentrations measured with microelectrode and Winkler-analyzed concentrations of anoxia-sulfide aquaria, were zero. pH increased slightly in anoxia and anoxia-sulfide treatment aquaria (8.2-8.4) by the end of the experiment from initial values of 7.8-7.9.
Fig. 3. Number of *Loimia* adults surviving as a function of time during exposure to anoxia with hydrogen sulfide, anoxia, and air-saturated seawater. $T=26\pm0.5^\circ$ C; $S=20\%$.
Number of worms surviving

Time (Hours)

TREATMENT

- ANOXA
- ANOXA AND SILFIDE

102% AIR SATURATION
DISCUSSION

The presence of hydrogen sulfide during anoxia did not significantly decrease time to mortality in the polychaetes Streblospio benedicti and Loimia medusa. This is surprising since hydrogen sulfide has been reported to decrease survival of other polychaete species (Theede et al. 1969, Groenendaal 1980, Bestwick et al. 1989, Vismann 1990). Sulfide concentrations (below 66 μM) to which S. benedicti and L. medusa were exposed, however, were smaller than concentrations used in experiments by other workers. Bestwick et al. (1989) studied the metabolic pathways involved in anaerobiosis, and sulfide tolerance and recovery from anaerobiosis in the intertidal polychaete Cirriformia tentaculata. They used 200 μM Na₂S and reported a time to 50% survival of 5 days, half the value of individuals exposed to anoxia. Groenendaal (1980) reported an average survival of Arenicola marina in hydrogen sulfide of 4.5 days at high sulfide concentrations (10 mM), a decrease of 10 days from reference individuals in anoxia. In the same study, survival of a variety of polychaetes (Nephthys cirrosa, N. hombergi, Scolelepis squamata, Pectinaria koreni and Owenia fusiformis) was in the range of 2-5.3 days at similar sulfide concentrations. Vismann (1990), studied tolerance and sulfide detoxification mechanisms of hydrogen sulfide in Nereis diversicolor and N. virens, using experimental sulfide concentrations of 172-187 μM in a hypoxia-sulfide flow-through system. Mortality was higher for sulfide-treated worms than for worms under hypoxia. Higher sulfide concentrations were used in short-term exposure experiments. Mortality was determined as a function of sulfide concentration per g of fresh weight. LD₅₀ values of 54 mM g⁻¹ for N. diversicolor and 26 mM g⁻¹ for N. virens were
reported. Other studies have used concentrations of 100 μM (Cuomo 1985) and 500 μM-2 mM (Dubiller 1988) in experiments designed to test sulfide as a larval settlement cue for Capitella sp.

Sulfide concentrations in fine sediments of Chesapeake Bay channels, where these two polychaetes are commonly found, range from a few micromolar during anoxia at the sediment surface to 100 micromolar in the top one centimeter of sediment, and to about 2 millimolar deeper than one centimeter (J. Cornwell, Univ. of Maryland, pers. comm.). Sulfide concentrations maintained in the present study are, therefore, within the environmental range to which Streblospio benedicti may be exposed. Loimia medusa inhabits tubes deeper in the sediment, but the tubes probably prevent contact of the animal with deep, sulfide-rich sediments. Irrigation activity allows replacement of water in the tube, preventing accumulation of sulfide from diffusion across tube wall. Even during anoxia, some ventilatory activity occurs, and may reduce exposure of the worm to high sediment sulfide concentrations. Sulfide was added to the water column in the present study. Diffusion of hydrogen sulfide from the sediment may elicit different behavioral effects than the observed (e.g., worms may avoid sulfide by moving away), but water-column sulfide assured adequate exposure to both tube-dwelling polychaetes.

The results of the experiments conducted here do not imply that Streblospio benedicti and Loimia medusa are not affected by hydrogen sulfide but that, in general, tolerance of anoxia is not decreased by the presence of sulfide at concentrations below 66 μM. Behavioral effects in Loimia medusa are indicated by the significantly higher number of moribund individuals on the sediment surface in anoxia plus sulfide than in anoxia alone. A similar behavior has been observed in Nereis diversicolor and Nereis virens (Vismann
1990). The percentage of those worms at the sediment surface increased from 8 and 35% in hypoxia to 17 and 100% in hypoxia plus sulfide, respectively.

The absence of response to moderate sulfide concentrations during anoxia may be predictable on the basis of the distribution of these two species. *Streblospio benedicti* inhabits the top thin oxidized layer of sediment, but the species is found in a variety of habitats. Some of these, such as marshes and deep muds, are unpredictable oxygen environments prone to sulfide accumulation. *Loimia medusa* also inhabits a variety of sediments, from coarse intertidal sands to clays of deep channels. Their broad distribution may be partially related to an ability to confront the rigorous physical factors of the estuarine ecosystem.

Exposure of *S. benedicti* to dissolved oxygen concentrations of 7% air saturation with the addition of hydrogen sulfide resulted in a mortality curve similar to the curve of worms exposed to anoxia, and anoxia-sulfide. The large difference in worm mortality between hypoxia and hypoxia-sulfide treatments suggests a real effect of hydrogen sulfide. This effect, however, is not conclusive, since prolonged periods of anoxia probably occurred, and their duration is unknown. Anoxia appears to be a critical factor determining the tremendous decrease in survival observed in *S. benedicti*. It is interesting to note the quick recovery of individuals of this species after severe hypoxia for 74 h.

The toxicity of hydrogen sulfide results from the inhibition of the electron transport chain in aerobic respiration (Torrans and Clemens 1982). Hydrogen sulfide, by binding to the heme of cytochrome-c-oxidase and other enzymes containing porphyrin-bound metals, such as haemoglobin, inhibits their function (Evans 1967). Tolerance of hydrogen sulfide, or even insensitivity,
can be achieved by mechanisms leading to the exclusion of sulfide from the body (Groenendaal 1981), or by its detoxification by sulfide oxidases (Powell and Somero 1986). Sulfide detoxification occurs in some invertebrates inhabiting sulfide-rich habitats (Vismann 1990), and has been demonstrated in some fish (Bagarinao and Vetter 1989). It can also be mediated in certain invertebrates, predominantly bivalves, by symbiotic bacteria which use the energy released from the oxidation of sulfide in their own anabolic processes (Dando et al. 1985, Doeller et al. 1988). Other invertebrates show tolerance of sulfide, or lack of response of their hemoglobin to sulfide (Wells and Pankhurst 1980), but have no sulfide detoxification (Groenendaal 1981, Degn and Kristensen 1981).

In the absence of these mechanisms, tolerance of hydrogen sulfide may be explained by reliance on anaerobic metabolism during anoxia (Degn and Kristensen 1981, Powell and Somero 1986). By switching from aerobic to anaerobic metabolism, the potential sulfide inhibition of respiratory enzymes remains without effect. The inhibitory effects of hydrogen sulfide are reversible once sulfide dissipates or is chemically oxidized (Evans 1967, Torrans and Clemens 1982). It is possible that the lack of response to hydrogen sulfide observed in the two polychaetes here studied, is related to a shift to anaerobiosis in the absence of oxygen. Nothing is known, however, about the metabolic physiology of these two species. The mechanisms by which Streblospio benedicti and Loimia medusa tolerate low dissolved oxygen, short periods of anoxia, and hydrogen sulfide, remain to be investigated.
CONCLUSIONS

1. The lower Rappahannock River is affected by seasonal hypoxic or anoxic events that vary in intensity and duration. In 1987, hypoxia was intermittent; the decrease in dissolved oxygen concentrations was greatest in deep channel stations (18 m) and upriver (20 km from the mouth).

2. Abundance and species number of macrobenthos were significantly lower in the channel and upriver than in a shallow station and at the mouth, in relation to the distribution of low dissolved oxygen concentrations.

3. Polychaetes accounted for 94% of the total number of individuals. The spionid *Streblospio benedicti* and the capitellid *Mediomastus ambiseta* were numerical dominants.

4. The effects of hypoxia were manifested through a sharp decline in species abundances and number. The magnitude of the responses of species to hypoxia appeared to vary.

5. The vertical distribution of macro-infauna in sediment exhibited significant patterns of temporal change, but without clear trends for most species in relation to low dissolved oxygen concentrations.

6. Exposures to 7.0 and 14.5% air saturation for 14 days had no significant effect on the survival of *Streblospio benedicti* adults in laboratory experiments. Larvae survived similar conditions for at least 4 days. In anoxia, adult survival was reduced to 2.3 days.

7. In contrast to *Streblospio benedicti*, survival of *Loimia medusa* was significantly reduced after prolonged exposures of 14 days to 7.2 and 14.3% air saturation. *L. medusa* tolerated anoxia for up to 5 days. Mortality of
half of the experimental population occurred in 3.3 days.

8. Exposures to moderate concentrations (below 66 μM) of hydrogen sulfide had no significant effect on mortality of *Streblospio benedicti* or *Loimia medusa* when compared to reference individuals in anoxia.

9. Under hypoxia, anoxia or hydrogen sulfide, feeding ceased in both species. *Streblospio benedicti* vacated its tube and died on the sediment surface in all treatments. On the other hand, the number of *Loimia medusa* dead on the sediment surface was significantly higher in anoxia with sulfide than in anoxia alone.

10. The present study confirms the dramatic effects that severe hypoxia and anoxia exert on macrobenthos. The results of this study indicate that the magnitude of the population response depends on the intensity, extent and frequency of low dissolved oxygen events. Small changes in dissolved oxygen concentrations below 10% air saturation are important, and may cause large mortalities.

11. The study suggests that species composition is changed as a result of low dissolved oxygen stress. This change in the composition of species may be a consequence (a) of the varying dissolved oxygen tolerances of species, as laboratory experiments here conducted suggest, (b) of life history traits that facilitate rapid colonization of some species in adverse oxygen environments, and (c) of the timing of hypoxic and anoxic events with respect to larval availability.
APPENDIX. Species collected in the lower Rappahannock River, April to October 1987. ni= not identified.

<table>
<thead>
<tr>
<th>Phylum/ Class/ Family</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cnidaria</td>
<td></td>
</tr>
<tr>
<td>Hydrozoa</td>
<td></td>
</tr>
<tr>
<td>Sertulariidae</td>
<td><em>Sertularia argentea</em> Linnaeus</td>
</tr>
<tr>
<td>Anthozoa</td>
<td></td>
</tr>
<tr>
<td>Edwardsiidae</td>
<td><em>Edwardsia elegans</em> Verrill</td>
</tr>
<tr>
<td>Diadumenidae</td>
<td><em>Diadumene leucolea</em> (Verrill)</td>
</tr>
<tr>
<td>Platyhelminthes</td>
<td></td>
</tr>
<tr>
<td>Turbellaria</td>
<td><em>Stylochus ellipticus</em> (Girard)</td>
</tr>
<tr>
<td>Nemertina</td>
<td></td>
</tr>
<tr>
<td>Anopla</td>
<td></td>
</tr>
<tr>
<td>Tubulanidae</td>
<td><em>Tubulanus pellucidus</em> (Coe)</td>
</tr>
<tr>
<td>Enopla</td>
<td><em>Amphiporus bioculatus</em> (McIntosh)</td>
</tr>
<tr>
<td>Annelida</td>
<td></td>
</tr>
<tr>
<td>Polychaeta</td>
<td></td>
</tr>
<tr>
<td>Polynoidae</td>
<td><em>Lepidametria commensalis</em> Webster</td>
</tr>
<tr>
<td>Chrysopetallidae</td>
<td><em>Bhawania heteroseta</em> (Hartman)</td>
</tr>
<tr>
<td>Phyllodocidae</td>
<td><em>Eteone heteropoda</em> Hartman</td>
</tr>
<tr>
<td>Hesionidae</td>
<td><em>Phyllodoce arenae</em> Webster</td>
</tr>
<tr>
<td><em>Podarke obscura</em> Verrill</td>
<td></td>
</tr>
<tr>
<td><em>Podarkeopsis levifuscina</em> Perkins</td>
<td></td>
</tr>
<tr>
<td>Pilargidae</td>
<td><em>Ancistroyllis jonesi</em> Pettibone</td>
</tr>
<tr>
<td>Syllidae</td>
<td></td>
</tr>
<tr>
<td>Nereididae</td>
<td><em>Nereis succinea</em> Frey &amp; Leuckart</td>
</tr>
<tr>
<td>Nephtyidae</td>
<td><em>Nephtys sp.</em></td>
</tr>
<tr>
<td>Glyceridae</td>
<td><em>Glycera americana</em> Leidy</td>
</tr>
<tr>
<td><em>Glycera dibranchiata</em> Ehlers</td>
<td></td>
</tr>
<tr>
<td>Goniadidae</td>
<td><em>Glycindae solitaria</em> (Webster)</td>
</tr>
<tr>
<td>Capitellidae</td>
<td><em>Capitella jonesi</em></td>
</tr>
<tr>
<td>Heteromastus filiformis* (Claparede)</td>
<td></td>
</tr>
<tr>
<td><em>Mediomastus ambiseta</em> Hartman</td>
<td></td>
</tr>
<tr>
<td>Maldanidae</td>
<td><em>Clymenella torquata</em> (Leidy)</td>
</tr>
<tr>
<td>Spionidae</td>
<td><em>Paraprionospio pinnata</em> (Ehlers)</td>
</tr>
<tr>
<td><em>Polydora cornuta</em> Bosc</td>
<td></td>
</tr>
</tbody>
</table>
### Appendix (cont.)

<table>
<thead>
<tr>
<th>Phylum/ Class/ Family</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbiniidae</td>
<td><em>Leitoscoloplos</em> sp.</td>
</tr>
<tr>
<td></td>
<td><em>Cirratulidae</em></td>
</tr>
<tr>
<td></td>
<td><em>Pectinariidae</em></td>
</tr>
<tr>
<td></td>
<td><em>Terebellidae</em></td>
</tr>
<tr>
<td></td>
<td><em>Sabellidae</em></td>
</tr>
<tr>
<td>Oligochaeta</td>
<td><em>Tubificidae</em></td>
</tr>
<tr>
<td></td>
<td><em>Naididae</em></td>
</tr>
<tr>
<td>Mollusca</td>
<td><em>Nassariidae</em></td>
</tr>
<tr>
<td></td>
<td><em>Acteonidae</em></td>
</tr>
<tr>
<td></td>
<td><em>Acteocinidae</em></td>
</tr>
<tr>
<td></td>
<td><em>Pyramidellidae</em></td>
</tr>
<tr>
<td>Gastropoda</td>
<td><em>Nassarius</em> sp.</td>
</tr>
<tr>
<td></td>
<td><em>Acteon punctostriatus</em> (C.B. Adams)</td>
</tr>
<tr>
<td></td>
<td><em>Acteocina canaliculata</em> (Say)</td>
</tr>
<tr>
<td>Pelecypoda</td>
<td><em>Odostomia</em> sp.</td>
</tr>
<tr>
<td></td>
<td><em>Odostomia engonia</em> Bush</td>
</tr>
<tr>
<td></td>
<td><em>Turbonilla interrupta</em> (Totten)</td>
</tr>
<tr>
<td>Ostreidae</td>
<td><em>Grassostrea virginica</em> (Gmelin)</td>
</tr>
<tr>
<td>Lucinidae</td>
<td><em>Lucina multilineata</em> Tuomey &amp; Holmes</td>
</tr>
<tr>
<td>Tellinidae</td>
<td><em>Macoma</em> sp.</td>
</tr>
<tr>
<td></td>
<td><em>Macoma tenta</em> Say</td>
</tr>
<tr>
<td>Mactridae</td>
<td><em>Mulinia lateralis</em> (Say)</td>
</tr>
<tr>
<td>Arthropoda</td>
<td><em>Leucon americanus</em> Zimmer</td>
</tr>
<tr>
<td>(Cumacea)</td>
<td><em>Edotea triloba</em> (Say)</td>
</tr>
<tr>
<td>(Isopoda)</td>
<td><em>Ampelisca</em> sp.</td>
</tr>
<tr>
<td>(Amphipoda)</td>
<td><em>Ampelisca abdita</em> Mills</td>
</tr>
<tr>
<td></td>
<td><em>Idunella barnardi</em> (Wigley)</td>
</tr>
<tr>
<td></td>
<td><em>Pleusymtes glaber</em> (Boeck)</td>
</tr>
<tr>
<td></td>
<td><em>Leptocheirus plumulosus</em> Shoemaker</td>
</tr>
<tr>
<td></td>
<td><em>Corophium tuberculatum</em> Shoemaker</td>
</tr>
<tr>
<td>(Decapoda)</td>
<td><em>Ogyrides alphaerostris</em> (Kingsley)</td>
</tr>
<tr>
<td>Ogyrididae</td>
<td><em>Upogebia affinis</em> (Say)</td>
</tr>
<tr>
<td>Upogebiidae</td>
<td><em>Xanthidae</em></td>
</tr>
<tr>
<td>ni</td>
<td><em>Pseudoerythoe paucibranchiata</em> Fauvel</td>
</tr>
<tr>
<td>ni</td>
<td><em>Prlonospio perkinsi</em> Maciolek</td>
</tr>
<tr>
<td>ni</td>
<td><em>Scolelepidides viridis</em> (Verrill)</td>
</tr>
<tr>
<td>ni</td>
<td><em>Streblospio benedicti</em> Webster</td>
</tr>
<tr>
<td>ni</td>
<td><em>Spiochaetopterus costarum</em> (Gitay)</td>
</tr>
<tr>
<td>ni</td>
<td><em>Sabellaria</em> sp.</td>
</tr>
<tr>
<td>ni</td>
<td><em>Leitoscoloplos</em> sp.</td>
</tr>
<tr>
<td>ni</td>
<td><em>Cirratulidae</em></td>
</tr>
<tr>
<td>ni</td>
<td><em>Pectinariidae</em></td>
</tr>
<tr>
<td>ni</td>
<td><em>Terebellidae</em></td>
</tr>
<tr>
<td>ni</td>
<td><em>Sabellidae</em></td>
</tr>
<tr>
<td>ni</td>
<td><em>Oligochaeta</em></td>
</tr>
<tr>
<td>ni</td>
<td><em>Tubificidae</em></td>
</tr>
<tr>
<td>ni</td>
<td><em>Naididae</em></td>
</tr>
<tr>
<td>Phylum/ Class/ Family</td>
<td>Species</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Phyllostomidae</strong></td>
<td>Pinnixa sp.</td>
</tr>
<tr>
<td></td>
<td><em>Pinnixa chaetopterana</em> Stimpson</td>
</tr>
<tr>
<td>Phoronida</td>
<td><em>Phoronis</em> sp.</td>
</tr>
<tr>
<td>Ectoprocta</td>
<td>ni</td>
</tr>
<tr>
<td>Echinodermata</td>
<td>ni</td>
</tr>
<tr>
<td>Ophiuroidea</td>
<td>ni</td>
</tr>
<tr>
<td>Hemichordata</td>
<td>ni</td>
</tr>
<tr>
<td>Enteropneusta</td>
<td>ni</td>
</tr>
<tr>
<td>Chordata</td>
<td><em>Holgula manhattensis</em> (Dekay)</td>
</tr>
</tbody>
</table>


Jørgensen, B.B. 1980. Seasonal oxygen depletion in the bottom waters of a Danish fjord and its effect on the benthic community. Oikos 34:68-76.


Olson and F.J. Burgess (eds.), Pollution and Marine Ecology, Interience Publisher, New York, N.Y., USA.


Whitlatch, R.B. 1977. Seasonal changes in the community structure of the

VITA

ROBERTO JAVIER LLANSO

Born in Palamós, Gerona (Spain), on 7 November 1955. Received B.S. in Zoology from Universidad Complutense de Madrid in 1979, and Grado de Licenciatura in 1980. Earned M.A. in Marine Science from College of William and Mary, School of Marine Science in 1985. Entered doctoral program as a graduate assistant in the Department of Geological and Benthic Oceanography, School of Marine Science, College of William and Mary, in 1986.