United States
Environmental Protection
Agency

Office of Water
4304

EPA-822-R-00-012
November 2000

Ambient Aquatic Life Water Quality Criteria for Dissolved Oxygen (Saltwater): Cape Cod to Cape Hatteras

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U.S. Environmental Protection Agency

Office of Water
Office of Science and Technology
Washington, DC

Office of Research and Development
National Health and Environmental Effects Research Laboratory
Atlantic Ecology Division
Narragansett, Rhode Island

Notices

This document has been reviewed by the Atlantic Ecology Division, Narragansett, RI (Office of Research and Development) and the Office of Science and Technology (Office of Water), U.S. Environmental Protection Agency, and approved for publication.

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Acknowledgments

This document was written by Glen Thursby, Don Miller, Sherry Poucher (Science Applications International Corporation), Laura Coiro, Wayne Munns, and Timothy Gleason. Comments on two earlier versions of this document by Richard Batiuk (EPA's Chesapeake Bay Program), Charles Delos, Keith Sappington (both from EPA's Office of Water), and Walter Berry, Wayne Davis, and Diane Nacci (all from EPA's Atlantic Ecology Division) improved the contents of the current version. The current version also addresses comments by six peer reviewers. These include Larry Brooke, Daniel Call, Gary Chapman, William Collins and Tyler Linton of the Great Lakes Environmental Center (GLEC), Traverse City, MI, and Stephan Jordan from the Maryland Department of Natural Resources. Useful discussions on several aspects of the final criteria also were held with David J. Hansen of GLEC. Several individuals were involved with the successful completion of many of the bioassays conducted at EPA's Atlantic Ecology Division. These include Steven Rego, Kathy Simmonin, and Nan Hayden. Kenneth A. Rahn provided valuable editorial comments for the final version.

Executive Summary

This document recommends an approach to deriving the lower limits of dissolved oxygen (DO) necessary to protect coastal and estuarine animals in the Virginian Province (Cape Cod, MA, to Cape Hatteras, NC). The information on hypoxic effects used here was obtained from studies conducted by the USEPA's Atlantic Ecology Division specifically for this purpose, and from all other available reports applicable to hypoxic issues of the Virginian Province. Hypoxia is defined here as concentrations of DO that are below saturation. Literature on the effects of anoxia, while applicable to certain ecological risk analyses, was not included in this document. This approach combines features of traditional water quality criteria with a new biological framework that integrates time (replacing the concept of an averaging period) and establishes separate criteria for different life stages (larvae versus juveniles and adults). Where practical, data were selected and analyzed in a manner consistent with the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan et al., 1985). This document considers how to protect three aspects of biological health: survival of juveniles and adults, growth, and larval recruitment (estimated with a generic model).

The recommended criteria described here apply to both continuous (persistent) and cyclic (diel, tidal, or episodic) hypoxia. If the DO exceeds the chronic protective value for growth (4.8 mg/L), the site meets objectives for protection. If the DO is below the limit for juvenile and adult survival (2.3 mg/L), the site does not meet objectives for protection. When the DO is between these values, the site requires evaluation of duration and intensity of hypoxia to determine suitability of habitat for the larval recruitment objective.

The limits identified are based entirely on laboratory findings but are supported in part by field observations. For example, juvenile and adult animals showed field acute effects at <2.0 mg/L, below the limit of 2.3 mg/L for juveniles and adults. Also, behavioral effects were generally seen in the range of laboratory sublethal effects. Unfortunately, however, no field observations are available for survival and growth of larvae that are sensitive to hypoxia. This type of information is critical because two of the three criteria are derived from laboratory responses of larvae.

Hypoxia as a stressor differs from chemical toxicants in that it can occur naturally and because it is not controlled directly, whereas toxic chemicals are. Instead, hypoxia is regulated primarily by controlling nutrients (largely nitrogen) and other oxygen-demanding wastes. Criteria for DO may be used appropriately in a risk assessment framework. The limits presented by the approach outlined here can be easily used to compare the abilities of different areas to support aquatic life. Environmental managers can determine which sites need the most attention, and how hypoxic problems vary in time and space from one year to the next. Finally, environmental planners can make better cost-benefit decisions by using this approach to evaluate how various management scenarios will improve conditions.

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Introduction

This document provides guidance to States and Tribes authorized to establish water quality standards under the Clean Water Act (CWA) concerning dissolved oxygen (DO) values that protect aquatic life from acute and chronic effects. Under the CWA, States and Tribes are to establish water quality criteria to protect designated uses. While this document constitutes the U.S. Environmental Protection Agency's (EPA's) scientific recommendations regarding ambient concentrations of dissolved oxygen that protect saltwater aquatic life in the Virginian Province, this document does not substitute for the CWA or EPA's regulations, nor is it a regulation itself. Thus, it cannot impose legally binding requirements on EPA, States, Tribes, or the regulated community, and may not apply to a particular situation based upon the circumstances. State and Tribal decisionmakers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate. EPA may change this guidance in the future.

Section 304 (a)(2) of the CWA calls for information on the conditions necessary "to restore and maintain biological integrity of all . . . waters, for the protection and propagation of shellfish, fish and wildlife, to allow recreational activities in and on the water, and to measure and classify water quality." EPA has not previously issued saltwater criteria for DO because the available information on effects was insufficient. This document is the result of a 10-year research effort to produce the required information to support the development of saltwater DO criteria. During that effort there were several technical work group meetings involving stakeholders and external scientists that helped to guide the process. The criteria presented herein represent the best estimates, based on the available data, of DO concentrations necessary to protect aquatic life and its uses.

These water quality criteria recommendations apply to coastal waters (waters within territorial seas, defined as within 3 miles from shore under Section 502(8) of the CWA) of the Virginian Province (southern Cape Cod to Cape Hatteras). However, with appropriate modification, they may be applied to other coastal regions of the United States. The document provides the information necessary for environmental planners and regulators in the Virginian Province to decide whether the DO at a given site can protect coastal or estuarine aquatic life. The approach can be used to evaluate existing localized DO goals (e.g., Jordan et al., 1992) or to establish new ones. This document does not address direct behavioral responses (i.e., avoiding low DO) or the ecological consequences of behavioral responses such as changes in predation rates or in community structures. The document also does not address the issue of spatial extent of a DO problem. A given site may have DO conditions expected to cause a significant effect on aquatic life, however; the environmental manager will have to judge whether the spatial extent of the low DO area is sufficient to warrant concern. The approach presented here for deriving criteria is expected to work for other regions. However, additional regionally specific data may be required in order to amend the database for use in other regions. Animals may have adapted to lower oxygen in locations where high temperatures have historically reduced concentrations, or in systems with natural high demands for oxygen.

In addition, effects of hypoxia[1] may vary latitudinally, or site-specifically, particularly as reproductive seasons determine risks of exposure for sensitive early life stages.

As with the freshwater DO document (U.S. EPA, 1986), all data and criteria are expressed in terms of the actual amount of DO available to aquatic organisms in milligrams per liter (mg/L). Unlike the freshwater document, which provides limits for DO in both warm and cold water, criteria are presented for warm saltwater only because hypoxia in Virginian Province coastal waters is restricted primarily to the warm water of summer. However, these warm-water limits can be considered protective for colder times of the year. Also, the freshwater criteria are based almost entirely on fish data even though insects were often more sensitive than fish. The saltwater limits, on the other hand, use data from fish and invertebrates.

The saltwater DO criteria described herein were derived using the *Guidelines*[2] and are intended to maintain and support aquatic life communities and their designated uses. Although the criteria are intended to protect aquatic communities, they rely primarily on data generated at the organism level, and emphasize data for the most sensitive life stage. But a population of a given species can potentially withstand some mortality to certain life stages without a significant long-term effect on the population. Hence, an assessment of criteria should preferably include population-level considerations. One nuance of population-level assessment is the fact that a population's sensitivity to hypoxia may depend on which stages have been exposed. For example, many populations of marine organisms may be more impacted by mortality occurring during the juvenile and adult stages than during the larval stage(s). In this regard, a particular individual larva is not as important to the population as a particular individual juvenile or adult. With this in mind, the saltwater criteria for DO segregate effects on juveniles and adults from those on larvae. The survival data on the sensitivity of the former are handled in a traditional *Guidelines* manner. The cumulative effects of low DO on larval recruitment to the juvenile life stage, on the other hand, address survival effects on larvae. The DO approach presented here uses a mathematical model to evaluate the effect on larvae by tracking intensity and duration effects across the larval recruitment season. The model is used to generate a DO criterion for larval survival as a function of time. It is recommended that the parameters for this model be evaluated and adjusted where necessary to meet site-specific conditions, especially those for length of recruitment season and larval development time.

For the reasons listed above, the approach recommended in this document to derive DO criteria for saltwater animals deviates from EPA's traditional approach for toxic chemicals outlined in the *Guidelines*. Where practical, however, data selection and analytical procedures are consistent with the *Guidelines*. Therefore, some of the

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[1]Hypoxia is defined in this document as the reduction of DO concentrations below air saturation.

[2]*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan et al., 1985—hereafter referred to as the *Guidelines*).

terminology and the calculation procedures are the same. Thus, knowing the *Guidelines* is useful (but not essential) for better understanding how the limits were derived. Terminology from the *Guidelines* used here includes species mean acute value (SMAV), genus mean acute value (GMAV), final acute value (FAV), genus mean chronic value (GMCV), and final chronic value (FCV). Procedures from the *Guidelines* include those for calculating FAVs, criterion maximum concentration[3] (CMC), and criterion continuous concentration (CCC).

Overview of the Problem

EPA's Environmental Monitoring and Assessment Program (EMAP) for the estuaries in the Virginian Province has shown that 25% of its area is exposed to some degree to DO concentrations less than 5 mg/L (Strobel et al., 1995). EMAP has also generated field observations that correlate biological degradation in many benthic areas with low DO in the lower water column (Paul et al., 1997). The two reports serve to emphasize that low DO is a major concern within the Virginian Province. Even though hypoxia is a major concern, a strong technical basis for developing benchmarks for effects of low DO have been lacking.

Hypoxia in the Virginian Province is essentially a warm-water phenomenon. In the southern portions of the Province, such as the Chesapeake Bay and its tributaries, DO may be reduced any time between May and October; in the more northern coastal and estuarine waters, any time from late June into September. Hypoxic events may be seasonal or diel. Seasonal hypoxia often develops as stratified water prevents the oxygenated surface water from mixing downward. Low DO then appears in the lower waters when respiration in the water and sediment depletes oxygen faster than it can be replenished. As summer progresses, the areas of hypoxia expand and intensify, then disappear as the water cools in the fall. The cooler temperatures eliminate the stratification and allow the surface and bottom waters to mix. Diel cycles of hypoxia often appear in unstratified shallow habitats where nighttime respiration can temporarily deplete DO.

Although the primary fauna at risk from exposure to hypoxia in the Virginian Province are summer inhabitants of subpycnocline[4] (i.e., bottom) waters, hypoxia can occur in other habitats as well. For example, upwelling may permit subpycnocline, oxygen-poor water to intrude into shallow areas. Hypoxia also may appear in the upper water of eutrophic water bodies on calm, cloudy days, when more oxygen is consumed than is produced by photosynthesis and when atmospheric reaeration is limited. In spite of this tendency, however, minima in DO are generally less severe above the pycnocline

[3]Although in the case of dissolved oxygen, CMC is more appropriately defined as the criterion *minimum* concentration.

[4]The pycnocline is the region of density discontinuity in a stratified water column between surface and bottom waters. The density difference between the two is primarily due to differences in temperature and salinity.

than below it. Hypoxia above the pycnocline also tends to be more transient because it largely depends on weather patterns.

Hypoxia may persist more or less continuously over a season (with or without a cyclic component) or be episodic (i.e., of irregular occurrence and indefinite duration). Continuous hypoxia without a cyclic component is exemplified in the subpycnocline waters of western Long Island Sound and off the New Jersey coast (Armstrong, 1979). Hypoxia in Long Island Sound may be interrupted temporarily by major storms, but returns 1 or 2 weeks later, when the waters again become stratified (Welsh et al., 1994).

Hypoxia may oscillate with tidal, diel, or lunar frequencies. Tidal hypoxia is common in subpycnocline waters of the mesohaline Chesapeake Bay main stem and the mouth of the adjacent tributaries during summer (Sanford et al., 1990; Diaz et al., 1992). In this case, DO concentrations oscillate as the tides alternately advect poorly oxygenated subpycnocline water from the mid-bay trough or tributaries and better oxygenated water from the lower bay. Diel cycles of hypoxia are found in small eutrophic embayments and harbors all along the coast of the Virginian Province, where oxygen is depleted overnight by respiration and replenished by photosynthesis after dawn. The Childs River is an example of diel hypoxia (D'Avanzo and Kremer, 1994). Lunar cycles of oxygen may occur in various systems but have been documented most clearly at the mouths of some Chesapeake Bay tributaries, where destratification from spring tides saturates the water with oxygen and stratification afterward depletes the oxygen (Haas, 1977; Kuo et al., 1991; Diaz et al., 1992).

Episodic hypoxia has been noted in shoal waters of mid-Chesapeake Bay (Breitburg, 1990) and in adjacent tributaries (Sanford et al., 1990). Persistent winds tilt the pycnocline laterally and displace low DO water onto the shoals or tributaries indefinitely. As noted above, DO may also be reduced episodically in eutrophic surface waters, particularly during calm and cloudy weather, when photosynthesis is slow and daytime reoxygenation is reduced.

Biological Effects of Low Dissolved Oxygen

Oxygen is essential in aerobic organisms for the electron transport system of mitochondria. Oxygen insufficiency at the mitochondria results in reduction in cellular energy and a subsequent loss of ion balance in cellular and circulatory fluids. If oxygen insufficiency persists, death will ultimately occur, although some aerobic animals also possess anaerobic metabolic pathways, which can delay lethality for short time periods (minutes to days). Anaerobiosis is well developed in some benthic animals, such as bivalve molluscs and polychaetes, but not in other groups, like fish and crustaceans (Hammen, 1976). There is no evidence that any free-living animal inhabiting coastal or estuarine waters can live without oxygen indefinitely.

Many aquatic animals have adapted to short periods of hypoxia and anaerobiosis by taking up more oxygen and transporting it more effectively to cells and mitochondria, that is, by ventilating its respiratory surfaces more intensely and increasing its heart rate. If

these responses are insufficient to maintain the blood's pH, the oxygen-carrying capacity of the respiratory pigment will decrease. An early behavioral response might be moving faster toward better oxygenated water. However, if the hypoxia persists, the animal may reduce its swimming and feeding, which will reduce its need for energy and hence oxygen. Such reduced motor activity may make the animal more tolerant over the short term, but will not solve its long-term problem. For example, even the modest reductions in locomotion required by mild hypoxia may make the animal more vulnerable to predators, and the reduced feeding may decrease its growth.

Compensatory adaptations are well developed in marine animals that commonly experience hypoxia, for example, intertidal and tide pool animals (McMahon, 1988) and burrowing animals, which partly explains their reported high tolerance to low DO. In contrast, compensatory adaptations are poorly developed in animals that inhabit well-oxygenated environments such as the upper water column. The animals most sensitive to hypoxia are among this latter group. Details on compensatory adaptations to hypoxia are provided in reviews for marine animals (Vernberg, 1972), aquatic invertebrates (Herreid, 1980), and fish (Holeton, 1980; Hughes, 1981; Kramer, 1987; Rombough, 1988a; Heath, 1995).

Overview of the Approach

The approach to determine the limits of DO that will protect saltwater animals within the Virginian Province considers both continuous (i.e., persistent) and cyclic (e.g., diel) exposures to low DO. The continuous situation is covered first, and deals with exposures longer than 24 hr. It is followed by sections on criteria for exposures of less than 24 hr but that may be repeated for days. Both scenarios cover three areas of protection (summarized here, and explained in more detail in the sections that follow):

1. *Juvenile and adult survival*—A lower limit is calculated for continuous exposures by using FAV calculation procedures outlined in the *Guidelines* (Stephan et al., 1985), but with data for only juvenile or adult stages. Limits for cyclic exposures are derived from an appropriate time-to-death curve for exposures less than 24 hr.

2. *Growth effects*—A threshold above which long-term, continuous exposures should not cause unacceptable effects is derived from growth data (mostly from bioassays using larvae). This FCV is calculated in the same manner as the FAV for juvenile and adult survival. This threshold limit as currently presented has no time component (it can be applied to exposures of any duration). Cyclic exposures are evaluated by comparing reductions in laboratory growth from cyclic and continuous exposures.

3. *Larval recruitment effects*—A larval recruitment model was developed to project cumulative loss caused by low DO. The effects depend on the intensity and the duration of adverse exposures. The maximum acceptable reduction in seasonal recruitment was set at 5% (although other percentages also may be

appropriate on a site-specific basis), which is equivalent to the protective limit for juvenile and adult survival. The number of acceptable days of seasonal exposure to low DO decreases as the severity of the hypoxic condition increases. The severity of cyclic exposure is evaluated with a time-to-death model (as in the protective limit for juveniles and adults).

Persistent Exposure to Low Dissolved Oxygen

Juvenile and Adult Survival

Data were used from tests with exposure ranging from 24 to 96 hr. This maximized the number of genera for the FAV calculation. Data for juveniles show that LC50 values calculated for 24 and 96 hr observations are very similar (Figure 1); therefore, all values are applied as 24 hr data. The restriction of the data set to tests of 96 hr duration or less was somewhat arbitrary; however, 96 hr is the duration used for most acute tests for traditional water quality criteria (Stephan et al., 1985). In addition, there are insufficient test data to compare 24 hr exposures versus those longer than 96 hr. Juvenile and adult mortality data from exposures longer than 96 hr are compared to the final criterion in the section, Other Laboratory Bioassay Data.

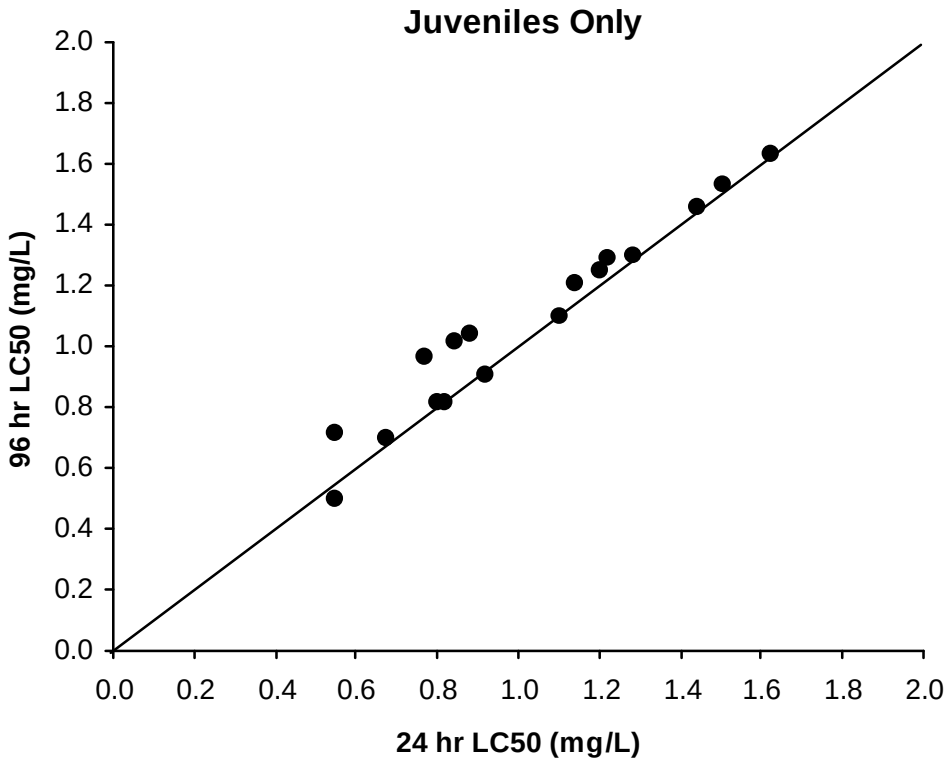

Figure 1. Relationship between 24 and 96 hr LC50 values for juvenile saltwater animals exposed to continuous low DO. Each point represents a paired set of values calculated from the same test run. The line drawn represents a one-to-one relationship. Data for the plot are summarized by species in Appendix A. Appendix A also contains data for test runs with larvae.

Data on the acute sensitivity of juvenile and adult saltwater animals to low DO are available for 12 invertebrate and 11 fish species (almost all of the data are for juveniles). The values are summarized in Table 1 and Appendix B. Overall GMAVs range from <0.34 mg/L for the green crab, *Carcinus maenas,* to 1.63 mg/L for the pipe fish, *Syngnathus fuscus*, a factor greater than 4.8. Juvenile fish are somewhat more sensitive than juvenile crustaceans (Table 1; Figure 2). In fact, the four most sensitive genera are all fish, and the range of values for these is 1.32 to 1.63 mg/L, a ratio of only 1.2.

As stated previously, the criterion for juveniles and adults exposed to continuous low DO was calculated using the *Guidelines* procedures for derivation of an FAV (Stephan et al., 1985). However, the procedures outlined in the *Guidelines* were created for toxicants. Since DO behaves in a manner opposite to that of toxicants (i.e., the greatest response is associated with the lowest concentrations), the calculation is reversed. The FAV calculation is essentially a linear regression using the LC50 values for the four most sensitive genera and their respective percentile ranks. The final FAV is the value representing the 95th percentile genus,[5] which for DO is 1.64 mg/L. This value is adjusted to a criterion of 2.27 mg DO/L by multiplying by 1.38, the average LC5 to LC50 ratio[6] for juveniles (Table 1). This value is analogous to the CMC in traditional Water Quality Criteria for toxicants.

Growth Effects

A threshold above which long-term, continuous exposures to low DO should not cause unacceptable effects was calculated with growth data (mostly from bioassays using larvae). Sublethal effects were evaluated with only growth data for two reasons. First, growth is generally more sensitive than survival to low DO. There were only two exceptions where survival was more sensitive to low DO than growth. One test was with *Dyspanopeus sayi*; however, growth was the more sensitive endpoint in eight other tests with this species (Appendix C). The results from this one test were not included in Table 2. The other exception was a 28-day early life stage test using the Atlantic silverside, *Menida menidia* (Appendix C). There was no effect at 4.8 mg/L DO, but there were 40% mortality and a 24% reduction in growth at a DO concentration of 3.9 mg/L. This 24% reduction in growth, however, was not statistically significant. There was essentially no growth of surviving *M. menidia* at a DO concentration of 2.8 mg/L. Only the growth data were summarized in Table 2.

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[5]The standard calculation for toxicants in the *Guidelines* uses the fifth percentile. The 95th percentile is used here because, unlike toxicants, DO effects decrease as the concentration of DO increases.

[6]The use of a ratio to adjust the FAV to a CMC is designed to estimate a negligible lethal effect concentration corresponding to the 5th percentile species. It may in fact represent an adverse effect concentration for species more sensitive than the 5th percentile. The *Guidelines* use a factor of 2; however, there were sufficient data available for low DO to use a factor specific to this stressor. There was not a significant relationship between genus sensitivity and the LC5/LC50 ratio; therefore, all ratios were included in the calculation of the final ratio.

Table 1. Acute sensitivity of juvenile and adult saltwater animals to low dissolved oxygen. Exposure durations ranged from 24 to 96 hr. Data from individual tests are presented in Appendix B.

Species	Common Name	Life Stage	SMAV LC50[a]	SMAV LC5	SMAV LC5/ LC50	GMAV LC50	GMAV LC5	GMAV LC5/LC50	GMAV Rank[b]
Carcinus maenus	green crab	Juvenile/Adult	< 0.34			< 0.34			1
Spisula solidissima	Atlantic surfclam	Juvenile	0.43	0.70	1.63	0.43	0.70	1.63	2
Rithropanopeus harrisii	Harris mud crab	Juvenile	0.51			0.51			3
Prionotus carolinus	northern sea robin	Juvenile	0.55	0.80	1.45	0.55	0.80	1.45	4
Eurypanopeus depressus	flat mud crab	Juvenile	0.57			0.57			5
Leiostomus xanthurus	spot	Juvenile	0.70	0.81	1.16	0.70	0.81	1.16	6
Tautoga onitis	tautog	Juvenile	0.82	1.15	1.40	0.82	1.15	1.40	7
Palaemonetes vulgaris	marsh grass shrimp	Juvenile	1.02	1.4	1.37	0.86	1.24	1.45	8
Palaemonetes pugio	daggerblade grass shrimp	Juvenile	0.72	1.1	1.53				
Ampelisca abdita	amphipod	Juvenile	< 0.9			< 0.9			9
Scopthalmus aquosus	windowpane flounder	Juvenile	0.81	1.20	1.48	0.90	1.20	1.48	10
Apeltes quadracus	fourspine stickleback	Juvenile/Adult	0.91	1.20	1.32	0.91	1.20	1.32	11
Homarus americanus	American lobster	Juvenile	0.91	1.6	1.76	0.91	1.6	1.76	12
Crangon septemspinosa	sand shrimp	Juvenile/Adult	0.97	1.6	1.65	0.97	1.6	1.65	13
Callinectes sapidus	blue crab	Adult	< 1.0			< 1.0			14
Brevoortia tyrannus	Atlantic menhaden	Juvenile	1.12	1.72	1.53	1.12	1.72	1.53	15
Crassostrea virginica	eastern oyster	Juvenile	< 1.15			< 1.15			16
Stenotomus chrysops	scup	Juvenile	1.25			1.25			17
Americamysis bahia	mysid	Juvenile	1.27	1.50	1.16	1.27	1.50	1.16	18
Paralichthys dentatus	summer flounder	Juvenile	1.32	1.57	1.19	1.32	1.57	1.19	19
Pleuronectes americanus	winter flounder	Juvenile	1.38	1.65	1.20	1.38	1.65	1.20	20
Morone saxatilis	striped bass	Juvenile	1.58	1.95	1.23	1.58	1.95	1.23	21
Syngnathus fuscus	pipe fish	Juvenile	1.63	1.9	1.17	1.63	1.9	1.17	22

Final Acute Value= 1.64 mg/L
Mean LC5/LC50 Ratio= 1.38
CMC = 1.64 mg/L x 1.38 = 2.27 mg/L

[a]SMAVs (Species Mean Acute Values) and GMAVs (Genus Mean Acute Values) are all geometric means (Stephan et al., 1985).

[b]Ranked by LC50 GMAV.

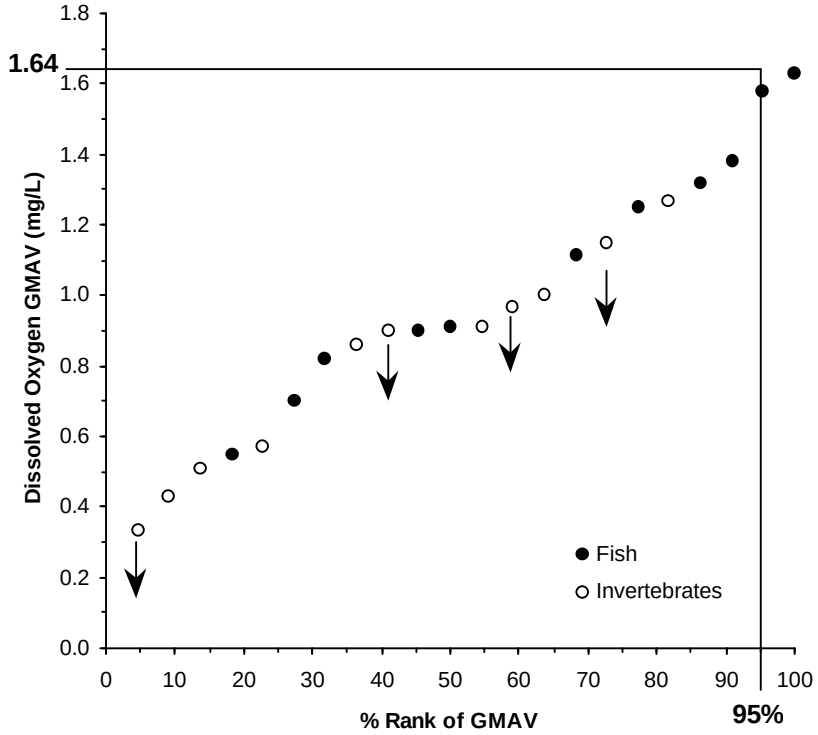

Figure 2. Plot of low DO effect (GMAVs for LC50s) against percentile rank of each value in the data set. Values for each genera are listed in Table 1. Results from individual tests for each species are listed in Appendix B. The value highlighted on the y-axis is the calculated FAV. This value is the LC50 that is higher than the values for 95% of the tested genera. The LC50 values for the four most sensitive genera are the only values used in the FAV calculation other than the total number ("n") of values. Arrows refer to those values that are less thans.

The second reason for restricting sublethal effects to growth is that results are available from only one saltwater test that measured reproductive effects. Data are presented in Appendix C from a 28-day life cycle test using the mysid, *Americamysis bahia*. Although growth was reduced 25% at 3.17 mg/L and was technically the most sensitive endpoint in this test, the percentage reduction in growth was essentially the same at 2.76 and 2.17 mg/L as it was at 3.17 mg/L (20% and 27%, respectively). Reproduction was reduced by 76% at 2.17 mg/L, the first treatment that resulted in a significant effect on this endpoint. Although this test suggests that growth is more sensitive than reproduction, there are insufficient data to confirm this conclusion for saltwater species. Data from two standardized freshwater tests, however, indicate that growth is more sensitive than reproduction for both fathead minnows (Brungs, 1971) and *Daphnia magna* (Homer and Waller, 1983). Thus, DO limits that protect against growth effects also may be protective for reproductive effects.

Table 2. Effects of low dissolved oxygen on growth of saltwater animals. Data from individual tests are presented in Appendix C.

Species	Common Name	Life Stage	Duration (days)	NOEC[a]	HOEC[a]	Chronic Value	Geo-Mean	Rank[b]
Cyprinodon variegatus	sheepshead minnow	larval	14	2.5	1.5	1.94	> 1.97	12
Cyprinodon variegatus	sheepshead minnow	larval	7	7.5	2.0	> 2.00		
Americamysis bahia	mysid	<48 hr old juvenile	10	2.4	1.6	1.96	2.67	13
Americamysis bahia	mysid	<48 hr old juvenile	28	4.17	3.17	3.64		
Morone saxatilis	striped bass	juvenile	21	2.8		< 2.8	< 2.8	14
Cancer irroratus	Atlantic rock crab	larval stage 5 to megalopa	7	3.42	2.41	2.87	2.87	15
Palaemonetes vulgaris	marsh grass shrimp	newly hatched	8	6.71	3.42	4.79	3.15	16
Palaemonetes vulgaris	marsh grass shrimp	<16 hr old	7	5.40	3.77	4.51		
Palaemonetes vulgaris	marsh grass shrimp	<16 hr old	8	6.94	3.20	4.71		
Palaemonetes vulgaris	marsh grass shrimp	larval stage 1 to 3	7	2.30	1.56	1.89		
Palaemonetes vulgaris	marsh grass shrimp	postlarval	14	3.57	2.59	3.04		
Palaemonetes vulgaris	marsh grass shrimp	postlarval	14	3.42	2.17	2.72		
Palaemonetes vulgaris	marsh grass shrimp	postlarval	14	2.5	1.51	1.94		
Mercenaria mercenaria	northern quahog	embryo	14	4.2	2.4	3.17	3.17	17
Menidia menidia	Atlantic silverside	embryo to larva	28	3.9	2.8	3.30	3.30	18
Paralichthys dentatus	summer flounder	newly metamorphosed juvenile	14	4.53	3.53	4.00	3.97	19
Paralichthys dentatus	summer flounder	newly metamorphosed juvenile	14	4.39	3.39	3.86		
Paralichthys dentatus	summer flounder	newly metamorphosed juvenile	14	7.23	4.49	5.70		
Paralichthys dentatus	summer flounder	newly metamorphosed juvenile	10	4.4	1.8	2.81		
Homarus americanus	American lobster	larval stage 2 to 3	4	5.4	3.9	4.59	4.47	20
Homarus americanus	American lobster	larval stage 2 to 3	4	5.0	3.7	4.30		
Homarus americanus	American lobster	larval stage 3 to 4	4	7.7	5.45	6.48		
Homarus americanus	American lobster	larval stage 3 to 4	4	4.9	3.8	4.32		
Homarus americanus	American lobster	larval stage 3 to 4	6	5.25	4.22	4.71		
Homarus americanus	American lobster	postlarval stage 4 to 5	20	7.51	3.45	5.09		
Homarus americanus	American lobster	juvenile stage 5 to 6	27	3.50	1.53	2.31		
Homarus americanus	American lobster	juvenile stage 5 to 6	29	7.61	3.54	5.19		
Dyspanopeus sayi	Say mud crab	<48 hr old	8	6.81	4.21	5.35	4.67	21
Dyspanopeus sayi	Say mud crab	larval stage 1 to 3	7	3.31	2.45	2.85		
Dyspanopeus sayi	Say mud crab	larval stage 1 to 3	7	7.65	3.39	5.09		
Dyspanopeus sayi	Say mud crab	larval stage 1 to 3	7	4.46	3.51	3.96		
Dyspanopeus sayi	Say mud crab	larval stage 3 to 4	7	6.27	5.00	5.60		
Dyspanopeus sayi	Say mud crab	larval stage 3 to megalopa	4	5.44	4.40	4.89		
Dyspanopeus sayi	Say mud crab	larval stage 3 to megalopa	8	5.78	4.68	5.20		
Dyspanopeus sayi	Say mud crab	larval stage 3 to megalopa	10	5.47	4.40	4.91		
Dyspanopeus sayi	Say mud crab	larval stage 3 to megalopa	11	7.54	3.23	4.93		
Labinia dubia	longnose spider crab	larval stage 1 to 2	7	5.30	4.11	4.67	4.67	22

[a]NOEC= no observed effect concentration; HOEC=highest observed effect concentration.

[b]Ranked by geometric means.

Data on the effects of hypoxia on growth are presented for 4 species of fish and 7 species of invertebrates from a total of 36 tests. Sensitivity of growth to low DO has been determined in only two standard 28-day tests that meet *Guidelines* requirements; the above life cycle test with *A. bahia* and the above early life stage test with *M. menidia*. Therefore, growth data from nonstandard tests (i.e., not life cycle, partial life cycle, or early life stage tests) were used to augment the chronic database. These nonstandard tests ranged from 4 to 29 days long. Data from short duration tests were included because effects of oxygen deprivation are assumed to be instantaneous. Oxygen is required continuously for the efficient production of cellular energy. Therefore, even modest reductions in DO may result in the redirection of energy use from growth to compensatory mechanisms. In addition, data from larval growth of two bivalves (Morrison, 1971; Wang and Widdows, 1991) and several fish and crustaceans (Appendix C) show that chronic values for DO do not change substantially for exposures ranging from a few days to several weeks for most of the species tested. The *Mercenaria mercenaria* (Morrison, 1981) and *Mytilis edulis* (Wang and Widdows, 1991) studies show that the effect on larval bivalve growth within the same test run is the same over a series of days (13 days for *M. mercenaria* and 6 to 10 days for *M. edulis*).

Overall GMCVs for effects on growth range from >1.97 for the sheepshead minnow, *Cyprinodon variegatus,* to 4.67 mg/L for the longnose spider crab, *Labinia dubia*, a ratio of <2.4. Three of the most sensitive species were crustaceans (Figure 3; Table 2). The range of chronic values for the four most sensitive genera is 3.97 to 4.67 species in the Virginian Province.[7] The consequences of reduced growth in the field, however, are uncertain.

Larval Recruitment Effects

A generic model has been developed that evaluates the cumulative effects of stresses on early life stages of aquatic organisms. Early life history information and exposure-response relationships are integrated with duration and intensity of exposure to provide an ecologically relevant measure of larval recruitment. There are existing recruitment models for marine organisms (e.g., Ricker, 1954; Beverton and Holt, 1957). However, these models address other processes such as parental stock size, population fecundity, and density-dependent processes such as cannibalism and intraspecific competition. These existing models therefore are not appropriate for the needs of the DO document, which requires incorporation of abiotic stressor effects.

Larvae are more acutely sensitive to low DO than juveniles (Figure 4). A method is provided that estimates how many days a given DO concentration can be tolerated

[7]However, the CCC represents the potential for an approximate 25% reduction in growth. The CCC for growth is based on statistically significant differences that result in chronic values similar to IC25s for growth of many organisms. IC25 values are listed as a part of Appendix C for four species of crustaceans and two species of fish. The geometric mean of these values (by species) correlates with the geometric mean of the chronic values. In fact, a CCC calculated using IC25 values is similar to the CCC calculated using statistically significant differences.

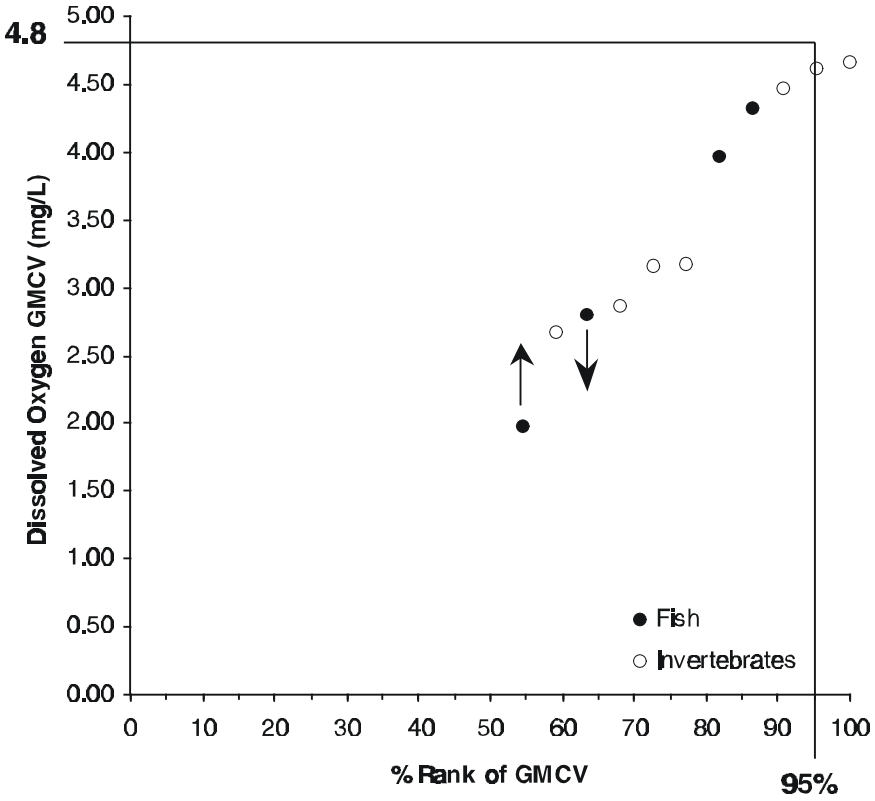

Figure 3. Plot of low DO effect (GMCVs for growth) against percentile rank of each value in the data set. Percentile rank was adjusted based on the total "n" from the acute data set (see text for explanation). Specific values for each genus included are listed in Table 2. Results from individual tests for each species are listed in Appendix C. The value highlighted on the y-axis is the calculated FCV. This value is the chronic value that is higher than the values for 95% of the species represented. The chronic values for the four most sensitive genera are the only values used in the FCV calculation other than the total number ("n") of values. Arrows refer to less than and greater than

without causing unacceptable effects on total larval survival for the entire recruitment season. This is accomplished with a larval recruitment model[8] and applying biological and hypoxic effect parameters for each species for which sufficient data are available. The level of impairment to cumulative seasonal larval recruitment that has been selected as acceptable is 5%. This does not mean that a population cannot withstand a greater percentage effect with no significant effect on recruitment. Rather, the 5% means that this level of effect should be insignificant relative to recruitment in the absence of hypoxic events. Many juveniles will eventually be eaten as prey or otherwise harvested as adults. The 5% impairment is intended to minimize the effect of hypoxia on the ultimate fate of juveniles. On the other hand, this may not be the case for certain highly sensitive species or populations that are already highly stressed, for example an endangered species. This

[8]Once the larvae are "recruited" into the juvenile life stage, the juvenile survival criterion established above is applied.

may also not be the case where there are other important natural or anthropogenic stressors that contribute to a loss of the larval life stage. In such situations, it may be that a 5% loss in larval recruitment from DO alone is not protective enough, and environmental risk managers may need to evaluate the province-wide 5% protection goal in light of their site-specific factors that may contribute to a cumulative loss in seasonal larval recruitment. States and authorized Tribes may choose a different level of acceptable impairment, but they must justify doing so and show that the new level of impairment still protects and maintains designated uses.

The equations that compose the model and the major assumptions used in its application are presented and explained in detail in Appendix E. The life history parameters in the model include larval development time, larval season, attrition rate, and vertical distribution. The magnitude of effects on recruitment is influenced by each of the four life history parameters. For instance, larval development time establishes the number of cohorts that entirely or partially co-occur with the interval of low DO stress. The second parameter, the length of the larval season, is a function of the spawning period, and also influences the relative number of cohorts that fall within the window of hypoxic stress. The third life history variable, natural attrition rate, gages the impact of slower growth and development of the larvae in response to low DO by tracking the associated increase in natural mortality (e.g., predation). The model assumes a constant rate of attrition, so increased residence time in the water column due to delayed development translates directly to decreased recruitment. Finally, the vertical distribution of larvae in

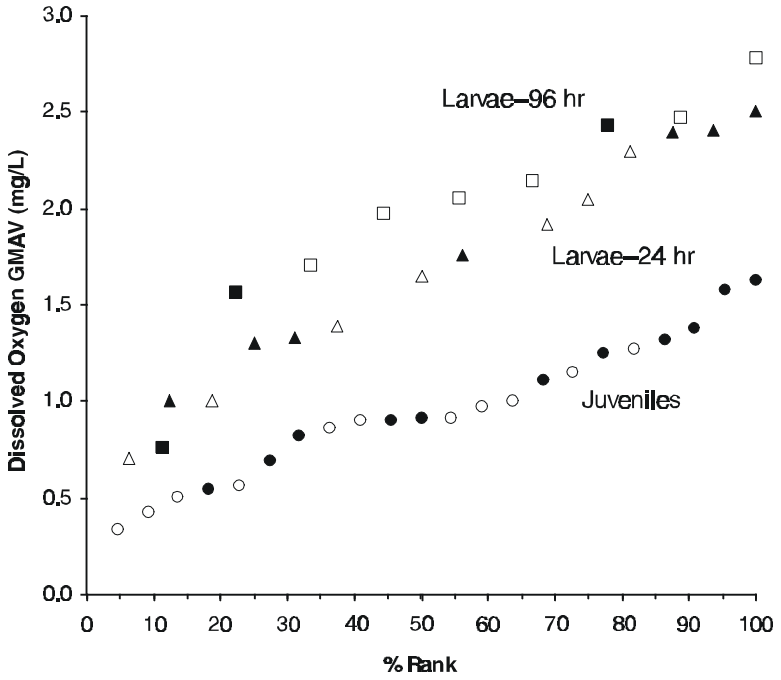

Figure 4. Plot of the GMAV data from Figure 2 (circles) along with 24 hr (triangles) and 96 hr (squares) LC50 values for larval life stages of various saltwater animals. The open symbols are for invertebrates and the closed for fish. The data for the juveniles are from Table 1. The data for the larvae are listed in Appendix D. Data points are plotted as absolute values even though some are less thans.

the water column determines the percentage of larvae that would be exposed to reduced DO under stratified conditions.

For the purpose of the Virginian Province criterion, certain simplifying assumptions have been made. The recruitment model assumes that the period of low DO occurs within the larval season (hypoxic events always begin at the end of the development time of the first larval cohort), and that hypoxic days are contiguous. The Province-wide application of the model also assumes that a new cohort occurs every day of the spawning season, and that each cohort is equal in size. These assumptions can be easily modified and the model rerun using site-specific information. The model does not require that a fresh cohort be available every day. If the model is run "longhand" as presented in Appendix E, then its use is very flexible. Successful calculation of the recruitment impairment only requires knowing the total number of cohorts available during a recruitment season (i.e., it does not matter whether they were created daily, weekly, monthly, etc.) and whether a cohort is exposed to hypoxia. If necessary, one also could use cohorts of various initial sizes. Assuming a fixed rate of cohort introduction and size simplifies the calculation of the total number of cohorts and the calculation of hypoxic effects on larval survival. The model application for the Virginian Province is further simplified by assuming that none of the life history parameters change in response to hypoxia. These parameters are only changed when a different species is modeled, although, as with cohort frequency and size, they can be easily changed for a site-specific application to adjust for latitudinal changes in life history requirements.

The dose-response data used in the model are presented in Figure 5. Data are available for nine genera and represent 24 hr exposure responses, except for the Say mud crab (*D. sayi*). These species were selected based in part on the ability to spawn and test them in the laboratory. In addition, they represent a range of sensitivities to hypoxia by water column species. The summary response curve for *D. sayi* represents the more sensitive transition from zoea to megalopa. These tests were necessarily longer (7 to 11 days) than the other tests to allow sufficient time for development to megalopa. Although some enhanced sensitivity in these tests may be from the longer exposures to low DO, mortality also appeared to be primarily associated with the molt to megalopa (which occurred over a 24 hr period for a given individual). When the model was run for *Dyspanopeus*, the assumption was made that the response of the late larvae in transition to megalopae could occur following a single day of exposure (i.e., this response is independent of exposure prior to the day of transition). Thus, the model applies this dose response as a 24 hr exposure. The model run for *Dyspanopeus* also includes a second, less sensitive, dose-response curve for the early life history larval stage for non-megalopa exposures of this species. Model runs for the other eight larval genera were conducted using only one life history stage.

Also included in Figure 5 is a final survival curve (FSC). The data points in the FSC are calculated in the same way that the FAVs and FCVs were calculated, using the data from the four most sensitive genera (*Cancer*, *Morone*, *Homarus*, and *Dyspanopeus*).

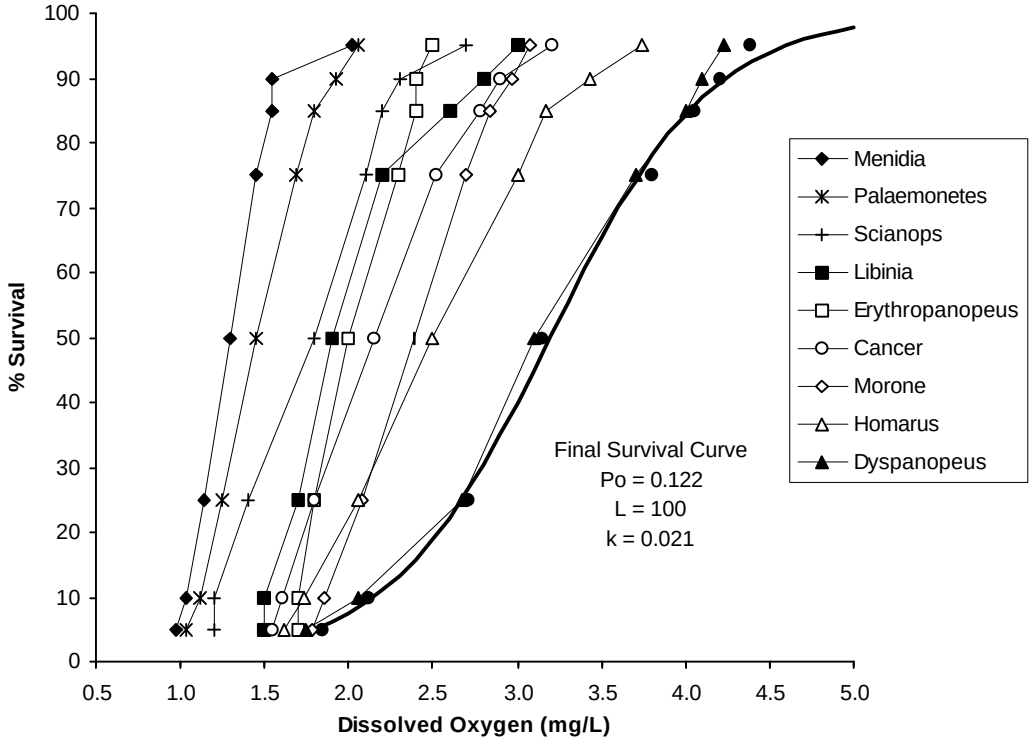

Figure 5. Twenty-four hr dose-response curves for nine genera used in the larval recruitment model. Dark solid line is the regression line of best fit for the FSC. See text for explanation of FSC and of P_0, L, and k. The Solver routine in Microsoft® Excel 97 was used to determine P_0 and k.

The FSC will be used later for establishing DO limits for larval survival during cyclic exposures.

The results of the model runs for each genus[9] are summarized in Figure 6. The complete data along with the biological parameters used for each genus are presented as part of Appendix E. For the purpose of the Virginian Province, many of the values for the biological parameters were selected to be deliberately conservative. For example, we have selected recruitment seasons and larval development times that more likely represent the northern portion of the Province. To support site-specific applications, Appendix F shows several examples of how recruitment curves would be expected to change based on changes to the model's biological parameters. Lengths of recruitment season and larval development are particularly important especially because they are expected to change

[9]Each genus, except for *Palaemonetes*, is represented by only one species. Final criteria values calculated using the 1985 *Guidelines* are based on genus mean values. Therefore, all references to final calculated values use genus rather than species.

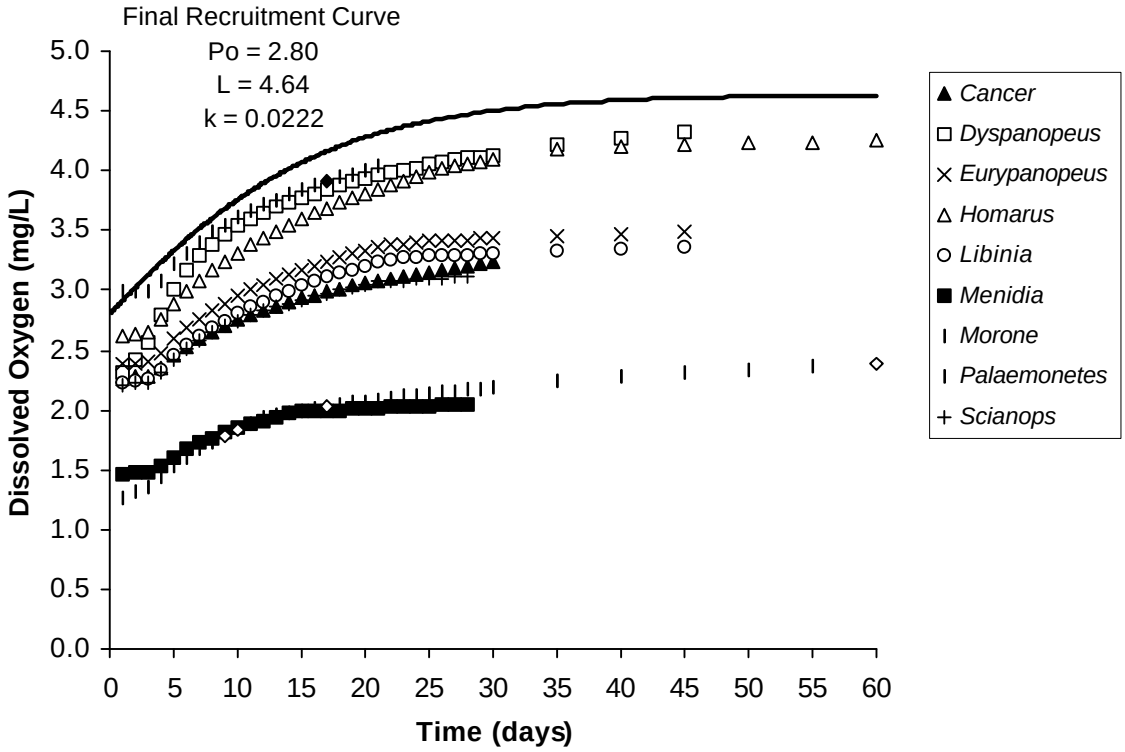

Figure 6. Plot of model outputs that protect against greater than 5% cumulative impairment of recruitment. Input parameters for each genus are explained in Appendix E. The solid line is the regression line of best fit for the FRC. See text for explanation of FRC and of P_0, L, and k. The Solver routine in Microsoft® Excel 97 was used to determine P_0, L, and k.

significantly with latitude. Recruitment season gets longer and development time often shortens as one moves south. This combination can significantly shift a recruitment curve down and to the right. For this reason, it is expected that the final recruitment curve (FRC) presented here for the Virginian Province may be overprotective for many sites. Therefore, FRCs using site-specific biological parameters are recommended.

An FRC was calculated in the same way as the FSC, using the four most sensitive recruitment curves out of the nine available curves. The four most sensitive curves were for the genera *Morone*, *Homarus*, *Dyspanopeus*, and *Eurypanopeus*. The equation for the FRC (and the FSC in Figure 5) was derived by an iterative process of fitting the best line through the points generated by the output of the recruitment model. The equation is a standard mathematical expression for inhibited growth (logistic function; Bittinger and Morrel, 1993). This equation is:

$$P(t) = \frac{P_0 L}{P_0 + e^{-Lkt}(L - P_0)} \qquad \text{Equation 1}$$

For Figure 6, P(t) is the DO concentration at time t, P_0 is the y-intercept, and L is the upper DO limit. P_0 and L were first estimated by eye from the original plot and then adjusted higher or lower to minimize the residuals between the real recruitment data and that estimated from the mathematical fit of the data. The rate constant k was similarly empirically derived. For Figure 5, the variables t and L represent DO concentration and the upper limit for survival (100%), respectively. In this latter case, L is always 100%, because this is always the upper limit for survival.

Application of Persistent Exposure Criteria

The final criteria for saltwater animals in the Virginian Province (Cape Cod to Cape Hatteras) are indicated in Figure 7 for the case of continuous (i.e., persistent) exposure to low dissolved oxygen. The most uncertainty with the application of these limits usually will be when DO conditions are between the juvenile survival and larval growth limits. Below the juvenile survival limit, DO conditions do not meet protective goals. Above the growth limit, conditions are likely to be sufficient to protect most aquatic life and its uses. Interpretation of acceptable hypoxic conditions when the DO values are between the

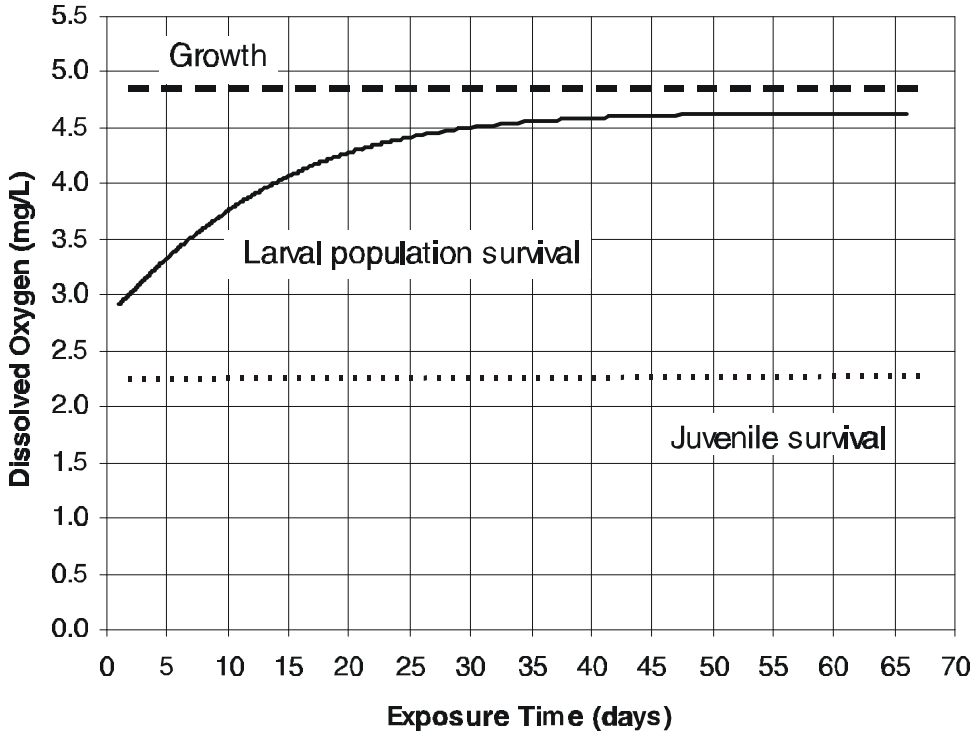

Figure 7. Plot of the final criteria for saltwater animals continuously exposed to low DO. The upper dashed line is the CCC for growth. The lower dotted line is the CMC for juvenile (and adult) survival, and the curve between the two is the FRC from Figure 6 representing protective for larval survival. All of the lines are truncated at 1 day. The cyclic portion of the criteria addresses exposure less than 24 hr.

juvenile survival and larval growth limits depends in part on characterization of the duration of the hypoxia. To determine whether a given site has a low DO problem, adequate monitoring data are required. The more frequently DO is measured the better will be the estimate of biological effects.

Figure 8 is a hypothetical time series for daily average DO. The portion of the data below the CCC is all that is considered. This area of the graph is first divided into several intervals. We recommend using no finer than 0.5 mg/L DO intervals because of limitations on most monitoring programs (see Implementation section). However, larger intervals may be necessary if monitoring data are not taken frequently enough. The resulting intervals in our example are (a) below 4.8 mg/L and above 4.3 mg/L, (b) below 4.3 and above 3.8, and so forth for intervals c and d. For each interval, the number of days is recorded that the DO is between the interval's limits. For example, in interval a, the DO is below 4.8 mg/L and above 4.3 mg/L from July 13 through 18 and again from July 23 through 25, for a total of 7 days. This number of days is then expressed as a fraction of the total number of days that would be allowed for the DO minimum for each interval. For interval a, the allowed number of days is 15 (using the FRC in Figure 6 at 4.3 mg/L). Table 3 lists the information for all four intervals from this hypothetical time series. The fractions of allowed days are totaled. If the sum is greater than 1 (as is the case in our example), then the DO conditions do not meet the desired protective goal for larval survival. If the sum is less than 1, then the protective goal has been met.

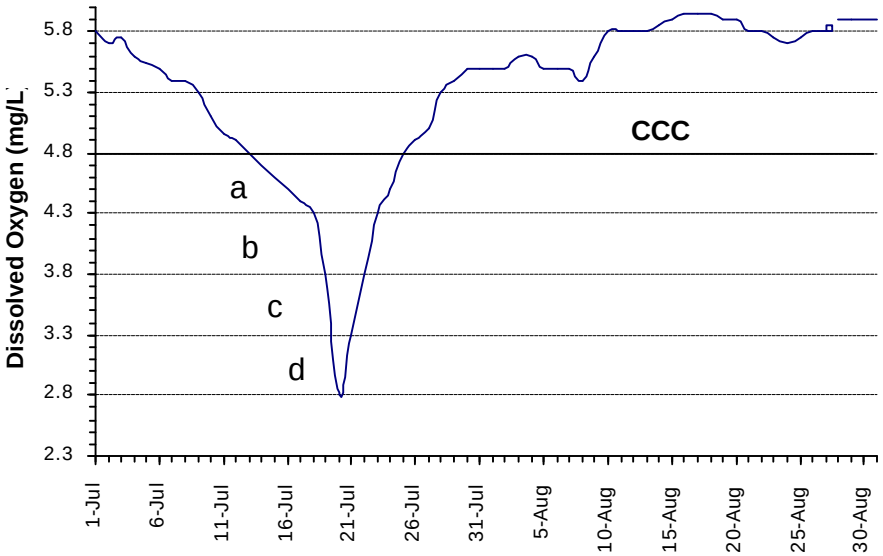

Figure 8. A hypothetical representative DO time series for one site. The horizontal line represents the CCC of 4.8 mg/L. The portion of the curve below 4.8 mg/L is divided into four arbitrary intervals (a,b,c,d) to estimate effects on larval recruitment. The DO minimum and the duration for each interval are determined for each interval.

Table 3. Dissolved oxygen and duration data from a hypothetical persistent time series (Figure 8).

	Range (mg/L)		No. Days Within Range	No. Days Allowed	Fraction of Allowed
Interval	Below	Above			
a	4.8	4.3	7	21	0.35
b	4.3	3.8	3	11	0.30
c	3.8	3.3	1	5	0.20
d	3.3	2.8	1	1	1.00
				TOTAL	**2.05**

The Below and Above columns show the range of DO covered by each interval. Number of Days Within Range refers to the duration that the observed DO is between the range given. In the last column this duration is expressed as a fraction of the number of days allowed by the recruitment model (Figure 6) for the DO minimum of the interval. These fractions are totaled to evaluate whether the larval survival protective goal has been met.

The current recruitment model is a first attempt at providing a method that incorporates duration of exposure in the derivation of DO criteria. A model that could integrate gradual change in daily DO concentrations is desirable. However, the current model may be adequate given the probable inaccuracies in assessments of DO conditions in coastal waters (Summers et al., 1997).

Less Than 24 Hr Episodic and Cyclic Exposure to Low Dissolved Oxygen

The criteria for continuous exposure to low DO do not cover exposure times less than 24 hr. This section addresses this topic by describing the available data and how they were used to evaluate the effect of low DO on exposure durations lasting less than 24 hr. These included one-time episodic events, as well as either tidal- or diel-influenced cycles where the DO concentrations cycle above and below the continuous CCC. The approaches described for treatment of nonconstant (e.g., cyclic) conditions are intended to provide protective goals that are equivalent to those established for persistent conditions. The data used come from two types of experiments. The first are those that provide time-to-death (TTD) data and are used to derive TTD curves. The second are experiments in which there were treatments consisting of a constant exposure to a given low DO concentration paired with a treatment in which the DO concentration cycled between that low concentration and a concentration near saturation (or at least well above concentrations that should cause significant effects). The data from both of these experiments are discussed below.

Cyclic Juvenile and Adult Survival

The persistent hypoxic criterion for juveniles and adults is 2.3 mg/L. A conservative estimate of the safe DO concentration for exposures less than 24 hr would be to simply use 2.3 mg/L. However, TTD data indicate that this would be overprotective. Data are available for two saltwater juvenile fish (*Brevoortia tyrannus* and *Leiostomus xanthurus*), one freshwater juvenile fish (*Salvelinus fontinalis*), and three larval saltwater crustaceans (*D. sayi*, *Palaemonetes vulgaris*, and *Homarus americanus*), providing a total of 33 TTD

curves (Appendix G). The curves represent a range of test conditions, including acclimation to hypoxia with *S. fontinalis*, and a range of lethal endpoints. Two general observations were made from these data. First, each curve can be modeled with the same mathematical expression, a logarithmic regression, of the form:

$$Y = m(\ln X)+b \qquad \text{Equation 2}$$

where X=time, Y=DO concentration, m=slope, and b=intercept where the line crosses the Y-axis at X=1.

Second, the shape of the curve (i.e., the slope and intercept) was governed by the sensitivity of the endpoint. This is true whether the sensitivity increase was due to interspecific differences (including saltwater and freshwater species) or the use of different endpoints (e.g., LC5 is a more sensitive endpoint than LC50).

Figure 9 shows the relationship between sensitivity (i.e., 24 hr LC values) and the slope (Figure 9A) and the intercept (Figure 9B) for all 33 TTD curves (Appendix G). The DO value from each TTD curve at 24 hr was used as a measure of sensitivity. Plots using other time intervals could have been used. The value at 24 hr was chosen in order to generate a curve for juveniles that meets the constant CMC at its 24 hr value (2.3 mg/L). The slope and intercept for a time-to-CMC curve were calculated using Figure 9 equations and the CMC 24 hr value of 2.3 mg/L. These were then used as the parameters in Equation 2 to generate a criterion for saltwater juvenile animals for exposures less than 24 hr (Figure 10).

Cyclic Growth Effects

The CCC for continuous exposure was derived based on growth effects data (mostly from bioassays on larvae, Table 2). The simplest way to determine effects from cyclic exposure to low DO is to compare growth of organisms under cyclic conditions to those for the same species under continuous conditions. Growth data are available from cyclic exposures to low DO for three species of saltwater animals, *D. sayi*, *P. vulgaris,* and *Paralichthys dentatus* (Coiro et al., 2000). These data are listed in Appendix H and summarized in Figure 11. Data are from experiments in which a low DO treatment was paired with a treatment cycling between the same low DO concentration and one that was above the continuous CCC (usually saturation). All cyclic treatments had 12 hr of low DO within any one 24 hr period. Most of the cycles consisted of 6 hr at the low concentration followed by 6 hr at the high concentration. Only two tests (both with *P. vulgaris*) were conducted using a 12hr:12hr cycle. There were a total of 20 paired treatments spread among the 3 species.

As expected, at the end of each test, cyclic exposures generally resulted in more growth than constant exposures to the minimum DO of the cycle (Figure 11). However, if the effects of DO on growth were instantaneous (i.e., growth reduction begins as soon as the DO concentration drops and growth rate returns to normal as soon as DO returns to above CCC concentrations), then the cyclic exposures in the above experiments would

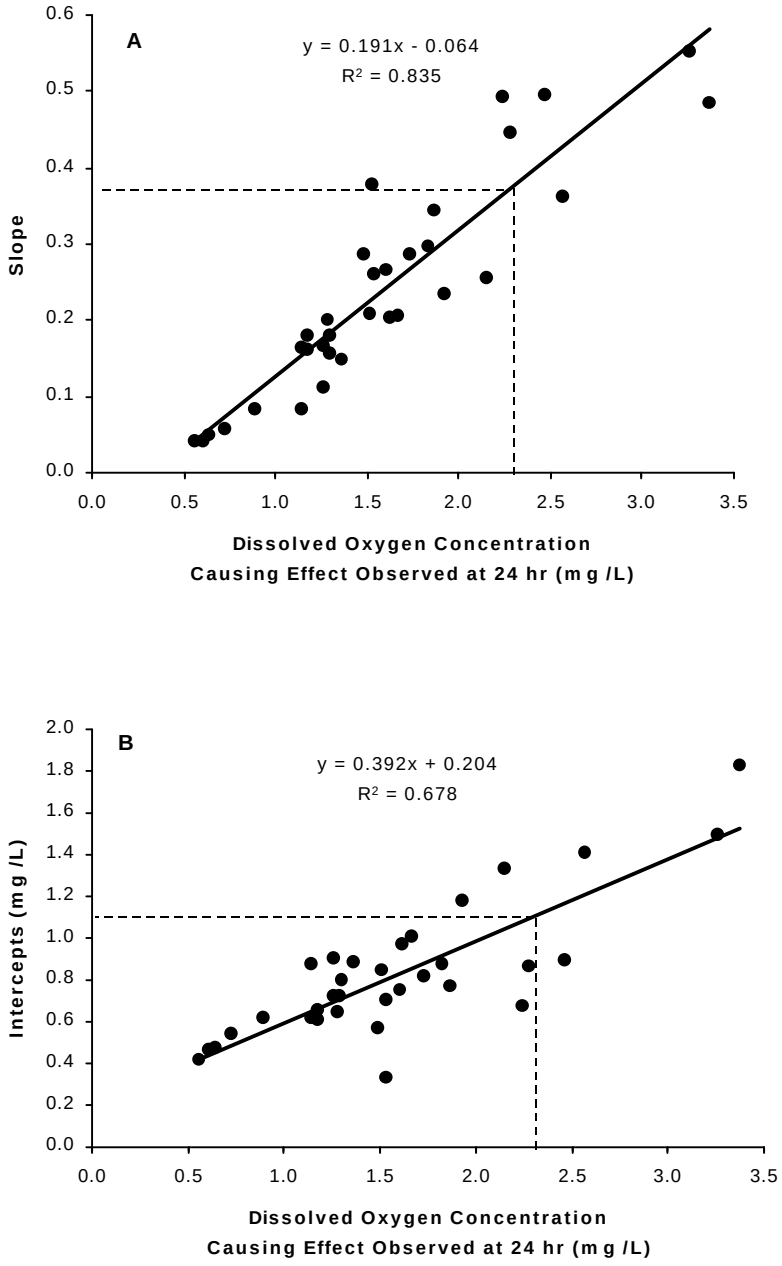

Figure 9. Slope (A) and intercept (B) versus low DO effect values at 24 hr from time-to-death (TTD) curves for two species of saltwater juvenile fish, one species of juvenile freshwater fish, and three species of saltwater larval crustaceans. Data used mostly represent LT50 curves, but values for other mortality curves are included. Species used and their associated TTD curves are presented in Appendix G. All TTD curves were fit with a logarithmic regression.

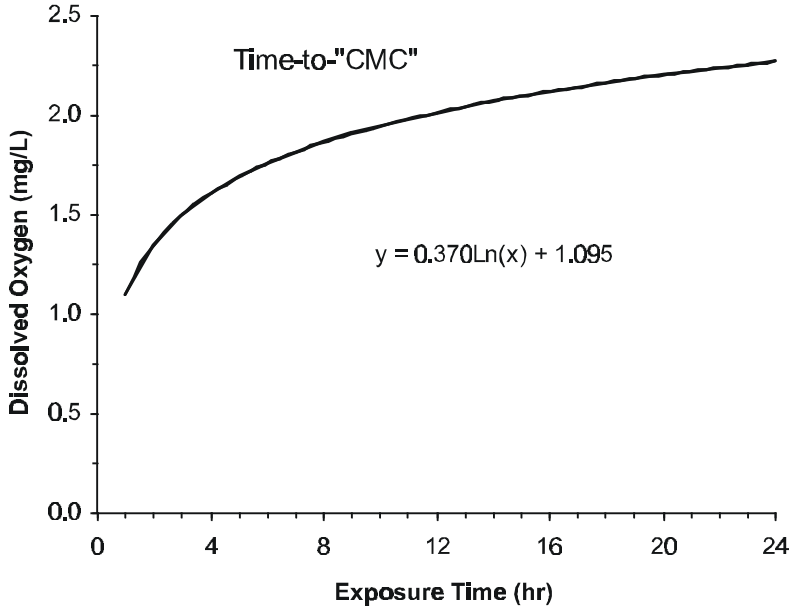

Figure 10. Criterion for juvenile saltwater animals exposed to low DO for 24 hr or less. The line represents the same protective limit as the CMC for juveniles for continuous exposure. The line is a logarithmic expression with a slope and intercept calculated from the regressions in Figure 9 at the DO concentration of 2.3 mg/L (the CMC).

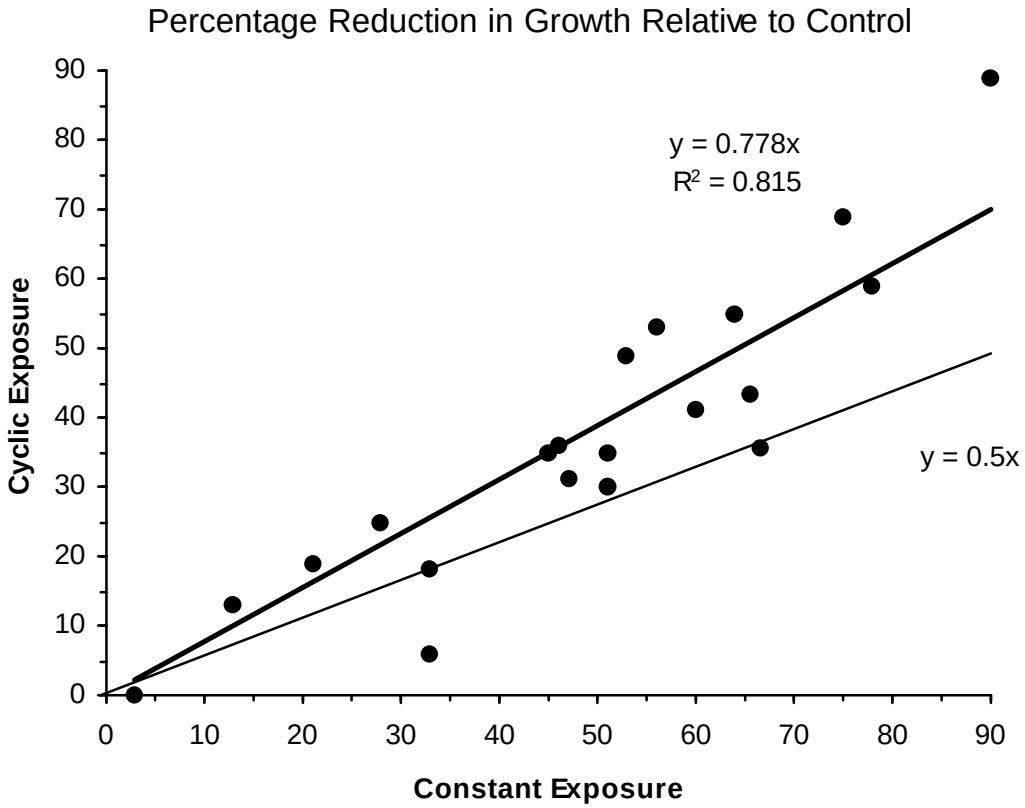

Figure 11. Plot of test results from growth experiments pairing constant low DO exposure with exposures to various cycles of low DO and concentrations above the CCC. The dark line is a linear regression of the data with the line forced through the origin. The lighter weight line is the "expected" relationship from a slope of 0.5 (see text for explanation). Species used and the experimental conditions are listed in Appendix H.

have been expected to cause one-half of the growth reduction observed in the constant treatment of each pair. (As noted above, the DO cycles had a total of 12 hr of low DO per day.) If this were true, then the slope of the line in Figure 11 would be 0.5. However, the slope of the line for the data (forced through the origin[10]) is 0.778, a factor of 1.56 greater. Thus greater growth impairment occurs from cyclic exposures than expected. One hypothesis for this discrepancy is that recovery from the low DO portion of the cycle is not instantaneous, and the actual low DO effect period is then greater than 12 hr within each day (by a factor of 1.56).[11]

Figure 12 shows a dose-response for growth of larval lobster (*H. americanus*) over a range of constant DO concentrations. The data are from 10 tests (see Appendix C) with durations ranging from 4 to 29 days. The percentage growth reduction is relative to a control response. Growth reduction effects are considered instantaneous; therefore, the percentage reduction can be applied to any time period. Data for the lobster are emphasized because it was the most sensitive species tested for which growth was measured. Its use is consistent with the 1985 *Guidelines* (Stephan et al., 1985), which allows a criterion to be established using data for a sensitive economically or ecologically important species.

To evaluate a cycle for chronic growth effects, the above relationship between cyclic and constant exposure is needed as well as monitoring data from a representative, or worst case, cycle of low DO for a given site. Figure 13 provides a hypothetical DO time series. To estimate the expected growth reduction during this cycle, the curve is divided into three DO intervals[12] for that portion of the cycle that falls below 4.8 mg/L (the CCC). The DO mean, and the total duration that the cycle is within the interval's range of DO, are determined for each interval. Data from this example are presented in Table 4. Interval c lasts a total of 5 hours. Interval b lasts a total of 3 hours (b1 before plus b2 after interval c). Similarly, interval a lasts for a total of 4½ hours. Each of these time intervals is multiplied by 1.56 to adjust for the cyclic effect.

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[10]A recent publication of these data (Coiro et al., 2000) clearly demonstrates that the growth reduction differences between constant and cyclic exposures are more or less constant across all of the DO concentrations tested. In other words, the ratio between constant and cyclic response should remain consistent across all concentrations. Thus the slope can be forced through zero.

[11]The data used to establish the relationship between cyclic and constant exposures (Figure 11) came from experiments with a total low DO exposure of 12 hr per 24 hr period. We assume that as the total time of exposure per 24 hr decreases, the discrepancy between expected and observed should also decrease. Thus the 12 hr data can be considered a worst case for any daily cycle of 12 hr or less exposure to low DO. There is insufficient information for cycles with greater than 12 hr exposure periods per day. We recommend assuming constant exposure conditions for these latter situations.

[12]Any number of intervals can be chosen, even one. For simplicity, different DO ranges can be selected for each interval so that each interval has approximately the same total time below the CCC. Alternatively, the cycle can be divided by selecting a constant DO range (e.g., 0.5 mg/L), giving each interval a different time value. Monitoring data, however, must be frequent enough to justify the chosen interval size.

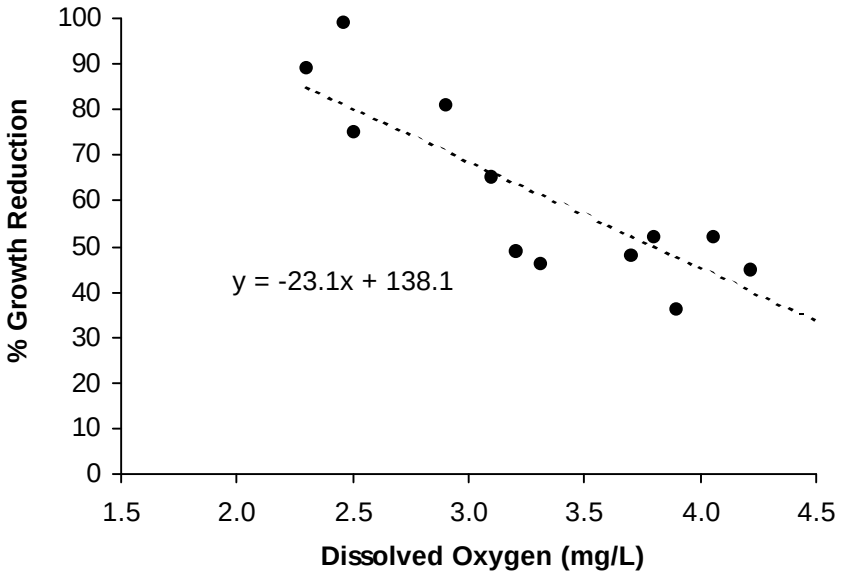

Figure 12. Plot of dose-response data for growth reduction in American lobster (*Homarus americanus*) exposed to various continuous low DO concentrations. Percentage growth reduction is relative to a control. The dashed line is a linear regression through the data points. Data are from Appendix C.

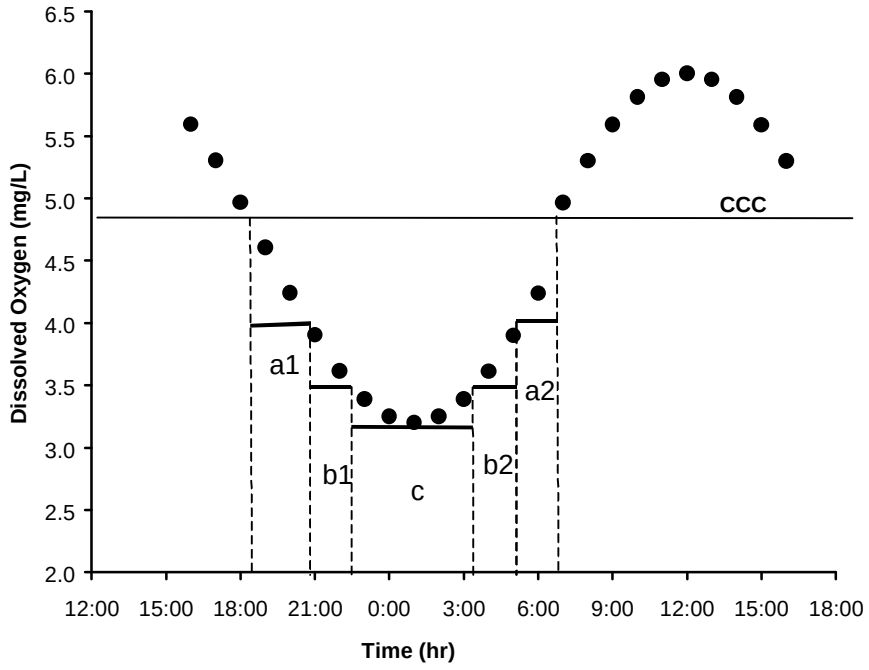

Figure 13. A hypothetical representative DO time series for one cycle. The horizontal line represents the CCC of 4.8 mg/L. The portion of the curve below 4.8 mg/L is divided into three arbitrary intervals (a,b,c) to estimate effects on growth. The range of DO, the mean DO, and the duration for each interval are listed in Table 4.

Table 4. Dissolved oxygen and duration data from a hypothetical cyclic time series (Figure 13).

Interval	DO Range (mg/L)	DO Mean (mg/L)	% Daily Reduction in Growth	Actual Duration (hr)	Cyclic Adjusted Duration (hr)	% Reduction for Duration
a1 – a2	4.8 – 4.0	4.40	36	4.5	7.0	11
b1 – b2	4.0 – 3.5	3.75	51	3	4.7	10
c	3.2 – 3.5	3.35	61	5	7.8	20

These data are used to estimate the growth reduction occurring for the recruitment modeled species during the cycle. Percentage reductions in growth for constant exposure are calculated with the equation in Figure 12. These in turn are normalized for the cyclic adjusted duration.

A DO mean concentration for each interval is used with the equation from Figure 12 to estimate a daily growth reduction that is expected for larval crustaceans during constant exposure to hypoxia. This value is then normalized for the interval's cyclic adjusted duration. The normalized reductions for all intervals are added (growth effects are cumulative) for an estimated growth reduction for the cycle. The total percentage reduction in our example is 44%. This reduction is greater than 25%;[13] thus our hypothetical cyclic hypoxic event does not meet the protective goal for growth.

Cyclic Larval Recruitment Effects

To evaluate cyclic exposures for their potential impact on larval recruitment to the juvenile life stage, two pieces of information are needed: (1) a set of larval TTD curves to estimate the expected daily mortality for a given low DO cyclic exposure and (2) a way to translate that predicted daily larval mortality into allowable days for the given low DO cycle using the constant exposure recruitment model output. Creation of the larval TTD curves is straightforward using the sensitivity information (dose-response curve) from the FSC in Figure 5 and the sensitivity-dependent relationships for TTD slopes and intercepts in Figure 9. Creation of a series of larval TTD curves followed the same procedure used to create the time-to-CMC curve for juveniles (Figure 10). Figure 14 shows the results for nine calculated curves for mortalities ranging from 5% to 95%.

Estimating the daily mortality expected to occur with the model species also is straightforward and, as with cyclic growth protection, requires representative or worst case DO monitoring data. Figure 15 is a hypothetical monitoring data set for a single cycle. As with growth, the portion of the cycle below the CCC is first divided into several intervals. The DO minimum is determined for each interval. It should not matter how the intervals are selected. All that is needed is a set of paired time and DO values. Table 5 lists the data for the intervals in this example. These data were plotted among the family of larval TTD curves (Figure 16). The greatest effect datum lies between the 15%

[13]See footnote 7.

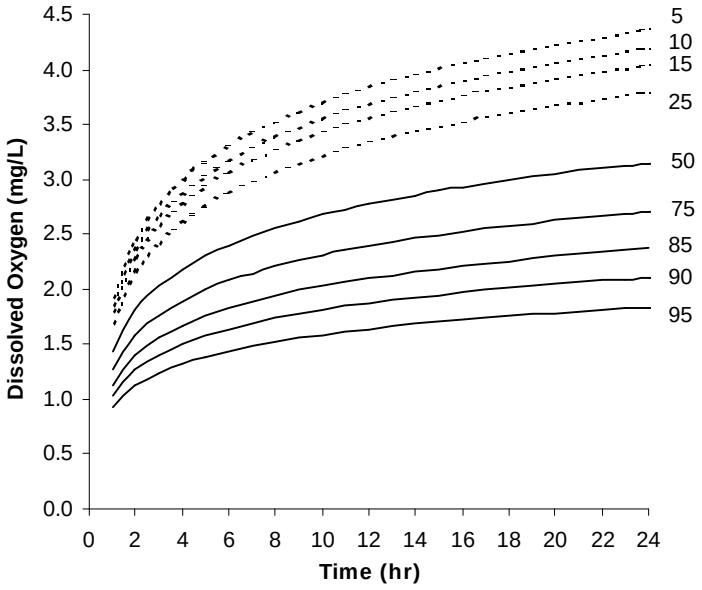

Figure 14. Time-to-death (TTD) curves generated for the Final Survival Curve "genus." Data to generate the curves were taken from Figures 5, 9A, and 9B. The numbers adjacent to each TTD curve are the percentage mortality that each curve represents. The dashed lines represent curves created with slopes and intercepts outside the range of the original data used in Figure 9.

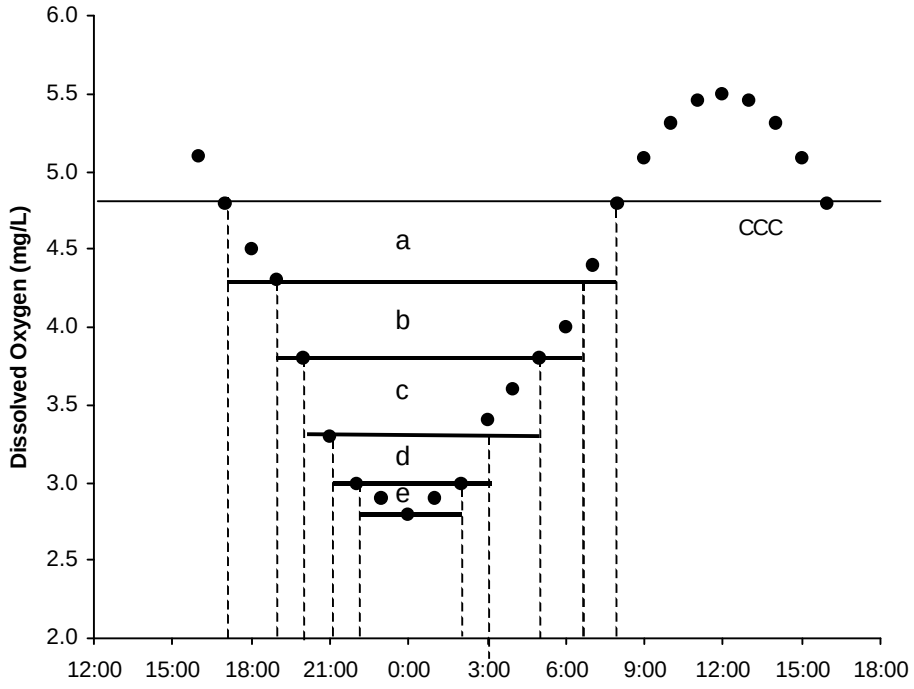

Figure 15. The same hypothetical DO time series as Figure 13. This time the portion of the curve below 4.8 mg/L is divided into several arbitrary intervals to estimate effects on mortality. The DO minimum and its duration for each interval are listed in Table 5.

Table 5. Dissolved oxygen and duration data from the intervals selected from the hypothetical cyclic time series in Figure 15.

Interval	DO Minimum for Interval (mg/L)	Duration of Interval (hr)
a	4.3	15
b	3.8	11.5
c	3.3	9
d	3.0	6
e	2.8	4

These data are plotted in Figure 16 to estimate the expected mortality occurring for recruitment modeled species during the cycle.

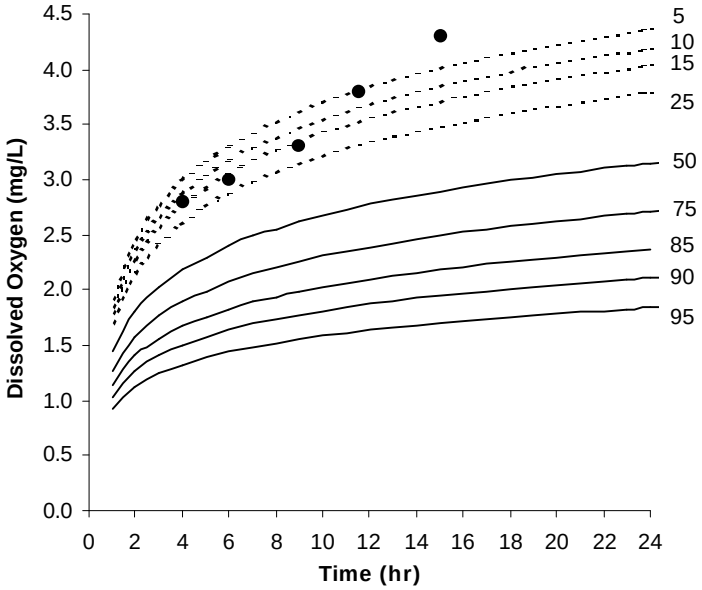

Figure 16. The DO minima and the durations listed in Table 5 superimposed on Figure 14 (solid circles). The expected mortality from the cyclic exposure is determined by the data point falling *closest to a TTD curve of greatest effect*; in this case 25% was selected.

and 25% mortality curves. For the purpose of this example, we will select the 25% mortality curve. Therefore, the hypothetical cycle of DO is expected to cause 25% daily mortality to the modeled larval crustacean. We are only concerned with the greatest effect datum because survival effects are not cumulative (i.e., an individual can die only once).

Now all that is needed is to translate the expected 25% mortality into the number of allowable days for this hypothetical cycle to occur. This is accomplished using the FSC and FRC curves in Figures 5 and 6, respectively. The information in Figure 5 is for percentage survival, but it can be converted easily into percentage mortality. Thus the

information shows the expected cohort mortality to occur for a given DO concentration. For the example, 25% mortality occurs at a DO concentration of 3.7 mg/L. From the equation used to fit the data in Figure 6, the 3.7 mg/L is allowed to occur for up to 9 days without significant impairment to seasonal recruitment. Thus the cycle that resulted in an estimated 25% daily mortality to larvae can be repeated for up to 9 consecutive days without exceeding a 5% reduction in seasonal larval recruitment. All of the above can be simplified by merging the information from the FSC and FRC into one cyclic translator figure using the DO axis that is common between Figures 5 and 6. This is shown in Figure 17.

Other Laboratory Bioassay Data

Additional available data on lethal and sublethal effects of hypoxia on saltwater animals (Appendix J) do not indicate significantly greater sensitivity than indicated previously. The other data are divided into effects on juveniles and adults, and effects on larvae. Figure 18 shows all of the juvenile mortality data from Appendix J plotted against the criteria for juvenile and adult survival (limits for both persistent and cyclic exposures are included). Most of the other survival data are well below the criteria, with three notable exceptions. The first is a single datum (LC50 of 1.9 mg/L) for the Atlantic

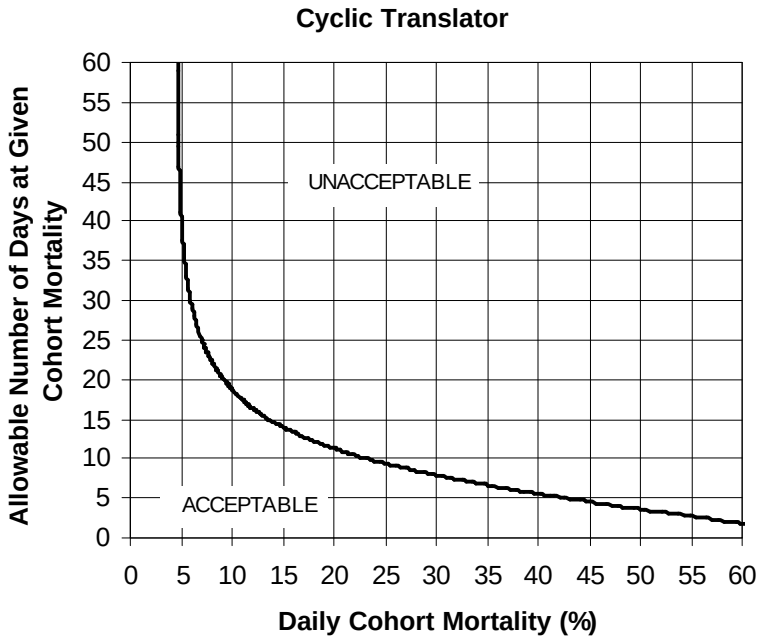

Figure 17. A plot that combines the information from Figures 5 (Final Survival Curve) and 6 (Final Recruitment Curve) into a single cyclic translator to convert expected daily mortality from cyclic exposures into allowable number of days of those cycles.

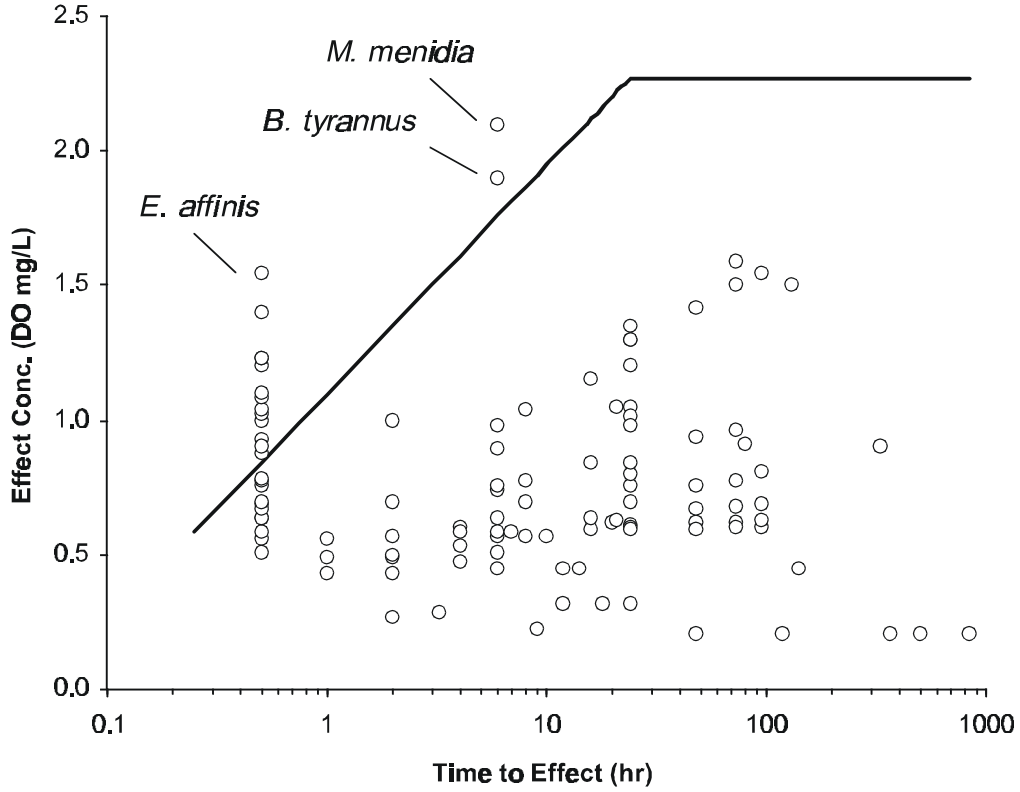

Figure 18. A plot of the other juvenile/adult mortality data from Appendix J (open symbols) along with the proposed DO criteria for juvenile/adult survival (solid line).

menhaden, *B. tyrannus,* at 6 hr (Voyer and Hennekey, 1972). However, several other LC50 values (Burton et al., 1980) for Atlantic menhaden with durations ranging from 2 to 72 hr were much less (0.70 to 0.96 mg/L). The second is a single datum for the Atlantic silverside *M. menidia* at 6 hr (also Voyer and Hennekey, 1972). There are no other data for juvenile Atlantic silversides, but the unusually high sensitivities reported by Voyer and Hennekey for the other species suggest that their exposure system might be a confounding factor. In addition, the authors provided no information on control response for either the Atlantic menhaden or the Atlantic silversides.

The third set of data above the criteria is a series of values at 0.5 hr for the copepod *Eurytemora affinis.* Some are below the criteria, but many are above it (Vargo and Sastry, 1978). However, the authors did not give any details on their experimental methods, including the number of replicates and the number of animals in each replicate, or on the response in the control. Thus, it is difficult to adequately assess the significance of these results. However, in the absence of data to the contrary, it is worth noting that the DO limit for juveniles and adults may not be protective of copepods. Alternatively, one could consider that short-lived species with high reproductive outputs (such as copepods) may be more appropriately protected in a manner similar to larval recruitment.

In this case, all of the *E. affinis* LC50 values would fall below the criterion provided by the larval recruitment (see explanation for Figure 19A below).

Figures 19A and 19B present all of the lethality data from Appendix J for tests using larval life stages. All of these data are from tests for effects on individuals, and the criterion for larval survival acknowledges that some larval mortality is acceptable. Most of the data for larvae are LC50 values for exposure durations other than 24 or 96 hr (these two durations are used elsewhere in the document). The LC50 data are plotted in Figure 19A. The most appropriate protective limit with which to compare these values is the TTD curve for 50% mortality from Figure 14. There are two series of data points for LC50 values for larval rock crab (*Cancer irroratus)* for exposure durations of 2 and 4 hours; each has some values above the 50% TTD curve (Vargo and Sastry, 1977). The more sensitive values in these sets are for tests run at 25°C; thus the animals were likely exposed to multiple stressors (temperature and low DO).

The rest of the other lethality data for larvae are plotted in Figure 19B. These data are separated into three categories, LC5 to LC35, LC40 to LC65, and LC90 to LC100. As with the LC50 values in Figure 19B, these values are plotted along with TTD curves (10%, 50%, and 90% mortality) from Figure 14. All of the LC5 to LC35 values are close to or below the 10% TTD curve. All of the LC40 to LC65 values are well below the 50% TTD curve. Finally, all but one of the LC90 to LC100 values are below the 90% TTD curve. This one value is for 100% mortality of striped bass larvae *(M. saxatilis)* that occurred after a 2 hr exposure to 1.90 mg/L DO. However, there are two other striped bass tests where 100% mortality of the larvae did not occur until 24 hr of exposure to similar low DO.

There are fewer other data on sublethal effects than on lethality effects (Appendix J). The sublethal effects included reduced feeding, growth, locomotion, and bivalve settlement, as well as delays in hatching and molting. However, none of these values indicate that the CCC would not be protective against these effects.

Laboratory Observed Behavioral Effects of Hypoxia

A number of laboratory studies report behavioral alterations following exposure to hypoxia. The effects include low DO avoidance, changes in locomotion, burrowing and feeding activity; and altered predator-prey behaviors. Because most of the effects observed occurred <2.3 mg/L, the 24 hr acute limit CMC would be protective. The most hypoxia-sensitive behavioral effect occurs in red hake (*Urophycis chuss*). In red hake, age 0+ fish leave their preferred bottom habitat and begin to swim continuously as DO concentrations fall below 4.2 mg/L (Bejda et al., 1987). Food search time is also reduced as a consequence. Below 1.0 mg/L, most locomotor and other behavioral activity ceases, and at 0.4 mg/L there is loss of equilibrium. Older red hake (age 1+ and 2-3+) did not exhibit these responses with low DO, except for loss of equilibrium at 0.6 mg/L.

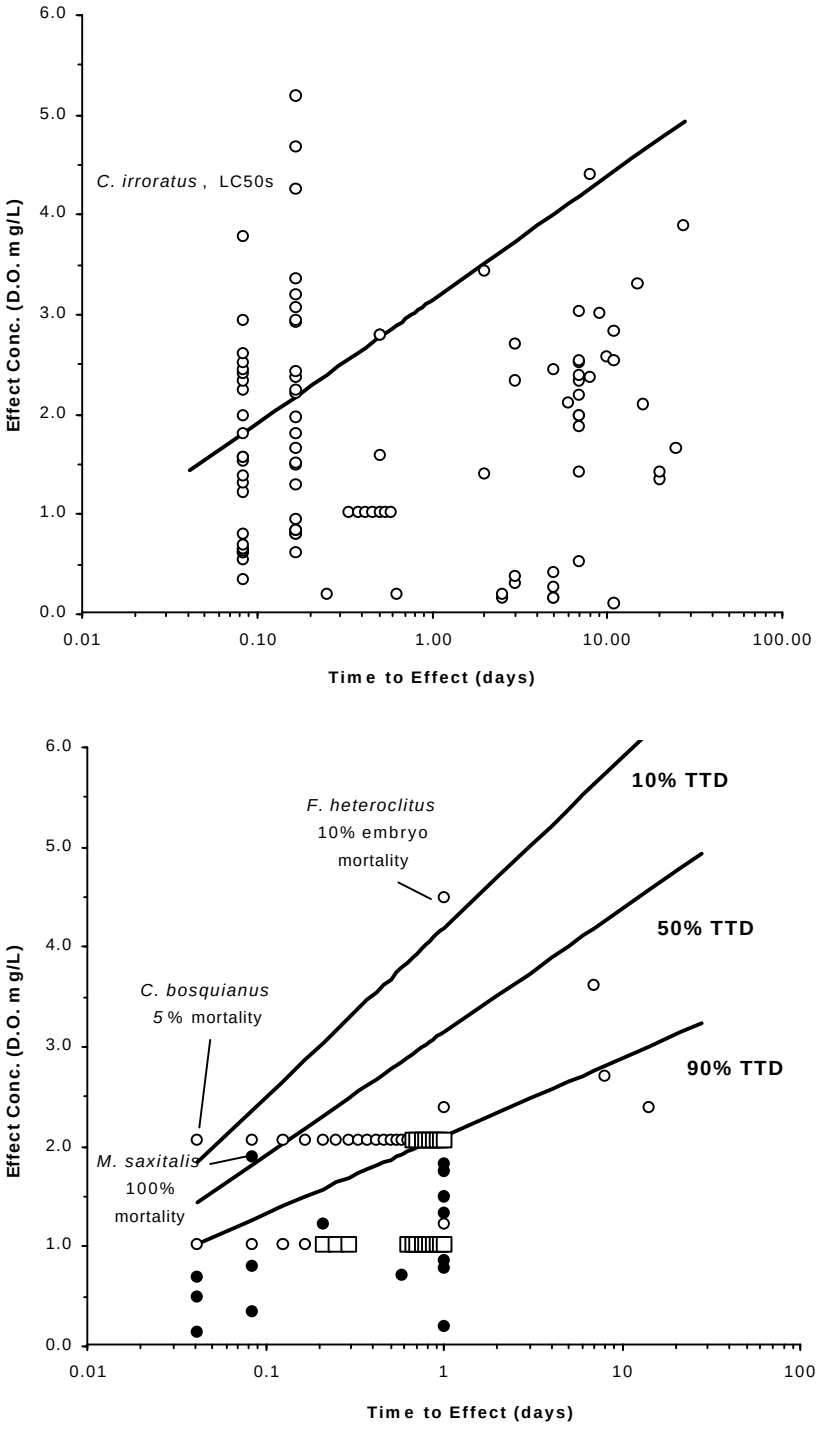

Figure 19. A plot of the other larval survival data from Appendix J. Figure 19A presents the available LC50 data (open circles) along with the 50% TTD curve from Figure 14. Figure 19B presents mortality data for other than 50%. Open circles represent 5% to 35% mortality, open squares 40% to 65% mortality, and closed circles 90% to 100% mortality. Figure 19B also includes the 10%, 50%, and 90% TTD curves from Figure 14.

The following effects are reported at less than the 2.3 mg/L protective limit. In the red morph of green crabs (*C. maenas*) the low DO avoidance EC25 was <2.3 mg/L and the EC50 was 1.8 (Reid and Aldrich, 1989). The green morph was less sensitive. In naked goby (*Gobiosoma bosc*) larvae, avoidance at 2.0 mg/L occurred with $1 hr exposure (Breitburg, 1994). No avoidance was observed at 3.0 mg/L. This same author reported 100% avoidance in larval bay anchovy (*Anchoa mitchilli*) at 0.75 mg/L following a 1 hr exposure. Reduced locomotor activity occurred in daggerblade grass shrimp (*P. pugio*) at 1.8 mg/L (Hutcheson et al., 1985). Burrowing in the northern quahog (*M. mercenaria*) was reduced 1.4- to 2-fold when exposed to 1.8 to 0.8 mg/L and slowed 4-fold in Atlantic surfclam (*Spisula solidissima*) at 1.4 mg/L (Savage, 1976). The polychaete, *Nereis virens*, EC25 for emergence from the sediment was 0.9 mg/L (Vismann, 1990). The shelter guarding and nest guarding behavior by adult male naked goby (*G. bosc*) was not altered at 0.7 mg/L, but they abandoned shelters at 0.38 mg/L and nests at 0.3 mg/L. Death occurred in these animals at 0.26 to 0.24 mg/L (Breitburg, 1992).

The following low DO effects on feeding are reported in a bivalve and four polychaetes. In eastern oyster (*Crassostrea virginica*) early postsettlement stage (436 :m mean shell height), exposure to 1.9 mg/L for 6 hr resulted in 54% to 61% reduction in feeding rate; at <0.4 mg/L for the same period, 86% to 99% reduction occurred (Baker and Mann, 1994b). In older postsettlement animals (651 :m mean shell height), feeding rate was not altered with 1.9 mg/L exposure for 6 hr, but at <0.4 mg/L it was reduced 97% to 99%. In the polychaetes, feeding stopped in *Nereis diversicolor* at 1.2 mg/L and in *N. virens* at 0.9 mg/L (Vismann, 1990). In adult *Loimia medusa*, feeding stopped at 1.0 mg/L during <20 hr exposure, then resumed in 42 to 113 hr in 42% of the animals (Llansó and Diaz, 1994). At 0.5 mg/L, there was no resumption of feeding after initially ceasing during the same initial exposure period. Following exposure in *Streblospio benedicti* adults, the initial response to 1.0 mg/L was cessation of feeding, but it resumed in 3.5 days; with 0.5 mg/L exposure, the initial response was the same, with feeding resuming in 4.5 days (Llansó, 1991).

Changes were observed in predator–prey activities in two fishes in low DO. In naked goby (*G. bosc*) larvae, avoidance of the sea nettle (*Chyrsaora quinquecirrha*) predator was reduced 60% following 3 hr exposure to 2.0 mg/L. In striped bass (*M. saxatilis*) juveniles, predation on naked goby larvae was reduced 50% following 1 hr 35 min exposure to 2.0 mg/L (Breitburg et al., 1994).

Observed Field Effects

Field reports of the biological consequences of hypoxia could be used to derive DO criteria if they include information to describe the exposure conditions. Yet sufficient data are rarely available. In most cases, DO conditions prior to observed effects are unknown, making it difficult to predict an exposure threshold for the observed effect. A field report of hypoxic effects must, at a minimum, provide a description of the concurrent DO exposure conditions if it is to be useful in deriving criteria. Ten studies in the Virginian

Province have provided concurrent DO measurements. The DO observations often are only point measurements, not continuous records, and they rarely provide information on DO conditions prior to the observed effects. The biological effects reported include alterations in the following: presence of fish and crustaceans, diel vertical migration of copepods, recruitment and population density of an oyster reef fish (naked goby), recruitment and growth of eastern oyster spat, and macrobenthic community parameters. Effects were usually not observed above 2 mg/L. Exceptions are the Long Island Sound trawl studies, where effects were reported in the 2.0 to 3.7 mg/L range.

The relationship between low DO and presence of fish and shellfish in Long Island Sound was examined in two trawl studies. Howell and Simpson (1994) reported marked declines in abundance and diversity in 15 of 18 study species when DO was below 2 mg/L. When DO was between 2 and 3 mg/L, there were significantly reduced abundances of three species: winter flounder, windowpane flounder, and butterfish. In a subsequent 3-year study, the aggregate data for 23 species of demersal finfish showed a decline for two community indices, total biomass and species richness, with declining DO (Simpson et al., 1995). The DO concentration that corresponded with a 5% decline below a response asymptote was 3.7 mg/L for total biomass and 3.5 mg/L for species richness. DO declines below these concentrations resulted in further exclusion of these animals, which has implications for the secondary productivity of these waters. Reduced species number implies reduction of community resilience, should this condition persist. The consequences of habitat crowding on animals occurring in adjacent waters are unknown.

Hypoxia-induced changes in the distribution of fish and crustaceans have also been reported in the lower York River, located in the Virginian portion of Chesapeake Bay (Pihl et al., 1991). Subpycnocline DO <2 mg/L developed during neap tide periods, and the study species (spot, croaker, hogchoker, blue crab, and mantis shrimp) migrated to shallower and better oxygenated habitats. The degree and order of vertical movement was believed to be a function of the water column DO concentration and species sensitivity to hypoxia; that is, croaker > spot = blue crab > hogchoker . mantis shrimp. Water column destratification and reaeration occurred with spring tide or strong winds, and all species except the burrowing mantis shrimp returned to the deeper strata, indicating a preference for the deeper habitats.

Diel vertical migration of copepods *Acartia tonsa* and *Oithona colcarva* was disrupted by hypoxia (Roman et al., 1993). In mid-Chesapeake Bay during the summer, these copepods typically occurred near the bottom during the day and migrated to the surface waters at night. However, when DO concentrations fell below 1 mg/L in subpycnocline waters, the copepods were displaced to the pycnocline, where the highest numbers were found both day and night. When mixing occurred during the summer, the bottom waters were reaerated, and the copepods once again were found at depth during the day. Vertical migration is believed adaptive in that it places the copepods in the chlorophyll maximum at night to maximize food intake, yet it provides daytime avoidance of the surface waters, protecting the copepods from visual feeding bay anchovy.

The consequences of hypoxia on recruitment were examined for two species at a mid-Chesapeake Bay site: the naked goby (*G. bosc*), a benthic oyster reef fish (Breitburg, 1992), and eastern oyster (*C. virginica*) (Osman and Abbe, 1994). In the naked goby study, low DO episodes were short-lived, but extreme (<0.5 mg/L), the result of movement of deep, oxygen-depleted bottom water into the near-shore reef habitat. Following each severe intrusion, the naked goby population density fell dramatically at the deeper stations, which experienced the lowest DO (0.4 mg/L). Small, newly recruited juveniles were absent, presumably due to extremely high mortality. There is evidence, based on observed densities, that older juveniles and adults survived these events by temporarily moving to inshore portions of the reef where DO was not as low, then returning during the weeks following the event. Embryonic development was also affected. Males abandoned egg-containing tubes placed at deeper sites, and the majority to all of the embryos were dead. In addition, the youngest embryos collected from the shallower, less hypoxia-stressed site developed abnormalities following laboratory incubation. The severe intrusions occurred during peak periods of recruitment, with the lowest DO occurring on portions of the reef where recruitment was expected to be highest. These adverse effects were not observed at sites having low DO $0.7 mg/L.

In the study with the eastern oyster (*C. virginica*) (Osman and Abbe, 1994), mortality was observed in newly set (2 to 4 days old) animals during periods of prolonged intrusions of low DO water (<1 mg/L 40% of the time in bottom water during the first 2 weeks of two experiments). Mortality was proportional with depth, which corresponded to severity of hypoxia. Growth rate of surviving spat decreased after 1, 2, and 4 weeks following deployment, with a greater effect also occurring at the deeper stations. Survival and growth of juvenile oysters were unaffected following simultaneous deployment at the same stations, indicating greater tolerance of the older animals. The authors concluded hypoxia to be a plausible causative factor, acting directly or indirectly, although other causative factors also are possible.

Responses of the macrobenthic community to DO <2 mg/L are reported for the lower Chesapeake Bay and tributaries (Dauer and Ranasinghe, 1992; Diaz et al., 1992; Llansó, 1992; Pihl et al., 1991, 1992). Two community effects are reduced species number and abundance, with these effects increasing spatially and temporally with increasing severity and duration of hypoxia. There also is a shift with hypoxia from dominance of longer-lived, deeper burrowing species of a mature community to short-lived, shallow burrowing opportunistic species. The response of benthic species, and their subsequent recoveries following hypoxia, depends on species tolerance, the timing of the hypoxic event relative to larval availability and settlement, and life history strategy. Some infaunal organisms migrate toward the sediment surface with hypoxia, beginning around 2 mg/L (Diaz et al., 1992). Animals that migrate to the surface are exposed to predation by hypoxia-tolerant fish and crustaceans (Pihl et al., 1992). Defaunation may only occur below 1 mg/L. These studies support 2 mg/L as the hypoxic effect threshold for the macrobenthos, which is consistent with the global literature (Diaz and Rosenberg, 1995).

To summarize, demersal finfish community biomass has been observed to diminish at DO <3.7 mg/L, and species richness to diminish at <3.5. These effects become increasingly pronounced with further DO decline. Below 2.0 mg/L, migration of the infaunal species to the sediment surface and movement of epifaunal species to better aerated water were observed. All effects reported at <1 mg/L DO concern hypoxia-tolerant species and life stages (i.e., disruption of diel vertical migration in copepods, reduced growth and survival of newly settled oysters, and lethality in larval goby) as demonstrated in parallel laboratory studies (Breitburg, 1992; Roman et al., 1993) or by other workers (Baker and Mann, 1992, 1994a).

Data Not Used

Data from a variety of published literature were not used. The literature on effects of anoxia was not used, as it provides negligible information on threshold requirements of aerobic animals. Information on anoxic effects may be found in a recent symposium (Tyson and Pearson, 1991) and a review (Diaz and Rosenberg, 1995) on this subject. Results of hypoxia effects studies were not cited for species that do not commonly occur in coastal and estuarine waters between southern Cape Cod, MA, and Cape Hatteras, NC, during the spring to autumn period that brackets the occurrence of hypoxia. Reports for occasional visitor species that occur in these waters during a favorably warm or cold summer were excluded.

Data were not cited if the test temperature was outside the temperature range of Virginian Province waters during the hypoxic season; for example, American lobsters tested at 5°C (McLeese, 1956). Data were not used if they are probably not reliable. Examples include indications that the test animals may have been stressed, for example, American lobster tested at 25°C that were not fed during an 8- to 10-week acclimation period (McLeese, 1956); excessive control mortality (>10% for juveniles or adults and >20% for early life stages); uncertain DO exposure concentration, whether due to questionable DO measurements or failure to directly measure test chamber DO conditions (e.g., Reish, 1966); or if test animals were removed and handled during the test to make other measurements, for example, for an energetics study (Das and Stickle, 1993). Literature on physiological responses of animals to hypoxia was reviewed but was not found useful to determine low DO effect thresholds. See Herreid (1980) for a discussion of difficulties in using oxygen consumption results to describe DO requirements of invertebrates. Rombough (1988b) has developed an approach to identify the DO requirements for fish embryos and larvae, but this approach has not been employed with species applicable to Virginian Province saltwaters.

Some data are not used for juvenile blue crabs, *C. sapidus* (Stickle, 1988; Stickle et al., 1989). Effect concentrations for this species from this laboratory are an order of magnitude higher than values from an earlier study using adult *C. sapidus* (Carpenter and Cargo, 1957). In addition, these effect concentrations for juvenile blue crabs are almost all higher than values for larvae of all tested species. Another study (DeFur et al., 1990) showed that adult *C. sapidus* make respiratory adjustments that allow them to tolerate

long-term (25 days at 22°C) exposure to 2.6 to 2.8 mg DO/L. These data for juvenile blue crabs are considered outliers until further testing shows otherwise.

Just prior to completion of this document, a paper appeared (Secor and Gunderson, 1998) describing the effects of hypoxia and temperature on juvenile Atlantic sturgeon, *Acipenser oxyrinchus*. There was 22% mortality at 19°C and an average within-tank DO concentration of 2.7 mg/L (within-tank data provided by author). This sensitivity is not that different from that of striped bass. However, a combination of low DO (ca. 3.5 mg/L) and high temperature (26°C) resulted in 100% mortality of *A. oxyrhincus* within approximately 24 hr. Because the greatest sensitivity was associated with the high temperature, the data were not included in this document. In addition, the salinity during the experiments only ranged between 1 and 3 ppt; therefore, it is likely that these data are more appropriately associated with freshwater criteria, which are higher than those for saltwater (see Implementation section).

Virginian Province Criteria

The recommended criteria for ambient DO for the protection of saltwater aquatic life in the Virginian Province: Cape Cod to Cape Hatteras are summarized in Table 6. These criteria are briefly described below:

(1) Protection of Juvenile and Adult Survival from Persistent Exposure

This limit is derived following the *Guidelines* procedures and is analogous to the CMC, except that a protective DO concentration limit is expressed as a minimum as opposed to a maximum, as would be the case for a toxicant. This limit represents the floor below which DO conditions (for periods of >24 hours) must not occur. Shorter durations of acceptable exposure to conditions less than the CMC have been derived from laboratory studies, as described in (4) below. Refer to Table 1 and Figure 2 for an explanation of the derivation of this limit.

(2) Protection of Growth Effects from Persistent Exposure

This limit is derived following the *Guidelines* procedures and is analogous to the CCC for a toxicant. This limit represents the ceiling above which DO conditions should support both survival and growth of most aquatic species from Cape Cod to Cape Hatteras. Refer to Table 2 and Figure 3 for an explanation of the derivation of this limit. This limit may be replaced with a limit derived in (3) as described below, when exposure data are adequate to derive an allowable number of days of persistent exposure.

Table 6. Summary of Virginian Province saltwater dissolved oxygen criteria.

Endpoint	Persistent Exposure (24 h or greater continuous low DO conditions)	Episodic and Cyclic Exposure (less than 24 h duration of low DO conditions)
Juvenile and Adult Survival (minimum allowable conditions)	(1) *a limit for continuous exposure* DO = 2.3 mg/L (criterion minimum concentration, CMC)	(4) *a limit based on the hourly duration of exposure* DO = 0.370*ln(t) + 1.095 where: DO = allowable concentration (mg/L) t = exposure duration (hours)
Growth Effects (maximum conditions required)	(2) *a limit for continuous exposure* DO = 4.8 mg/L (criterion continuous concentration, CCC)	(5) *a limit based on the intensity and hourly duration of exposure* Cumulative cyclic adjusted percent daily reduction in growth must not exceed 25% $\sum_{1}^{n} \frac{t_i * 1.56 * Gred_i}{24} < 25\%$ and $Gred_i = -23.1 * DO_i + 138.1$ where: $Gred_i$ = growth reduction (%) DO_i = allowable concentration (mg/L) t_i = exposure interval duration (hours) i = exposure interval
Larval Recruitment Effects[a] (specific allowable conditions)	(3) *a limit based on the number of days a continuous exposure can occur* Cumulative fraction of allowable days above a given daily mean DO must not exceed 1.0 $\sum \frac{t_i(actual)}{t_i(allowed)} < 1.0$ and $DO_i = \frac{13.0}{(2.80 + 1.84e^{-0.10 tsubi})}$ where: DO_i = allowable concentration (mg/L) t_i = exposure interval duration (days) i = exposure interval	(6) *a limit based on the number of days an intensity and hourly duration pattern of exposure can occur* Maximum daily cohort mortality for any hourly duration interval of a DO minimum must not exceed a corresponding allowable days of occurrence where: Allowable number of days is a function of maximum daily cohort mortality (%) Maximum daily cohort mortality (%) is a function of DO minimum for any exposure interval (mg/L) and the duration of the interval (hours)

[a] Model integrating survival effects to maintain minimally impaired larval populations.

(3) Protection of Larval Recruitment Effects from Persistent Exposure

This limit is derived from a generic larval recruitment model. The limit represents allowable DO conditions below the CCC, provided the exposure duration does not exceed a corresponding allowable number of days that ensure adequate recruitment during the larval recruitment season. The cumulative effects of all exposure interval durations at a given DO below the CCC can be accounted for by totaling the fractions of the actual (or projected) exposure duration (in days) divided by the allowable exposure duration for each interval of a specific DO concentration. Refer to Table 3 and Figure 6 of this document for an explanation of the derivation of this limit.

(4) Protection of Juvenile and Adult Survival from Episodic or Cyclic Exposure

This time-dependent limit was derived to represent the responses of the most sensitive juveniles tested in the laboratory. It provides a degree of protection equivalent to the CMC, but for shorter exposure durations than a day. It is assumed that adults are no more sensitive than juveniles. This limit represents the minimum DO conditions that must be maintained on an hourly basis (e.g., 1-hour minimum, 2-hour minimum). The limit applies to conditions occurring on a single given day; even if this limit is met, recurring exposure patterns still must be checked for agreement with the larval recruitment limit described in (6) below. Refer to Figure 10 of this document for an explanation of the derivation of this limit.

(5) Protection of Growth Effects from Episodic or Cyclic Exposure

This limit is derived from the dose-response relationship for DO vs. growth reduction for the American lobster, and comparisons of the effects of cyclic exposure versus constant exposure on growth for a variety of species. It provides a degree of protection equivalent to the CCC, but for exposure durations shorter than a day. The limit represents the DO conditions that maintains a daily percent growth reduction not greater than 25%. The cumulative effects of all exposure interval durations at a given DO below the CCC are accounted for by summing the percent reductions for time intervals at representative DO concentrations. An adjustment factor of 1.56 was derived to estimate time-variable effects from intermittent exposure tests that indicated residual, or delayed, recovery effects from various growth-inhibiting conditions. The limit applies to DO conditions that may occur as a recurring pattern throughout the year without adverse growth effects at the CCC level of protection. However, a recurring pattern of exposure may be limited for a certain number of days based on the larval recruitment limit (6). Recurring patterns of DO conditions that do not meet the growth limit may be allowed for a limited number of days in a recruitment season, provided the larval recruitment limit is met according to (6). Refer to Table 4 and Figure 12 of this document for an explanation of the derivation of this growth limit. The larval recruitment limit can be substituted in whole for the growth limit.

(6) Protection of Larval Recruitment Effects from Episodic or Cyclic Exposure

This limit is derived from the modeled relationships between daily cohort mortality and the allowable number of days at a given maximum daily larval cohort mortality that protects against greater than 5% cumulative impairment of recruitment over a recruitment season. It provides a degree of protection equivalent to the limits described in (3) above, but for recurring patterns of low DO as opposed to continuous low DO conditions. Figure 16 of this document illustrates how to determine the maximum daily cohort mortality from duration intervals of DO minima. Figure 17 of this document illustrates how to determine the allowable number of days of cyclic exposure for a given maximum daily cohort mortality. This limit provides additional information that should be used in conjunction with the limits described in (4) and (5) above. The limit determines the number of days that recurring episodic or cyclic conditions may occur, including whether the pattern may occur for an unlimited number of days. For example, a cyclic pattern that includes a DO minimum of 3.0 mg/L for 6 hours results in a daily cohort mortality of almost 25% (see Figure 16). Assuming this represents the maximum daily cohort mortality for the cyclic pattern, the allowable number of days for the cyclic exposure is 9 (see Figure 17). Refer to pages 31-34 of this document for a detailed explanation of the derivation of this limit.

In summary, limits (1) and (4) establish 1-day and hourly minimum conditions that should be maintained for persistent and cyclic exposures, respectively; limits (3) and (6) establish conditions that may occur for a limited number of days for persistent and cyclic exposures, respectively; and limits (2) and (5) establish long-term conditions that should be maintained for the remaining number of days for persistent and cyclic exposures, respectively.

Implementation

Dissolved oxygen criteria should be implemented differently from those of toxicants, but not for reasons associated with biological effects or exposure. Uncertainties associated with aquatic effects of DO, such as behavior, synergistic relationships with temperature, salinity, or toxics, apply to toxics as well. Dissolved oxygen also does not differ from toxics for reasons associated with exposure. Dissolved oxygen can vary greatly in the environment, but so can toxics. Effluents and their receiving waters can vary daily, even hourly, in their toxicity to aquatic life. Toxicity of saltwater-receiving waters also can vary with the tide and the depth of water (Thursby et al., 2000). It may be mistakenly perceived that DO varies more in concentration simply because it can be measured easily and nearly continuously.

From the standpoint of environmental management, DO differs from toxic compounds primarily because it is not regulated directly. Hypoxia is a symptom of a problem, not a direct problem. Dissolved oxygen is regulated primarily by controlling discharges of nutrients (in the marine environment, most commonly nitrogen). Dissolved oxygen also differs from most toxic compounds because hypoxia can have a large natural

component. Therefore, criteria for hypoxia should not automatically be applied in the same way as limits for toxicants are.

This document provides the information necessary for environmental planners and regulators in the Virginian Province to address the question of whether DO at a given site is sufficient to protect coastal or estuarine aquatic life. The document does not address how compensatory mechanisms such as avoidance can influence the response of local populations to seemingly adverse DO conditions. The document also does not address the issue of spatial extent of a DO problem. In other words, even if the DO at a site is low enough to significantly affect aquatic life, the environmental manager will have to judge whether the hypoxia is widespread enough for concern. Finally, as with all criteria, this document does not address changes in sensitivity to low DO that accompany other stresses such as high temperature, extremes of salinity, or toxicants. Chief among these concerns would be high temperature because high temperature and low DO often appear together. Low DO will be more lethal at water temperatures approaching the upper thermal limit for species. This effect has been seen for freshwater species (U.S. EPA, 1986; Secor and Gunderson, 1998), and saltwater species (e.g., *C. irroratus* and *E. affinis*). The limits provided here should be sufficient under most conditions where aquatic organisms are not otherwise unduly stressed.

Many programs that monitor coastal DO with electronic equipment cannot measure DO to better than 0.5 mg/L due to limitations of instrument accuracy and resolution (e.g., Strobel et al., 1995; Strobel and Heltshe, 1999) or sampling design (Summers et al., 1997). Attempts to refine the limits presented here or to apply these limits in assessing field DO conditions should take this into account. Criteria for DO can be appropriately used in a risk assessment framework. The approach outlined in this document can be easily used to compare DO conditions among areas, and determine if the DO conditions are adequate to support aquatic life. Environmental managers can determine which sites need the most attention, and evaluate the spatial and temporal extent of hypoxic problems from one year to the next for sites of concern.

Environmental managers who wish to use the protective approach presented here will have to decide several questions about how the limits will be used, five of which are described below.

1. *Accuracy of monitoring data*—The most important decision is to determine how accurate the monitoring data are—the better that hypoxia is characterized, the more reliably one can decide whether it meets the criteria. Data from existing monitoring programs may not always be accurate enough to take full advantage of the approach provided here. For example, a recent assessment of conventional sampling procedures along the Atlantic and Gulf coasts has suggested that hypoxia in their estuarine waters is substantially more widespread than previously believed (Summers et al., 1997). Deciding what data can adequately characterize hypoxia is a matter of risk management. Cyclic conditions may require measurements every 30 min for several days, whereas persistent hypoxia may need only several measurement a week.

Decisions also have to be made about the number and locations of sampling sites to properly represent a given area.

2. *Biological effects*—Potential biological effects are most difficult to predict when DO lies between the limits for juvenile and adult survival and larval growth. Deciding whether concentrations between these limits are acceptable will depend in part on several biological parameters related to the recruitment model. How to best represent these issues is a risk-management decision. The 5% impairment level for seasonal larval recruitment was selected to be consistent with the protection provided to juvenile and adult life stages, but a different percentage (higher or lower) may be valid for a site-specific DO criteria. The biological effects data represent the expected range of sensitivity to hypoxia for the Virginian Province. In certain site-specific situations, data on additional species more representative of the site may be desirable. Deletion of data from the current data set, however, should be done with caution. The fact that a species (e.g., American lobster) may not be present at a more southern site does not mean that it does not represent sensitive species in the community that could not be tested. In addition, the lengths of recruitment season and larval development period may be adjusted to be consistent with conditions expected at a site.

3. *Spatial extent*—After environmental managers have found a hypoxic area, they must decide whether it is small enough relative to nearby unaffected areas to allow the coastal region as a whole to meet the criteria.

4. *Freshwater versus saltwater*—It is not trivial to decide whether the DO in certain parts of estuaries should be judged by freshwater criteria or saltwater criteria, particularly where the tides vary the salinity between near fresh and a few parts per thousand. This decision is important because the criteria for freshwater are greater than the saltwater limits developed here, depending on water temperature and the life stage being protected (U.S. EPA, 1986). A reasonable way to start is by considering their biological communities. If they are more like freshwater organisms, freshwater criteria should be applied. If they are more like saltwater, then saltwater criteria apply.

5. *Threatened and endangered species*—In cases where a threatened or endangered species occurs at a site, and sufficient data exist to suggest that it is more sensitive at concentrations above the criteria, it is appropriate to consider development of site-specific criteria based on this species.

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