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Determination of lethal dissolved oxygen levels for selected marine and estuarine fishes, crustaceans, and a bivalve

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Abstract The objective of this study was to provide a database of the incipient lethal concentrations for reduced dissolved oxygen (DO) for selected marine and estuarine species including 12 species of fish, 9 crustaceans, and 1 bivalve. All species occur in the Virginian Province, USA, which is a cold temperate region. The study period was August 1987 to September 1995. Standard bioassay procedures were employed, with most tests being of 4-day duration. Up to eight lethal concentrations (LCs) between LC_{05} and LC_{95} were estimated. The study provides four general conclusions about determining lethal thresholds of low DO for these organisms. First, the concentration response curve of most species did not change greatly beyond day 1 of the exposure with the exception of crustacean larvae, which were usually more sensitive on day 4, possibly due to molting. Second, acute LC_{50} values (1- to 4-day) for low DO were influenced by life-stage and habitat, with pelagic larvae generally being the most sensitive and benthic juveniles the least. Species mean LC_{50} values ranged from 1.4 to 3.3 mg l^{-1} for larvae, 1.0 to 2.2 mg l^{-1} for postlarvae, and 0.5 to 1.6 mg l^{-1} for juveniles. No intraspecific differences in LC_{50} were detected between larval stages in crustaceans or with age in larval fishes. The response range between LC_{05} and LC_{95} was narrowest for the least sensitive organisms (0.6 mg l^{-1}), and broadened with sensitivity. The mean LC_{10}:LC_{50} ratio for all species was 1.32 for larvae and juveniles, and 1.36 for postlarvae. The ratio for postlarvae represents only four species, and hence is not considered different from the other life stages. Third, variability increased with increased species and life stage sensitivity to low DO, and with endpoints of LC_{15} and below, which reduces the certainty of some of these results. Lastly, no influence of temperatures between 20°C and 30°C was detected in a small set of tests with thermally acclimated crustacean larvae. This data set has been used to describe protection limits for juvenile and adult survival, and for larval recruitment for the case of persistent (≥24 h) low DO for estuarine and coastal waters of the Virginian Province, USA.

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Introduction

Eutrophication is a problem of increasing importance in estuarine and coastal waters, a result of marked increases in the human population of coastal regions (de Jonge et al. 1994). One frequent consequence is the reduction of dissolved oxygen (DO) in these waters.

The deleterious effects of low DO on the fauna must be known in order to assess ecological risk and estimate protective limits. Unfortunately, only a small portion of the sizable literature on effects of reduced DO deals with saltwater species (Douderoff and Shumway 1970; Davis 1975; Renaud 1985). Perhaps the best studied assemblage is the macrobenthos (Diaz and Rosenberg 1995).

These data are of limited value for assessing damage, however, because the macrobenthos is not particularly sensitive to low DO: its general threshold is 2 mg l^{-1}, and some species can tolerate half that for several days (Diaz and Rosenberg 1995). Little is known about thresholds for other estuarine and coastal assemblages. Relatively few species have been studied, and early life stages (ELS) little considered, even though ELS are now known to be particularly sensitive (US EPA 1986; Rombough 1988). Also, many of the pertinent laboratory studies are difficult to interpret and reconcile because of differences in design, exposure, and endpoints. Hence, the incipient lethal and sublethal thresholds for low DO in ELS of freshwater and saltwater fish are poorly defined (Rombough 1988). The same can be said for other classes and life stages.

The present study was designed to provide a database on lethality of low DO for coastal and estuarine species. It focused on short-term sensitivities (1–8 days). It emphasized sensitive species and life stages in order to describe the upper portion of the lethal range. Fishes, crustaceans, and one bivalve were examined. Species were limited to those occurring in cold temperate waters in order to eliminate the possibility of regional differences in response. Standardized bioassay methods (ASTM 1989) were used to enhance the precision and quality of the tests and to allow results within the data set to be fully comparable. The results were analyzed in a format amenable to assessing ecological risk of low DO in addition to providing the traditional information on median responses.

Materials and methods

Test organisms

A total of 22 species were tested; the classes, names, and general sources are indicated in Table 1. The organisms used were obtained from the East and Gulf coasts of the United States of America, and the western Atlantic. Most field-collected organisms were from Narragansett Bay, Rhode Island, its immediate tributaries, and adjacent ponds. *Stenotomus chrysops* and *Tautoga onitis* were collected in Long Island Sound. Most spawning stocks were obtained from waters in Rhode Island or Connecticut. Embryos or gravid stocks obtained from other locations were: *Morone saxatilis* embryos from the Choptank River (Maryland) and the Hudson River (Verplank, New York); gravid *Homarus americanus* from Veatch and Oceanographer Canyon (western Atlantic); *Sciaenops ocellatus* embryos from St. Petersburg, Florida; and A*mericamysis bahia* (= *Mysidopsis bahia*, Price et al. 1994) from Gulf Breeze, Florida.

Juvenile and brood stock organisms were held in flow-through circular tanks (fishes) or troughs (crustaceans) receiving water from Narragansett Bay. Juvenile *Palaemonetes* spp. and *Crangon septemspinosa* were held in aerated static aquaria the seawater in which was partially renewed daily. Laboratory-cultured organisms were held in flow-through or static renewal systems (Appendix 1, electronic supplementary material available at http://dx.doi.org/10.1007/s002270100702).

Published culture methods were used for five species (Appendix 1, electronic supplementary material). The methods for the remaining fishes, and the remaining crustaceans reared in static renewal systems, were similar to the referenced procedures for the respective water-exchange regimes. The method for the other crustaceans cultured in flow-through systems involved washing newly hatched larvae from the parental holding trough into nytex-screened catch chambers. These chambers were then suspended in a flow-through trough where development took place. The age, size, or instar of the cultured organisms used in the tests are indicated in Appendix 1, electronic supplementary material.

All laboratory-held and laboratory-cultured organisms were fed daily, with larval cultures fed ad libitum. *Sciaenops ocellatus* larvae,

Table 1 Names, life stages, and source of species tested. Life stages with *asterisk* were laboratory-cultured; the remaining were field-collected (*l* larva; *pl* postlarva; *j* juvenile; *a* adult)

Scientific name	Common name	Life stage
Osteichthyes		
Apeltes quadracus (Mitchill)	Fourspine stickleback	j, a
Brevoortia tyrannus (Latrobe)	Atlantic menhaden	j
Menidia beryllina (Cope)	Inland silverside	l*
Morone saxatilis (Walbaum)	Striped bass	pl*, j*
Paralichthys dentatus (Linnaeus)	Summer flounder	j*
Pleuronectes americanus (Walbaum)	Winter flounder	j[a]
Prionotus carolinus (Linnaeus)	Northern sea robin	j
Sciaenops ocellatus (Linnaeus)	Red drum	l*
Scophthalmus aquosus (Mitchill)	Windowpane flounder	j
Stenotomus chrysops (Linnaeus)	Scup	j
Syngnathus fuscus Storer	Pipe fish	j*
Tautoga onitis (Linnaeus)	Tautog	j
Crustacea		
Americamysis bahia (Molenock)	Mysid	j*
Cancer irroratus Say	Atlantic rock crab	l*, pl*
Crangon septemspinosa Say	Sand shrimp	j
Dyspanopeus sayi (Smith)	Say mud crab	l*
Eurypanopeus depressus (Smith)	Flat mud crab	l*
Homarus americanus Milne Edwards	American lobster	l*, pl*, j*
Libinia dubia Milne Edwards	Longnose spider crab	l*
Palaemonetes vulgaris (Say)	Marsh grass shrimp	l*, pl*, j
Palaemonetes pugio (Holthuis)	Daggerblade grass shrimp	l*, j
Bivalvia		
Spisula solidissima (Dillwyn)	Atlantic surfclam	j*

[a]One test group, field-collected; one, laboratory-cultured

and 4- to 7-day-old *Menidia beryllina* were fed rotifers. The remaining fish ELS and all crustacean ELS were fed *Artemia* sp. nauplii hatched from reference *Artemia* sp. cysts of lot II or III (Bengtson et al. 1984; Collins et al. 1991). Juvenile fishes were fed flake food (Tetra SM80, Tetra Werke Baensch, Melle, Germany). Cultured *Homarus americanus* and cultured juvenile fish also received frozen adult brine shrimp (Pro Salt brand, Mid Jersey Pet Supply, Carteret, N.J., USA). Brood stock *H. americanus* were fed mussels. The *Spisula solidisima* tanks were supplemented with algae (*Isocrysis galbana*).

All organisms were held within 1°C of the test temperature for at least 2 days prior to testing. Temperatures before the tests were adjusted by no more than 1°C day^{-1}.

Testing

Tests were conducted between August 1987 and September 1995. All tests used a single-pass, flow-through exposure system (Miller et al. 1994) and water from Narragansett Bay filtered to ≤ 5 μm. Low-DO water was generated by vacuum degassing. The pH was maintained during testing by adding CO_2 to the degassed water. The concentration of DO in the system was controlled electronically to a standard deviation (SD) of 0.2 mg l^{-1}, and temperature to ± 1°C. Salinity was controlled only in tests with postlarval striped bass (*Morone saxatilis*), which were exposed at 5 g kg^{-1}. Otherwise, it was left at the local 28–32 g kg^{-1}. Water temperature of the tests was normally within the local summer temperature range; specific temperatures are indicated in Table 2. The effect of temperature on the lethal response was investigated in additional acute tests with larval *Palaemonetes vulgaris*, *Dyspanopeus sayi*, and *Menidia beryllina*. Test larvae were fed daily ad libitum.

The short-term lethal tests used laboratory practices and a standardized bioassay design adapted from the ASTM (1989) standard guide for acute toxicity tests. Exceptions are noted below. Wherever possible, the statistical resolution of the testing was improved by exceeding the number of recommended test organisms and replicating the tests.

Most tests were run for 4 days because preliminary information indicated that acute responses stabilize by then. Some tests with fishes were terminated early to eliminate results for days when the mortality of the control fish exceeded the standards we applied. Some tests with crustaceans were carried out >4 days in order to observe the effects of one or more molts on the lethal responses. In these cases, the 4-day responses were also recorded. A test generally included five reduced DO concentrations and an air-saturated control, with two to four replicates per treatment. Most tests on fishes exposed 20 individuals (n) per treatment. Exceptions were *Scophthalmus aquosus* ($n=15$) and *Brevoortia tyrannus* ($n=16$). Postlarval and juvenile fishes were placed it test chambers 18–24 h prior to the test, and the chambers were covered with black plastic to minimize visual disturbance. Tests with crustaceans used 20–24 individuals for postlarvae and juveniles, and 30–80 for the larvae. These organisms were placed in air-saturated water in the test chamber, then exposed to reduced DO soon after the chambers were closed. The transition period usually lasted <30 min, and depended on the flow rate into the chamber. Larvae and juveniles of *Homarus americanus*, and juveniles of *Palaemonetes vulgaris*, *P. pugio*, and *Crangon septemspinosa* were exposed individually in 180-ml polycarbonate chambers to prevent cannibalism. Juvenile fishes were exposed in 7-l glass aquaria, while the remaining organisms were in 500-ml polycarbonate chambers. Each chamber was covered and equipped with an in- and out-flow port and a port to receive the DO probe. Flow rate for individually exposed organisms and early larvae was targeted at 50 ml min^{-1}; for later larvae and postlarvae, 100 ml min^{-1}; and for juveniles, 250–500 ml min^{-1}. The rate selected for juveniles considered relative biomass and activity.

Different methods were used in the four tests where crab larvae were exposed from the last molt to the postlarval stage, here called larval-to-postlarval tests (L-PL). The single L-PL test on *Cancer irroratus* was for 7 days in order for the control group to fully molt to postlarvae. One L-PL test on *Dyspanopeus sayi* (no. 1 in Fig. 2c) used organisms from the field rather than the laboratory. The two other L-PL tests on *D. sayi* (nos. 2 and 3 in Fig. 2c) differed in three ways: (1) they began with stage 3 zoea, and hence (2) were exposed to low DO over two molts before reaching the postlarval stage; and (3) the low DO in each treatment continued until 90% of the test organisms had molted to postlarvae or died. Thus the exposure time depended on the treatment. In addition, in all four tests, individuals that successfully molted to postlarvae were removed daily.

All tests were monitored daily. Surviving organisms were counted, the temperature, salinity, and DO measured, and excess food was removed and new food provided (generally twice daily for larvae). Flow rate and pH were measured at the beginning and end of each test, and weekly in tests exceeding 7 days. Flow rate was usually also checked visually each day and adjusted to the target rate as necessary. The daily DO measurements were made directly in the organism exposure chambers using a Nester model 8500 meter and a biological oxygen demand probe that was calibrated daily against air-saturated seawater. Winkler measurements of each treatment were made at the beginning and end of each test, and weekly in tests exceeding 7 days. DO was generally kept within 0.1 mg l^{-1} of the target. The criterion for test acceptability was a SD of 0.2 mg l^{-1} for the average of the daily observations.

At least 90% of the control organisms typically survived. For crustacean larvae, fewer controls survived in seven of the tests; five had values of 83–86% (one test each for *Libinia dubia*, *Palaemonetes vulgaris*, and *Cancer irroratus*, and two tests for *Homarus americanus*), and two were <80% [79% for one larval test for *C. irroratus* and 78% for one L-PL test of *Dyspanopeus sayi* (no. 2 in Fig. 2)].

Analysis

Lethal concentrations (LCs) of DO were estimated for eight degrees of lethality between 5% and 95% using a non-parametric, linear-interpolation procedure (Norberg-King 1993; Chapman et al. 1995). This graphical method estimates the points from segmented plotting (similar to the more familiar Spearman–Karber method of Hamilton et al. 1977). Its only transformation is to smooth the data monotonically. Confidence intervals of the estimates were calculated by a bootstrap procedure (Norberg-King 1993), and the significance of each estimate evaluated by hypothesis-testing using ANOVA and the post hoc tests of Williams (1971, 1972). The analyses were run with ToxCalc software version 5.0.15 (TidePool Scientific Software 1994).

Results and Discussion

The 67 tests on 22 species, primarily fishes and crustaceans, are summarized in Table 2. Nine species were tested as larvae, three as larvae molting to postlarvae, four as postlarvae, and 16 as juveniles. The results are given for LC_{90}, LC_{50}, and LC_{10}. The LC_{50} represents one of the less variable endpoints, and the LC_{90} and LC_{10} give a sense of the range of the lethal response. The results are grouped by class and life stage; within a life stage, species are ranked by their LC_{50}. Replicate tests were usually averaged (see below). The response curves (lethality vs. DO) in Figs. 1, 2 offer more detailed results by incorporating up to eight levels of response.

Uncertainty

Within-test uncertainty for Table 2 endpoints is indicated by the median 95% confidence interval (CI) for

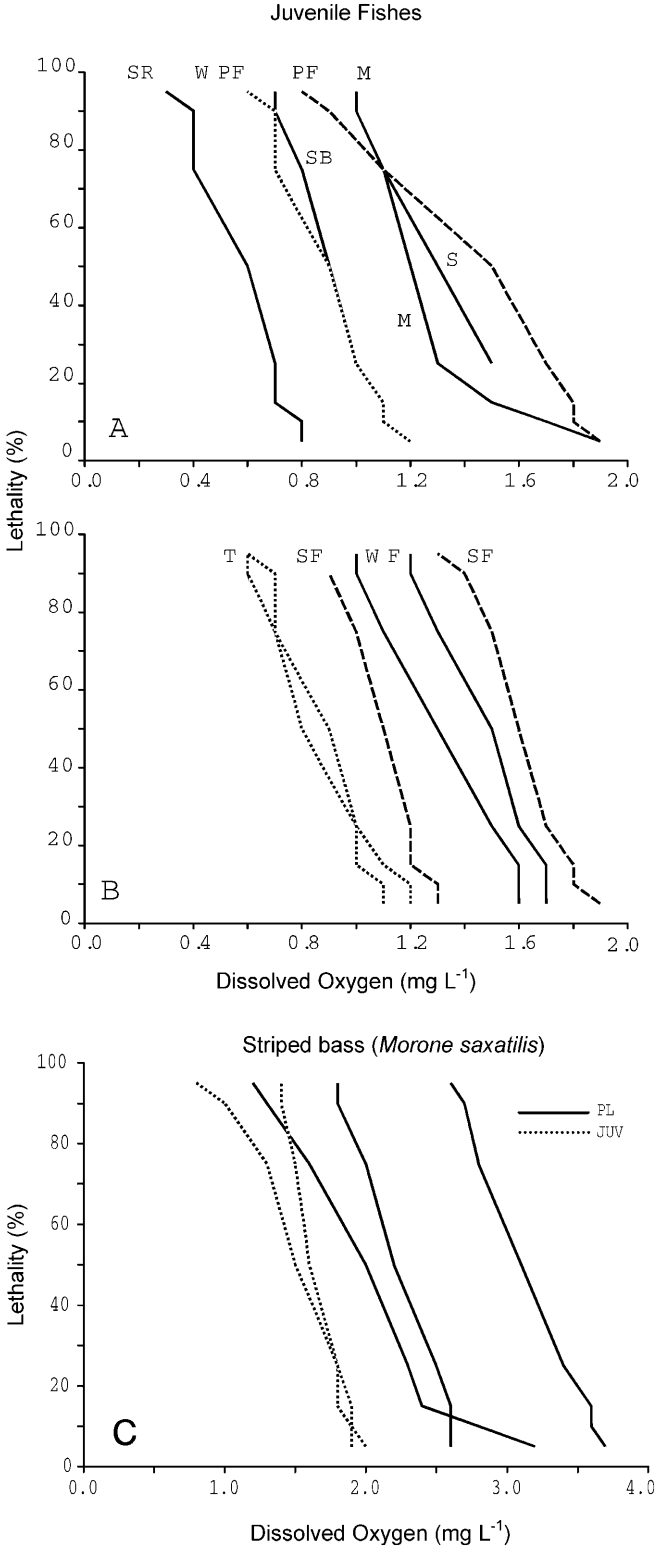
Table 2 Summary of LC_{90}, LC_{50}, and LC_{10} (all in mg l^{-1}) from short-term tests with low DO (*n* number of tests; *CI* confidence interval; *MSD* minimum significant difference). Median CIs and MSD are cited for multiple tests. Species ranked by LC_{50} within a life stage. If test temperatures differ appreciably for a species and life stage, the number of tests per temperature is indicated in *parentheses* (*NC* CI not calculated since assumptions not met)

Class, life stage, species	Test period (days)	Temp. (°C)	Mean LC_{90} (*n*)	CI	Mean LC_{50} (*n*)	CI	Mean LC_{10} (*n*)	CI	MSD (%)
Osteichthyes									
Larva									
Sciaenops ocellatus	1	29	1.2 (1)	0.1	1.8 (1)	0.3	2.3 (1)	1.0	7
Menidia beryllina	1–4	20 (1), 25 (3), 28 (1)	1.3 (4)	0.2	1.4 (5)	0.3	1.7 (5)	1.0	24
Postlarva									
Morone saxatilis	1–4	19–20	1.9 (3)	0.5	2.4 (3)	0.2	3.0 (3)	0.0	7
Juvenile									
Morone saxatilis	4	19–21	1.2 (2)	0.4	1.6 (2)	0.2	1.9 (2)	0.1	9
Syngnathus fuscus	1	20	0.9 (1)	0.9	1.5 (1)	0.1	1.8 (1)	0.3	25
Pleuronectes americanus	4	20	1.1 (2)	0.2	1.4 (2)	0.2	1.6 (2)	0.4	8
Paralichthys dentatus	3	24	1.8 (1)		1.6 (1)		1.4 (1)		14
	4	20	1.3 (1)		1.1 (1)		0.9 (1)		9
Stenotomus chrysops	1	20	1.0 (1)	0.1	1.3 (1)	0.1	–		13
Brevoortia tyrannus	4	19	1.0 (1)	< 0.1	1.2 (1)	< 0.1	1.7 (1)	0.8	12
Apeltes quadracus[a]	4	19	0.7 (1)	0.1	0.9 (1)	0.4	1.1 (1)	4.6	NC
Scophthalmus aquosus	1	20	0.7 (1)	0.1	0.9 (1)	0.1	1.1 (1)	0.2	18
Tautoga onitus	4	24	0.6 (2)	< 0.1	0.8 (2)	< 0.1	1.2 (2)	< 0.1	8[b]
Prionotus carolinus	4	19	0.4 (1)	0.0	0.6 (1)	0.0	0.8 (1)	0.0	NC
Crustacea									
Larva (molting to larva)									
Homarus americanus	4	18–20	2.2 (5)	1.0	3.1 (6)	2.1	4.1 (2)	3.2	40
Libinia dubia	4	20	2.3 (1)	< 0.1	2.7 (1)	0.1	3.1 (1)	4.4	11
Cancer irroratus	4	20	2.1 (2)	0.9	2.6 (4)	0.4	3.8 (4)	2.7	19
Eurypanopeus depressus	4	20–21	2.0 (2)	0.1	2.2 (2)	0.2	2.6 (2)	0.8	15
Palaemonetes vulgaris	4	24–26 (6), 21 (1), 30 (1)	1.6 (4)	0.4	2.1 (8)	0.4	2.7 (6)	1.3	12
Dyspanopeus sayi	4	25–26 (4), 20–21 (2)	1.4 (4)	0.2[c]	1.9 (6)	0.7	3.4 (6)	2.6	18
Palaemonetes pugio	4	25	1.1 (1)	1.7	1.6 (1)	0.3	2.2 (1)	2.8	18
Larva (molting to postlarva)									
D. sayi	≤ 8, ≤ 10	25	2.4 (2)	1.4	3.7 (2)	1.0	4.1 (1)	3.2	25
	4	20	1.7 (1)	2.4	2.5 (1)	0.8	3.0 (1)	0.4	16
C. irroratus	7	20	–		3.0 (1)	0.6	–		30
H. americanus	4, 5	19–20	1.8 (1)	0.1	2.8 (2)	1.4	3.4 (2)	9.0	27
Postlarva									
C. irroratus	4	20	1.7 (1)	0.6	2.2 (1)	4.2	3.3 (1)	13.3	44
H. americanus	4	19	1.2 (1)	< 0.1	1.4 (1)	0.3	1.8 (1)	0.3	5
P. vulgaris	4	18	0.6 (1)	< 0.1	1.0 (1)	0.1	1.4 (1)	0.2	13
Juvenile									
Americamysis bahia	4	26	1.0 (2)	0.2	1.2 (2)	0.2	1.9 (2)	1.9	12
H. americanus	4	20	0.8 (1)	< 0.1	1.0 (1)	0.1	1.4 (1)	1.5	13
P. vulgaris	4	24	0.7 (1)	< 0.1	1.0 (1)	0.0	1.4 (1)	0.3	7
Crangon septemspinosa	4	20	–		1.0 (1)	0.3	1.4 (1)	0.6	10
P. pugio	4	20	0.6 (1)	< 0.1	0.7 (1)	< 0.1	0.9 (1)	< 0.1	8
Bivalvia									
Juvenile									
Spisula solidissima	4	23	0.3 (1)	–	0.5 (1)	< 0.1	0.7 (1)	0.5	10

[a]Juvenile and adult stages
[b]CI calculated for one test only

each data set (Table 2), and between-test variance by the coefficient of variation (CV) (Table 3). The minimum significant difference (MSD) expresses the smallest difference from the controls that can be detected statistically (Table 2). These three measures represent the level of confidence in the results, and should be kept in mind when interpreting and applying the results. For example, even though the LC_{50} was usually less variable than the LC_{10}, LC_{50} variation in some groups was also high. These included tests with juvenile *Apeltes quadracus* and *Paralichthys dentatus*, postlarval *Cancer irroratus* and *Morone saxatilis*, larval *Dyspanopeus sayi* and *Homarus americanus*, and larvae of *D. sayi* and *C. irroratus* molting to postlarvae. The CI for larvae was often wider than for juveniles and wider for LC_{15}, LC_{10}, and LC_{05} than for the higher LCs. The CI for the LC_{10} was usually not much larger than for the LC_{15}, but for the LC_{05} it was often twice that of the LC_{10}. This is the reason the LC_{05} was not used for the lower-bound endpoint (Table 2). The MSD also showed the lower confidence

for endpoints below LC_{15}; 12 of the 32 MSDs in Table 2 exceeded 15%. The CV of replicate tests ranged from 4% to 37%, with appreciable differences between the LC_{10}, LC_{50}, and LC_{90} (Table 3). Between-test variance was large (e.g. CV > 20%) for all three endpoints in the tests of juvenile *P. dentatus*, for two endpoints with *M. saxatilis* postlarvae and *D. sayi* larvae molting to postlarvae, and for one endpoint in five other cases (Table 3).



Conditions between averaged tests

Some of the averages in Table 2 were derived from tests under dissimilar conditions. Conditions that differed for one or more species included temperature, test duration, age, and source. Only some of these differences appeared to affect the results appreciably, however.

Differing temperatures did not usually affect the results. For example, *Menidia beryllina*, *Dyspanopeus sayi*, *Palaemonetes vulgaris*, and juvenile *Paralichthys dentatus* were routinely tested at 25°C. Results from tests nearer 20°C and/or 30°C for the first three of these species were essentially the same as at the nominal temperature, and so were averaged with them to give the values shown in Table 2. One clear exception was the LC_{50} from one test on juvenile *P. dentatus* at 20°C, which was 0.5 mg l^{-1} less than at 24°C. This difference may have been a direct effect of temperature on the lethal response, or indirectly due to developmental differences between *P. dentatus* cultured at different temperatures (20°C and 24°C). The more-sensitive 24°C group was tested 2 weeks after metamorphosis; the less-sensitive 20°C group, 3 weeks after metamorphosis. Because the reason for these different results was not clear, they were not averaged in Table 2.

Differing durations of tests affected crustaceans but not fish. For fish, most of the LCs at days 2–4 fell within 0.1 mg l^{-1} of the values for day 1. In only three tests did the sensitivities change (increase) with exposure time, i.e. larval *Menidia beryllina* (LC_{10}, LC_{50}, LC_{90}) and juvenile *Morone saxatilis* and *Brevoortia tyrannus* (LC_{10}), and then by only 0.2–0.5 mg l^{-1}. These similarities justify averaging the results from tests of different durations (Table 2). Different test durations were important for crustaceans, however, as discussed below under molting.

Neither age nor source of fish larvae seemed to affect their sensitivity to low DO: larvae of *Menidia beryllina* at 12 days post-hatch (dph) (one test) were intermediately sensitive relative to those at < 1 dph (four tests), and results for *Morone saxatilis* from the Choptank River were similar to those from the Hudson River.

The largest effect of dissimilar test conditions was for exposure time and molting on crustacean larvae: most

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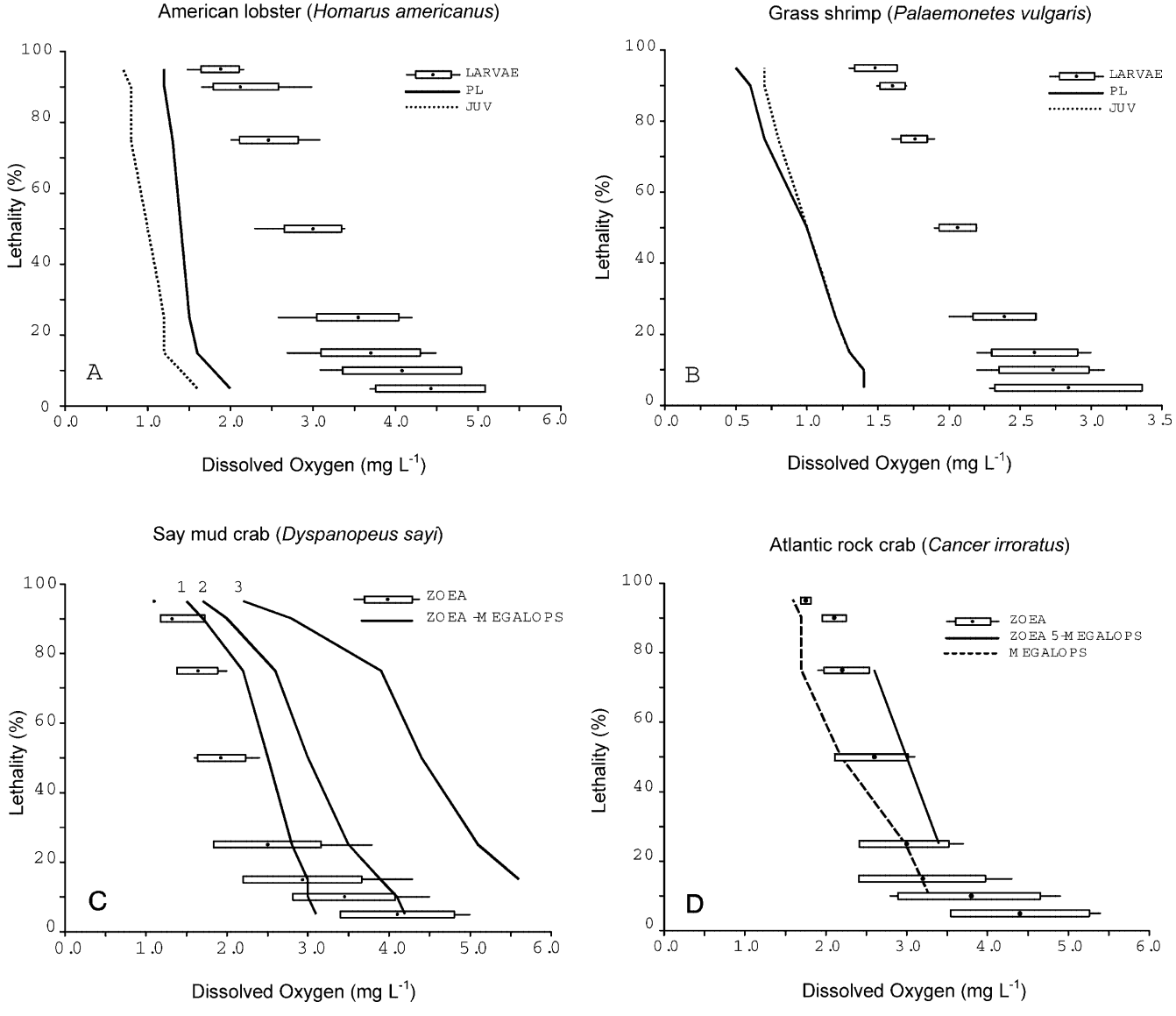
Fig. 1a–c Osteichthyes. Lethal responses of fishes to low dissolved oxygen based on eight endpoints between LC_{05} and LC_{95}; individual test results shown. *Vertical lines* between some estimated points are from rounding. a Juveniles [*SR* northern sea robin (*Prionotus carolinus*); *WPF* windowpane flounder (*Scophthalmus aquosus*); *SB* fourspine stickleback (*Apeltes quadracus*); *PF* pipe fish (*Syngnathyus fuscus*); *M* Atlantic menhaden (*Brevoortia tyrannus*); *S* scup (*Stenotomus chrysops*)]. b Juveniles [*T* tautog (*Tautoga onitus*); *SF* summer flounder (*Paralichthys dentatus*); *WF* winter flounder (*Pleuronectes americanus*)]. c Postlarval (*PL*) and juvenile (*JUV*) striped bass (*Morone saxatilis*)

LC_{50} values for six of the seven species were greater by 0.3–0.8 mg l^{-1} on day 4 than on day 1 (Fig. 3). The reason was investigated in six longer tests on larval *Palaemonetes vulgaris* and *Dyspanopeus sayi* that continued to day 7 or 8 and involved a second or third larval molt. Three patterns of LC_{50} with time (or LC_{25} if the LC_{50} was not reached) were found: (1) no change (*P. vulgaris* and *D. sayi*); (2) an increase of 0.2 mg l^{-1} from day 2 to day 3 and steady after that (*P. vulgaris*); and (3) an increase of 0.1–0.4 mg l^{-1} between days 1 and 4, followed by only a slight increase (0.1 mg l^{-1}) by day 7 or 8 (*P. vulgaris* and *D. sayi*). These results indicate that larvae of these two crustaceans may become more sensitive to low DO when exposed over the first molt cycle, but change little when exposed for an additional period of hypoxia or at least one more molt cycle. This may translate to differences in survivability of crustacean larvae in the field, which are dependent on the molt cycle of the larvae at the time of the hypoxic event.

Three later-stage crustaceans were little influenced by exposures longer than 1 day or through one molt. Postlarvae of *Homarus americanus* remained equally sensitive at 1, 4, and 20 days (with most individuals exposed at concentrations above the LC_{25} having molted by day 20); juvenile *Palaemonetes vulgaris* and *P. pugio* became more sensitive by only 0.1 mg l^{-1} between days 1 and 4; and juveniles of *H. americanus* became more sensitive by 0.2 mg l^{-1} between days 1 and 4, but then remained the same until the test group finished molting by day 10.

Fig. 2a–d Crustacea. Lethal responses of four crustaceans to low dissolved oxygen based on eight endpoints between LC_{05} and LC_{95}. *Vertical lines* between some estimated points are from rounding. a American lobster (*Homarus americanus*), b marsh grass shrimp (*Palaemonetes vulgaris*), c Say mud crab (*Dyspanopeus sayi*), d Atlantic rock crab (*Cancer irroratus*). *Solid and dashed lines* represent individual test results; *box and whisker symbols* represent mean, standard deviation, and range of multiple test results. Three *D. sayi* zoea–megalops (i.e. larval to postlarval) tests are identified numerically (*PL* postlarval; *JUV* juvenile)



Influence of life stage

For crustaceans, the tests with larvae molting to the next larval stage (L-L) are separated from the tests with larvae molting to the postlarval stage (L-PL) in Table 2. Multiple L-L tests for a species usually involved tests with different larval stages. No stage-specific differences could be detected in the L-L tests, however, because within-stage variation of repeated tests was greater than the between-stage variation. Accordingly, the L-L tests were averaged.

The L-PL tests on the crabs *Dyspanopeus sayi* and *Cancer irroratus*, and the American lobster, *Homarus americanus*, were conducted to see whether the sensitivity of crustacean larvae changed as they developed into postlarvae. The mean LC_{50} for two L-PL tests of the lobster fell within the range of the L-L results, but the LC_{50} values for the crabs reached or exceeded the highest 4-day L-L results (Table 2; Fig. 2c, d). These results infer that crabs are more sensitive to low DO during the molt to megalops (the postlarval stage), perhaps because there is a morphological transition. However, this is not considered conclusive because of high variance within and between these tests, the small number of L-PL tests conducted at the nominal temperature, and differences in the L-PL test methods [three lasted 7–10 days, *D. sayi* L-PL tests 2 and 3 (Fig. 2c) were exposed through two molts, and the surviving megalops were removed before the test ended].

The LC_{50} values clearly depended on life stage. For the crustaceans *Homarus americanus*, *Palaemonetes vulgaris*, and *P. pugio*, larvae were the most sensitive, juveniles the least sensitive, and postlarvae intermediate. The postlarval and juvenile stages of *Morone saxatilis* also followed this pattern. But when results for all species were pooled by life stage, the sensitivities of adjacent stages generally overlapped. Species-average LC_{50}s from tests of 1–4 days on the crustaceans ranged from 1.6 to 3.1 mg l^{-1} for larvae (assuming brachyuran larvae molting to postlarvae are not a distinct group), 1.0 to 2.2 mg l^{-1} for postlarvae, and 0.7 to 1.2 mg l^{-1} for juveniles (Table 2). Adding fishes and the bivalve mollusc broadened the ranges of the mean LC_{50} values only slightly, to 1.4–3.1 mg l^{-1} for larvae, 1.0–2.4 mg l^{-1} for postlarvae, and 0.5–1.6 mg l^{-1} for juveniles.

Response curves

Response curves to low DO are given for postlarval and juvenile fishes (Fig. 1) and for the better-studied crusta-

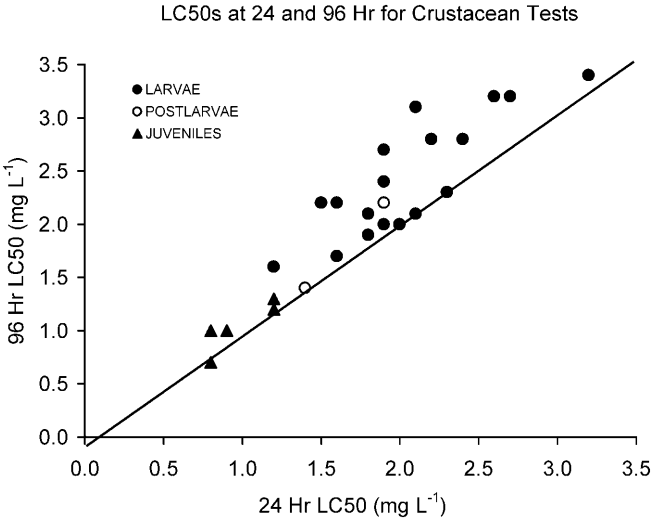


Fig. 3 Relationship between 24- and 96-h LC_{50} values for crustacean tests with low dissolved oxygen. *Each point* represents a paired set of values calculated from the same test; the *line* represents a one-to-one relationship

Table 3 Coefficient of variation (*CV*) of mean LC_{90}, LC_{50}, and LC_{10} endpoints for repeated short-term tests. Species ranked by decreasing LC_{50} within each life stage category

Class, life stage, species	CV, mean LC_{90}	CV, mean LC_{50}	CV, mean LC_{10}
Osteichthyes			
Larva			
Menidia beryllina	9.7	10.1	21.6
Postlarva			
Morone saxatilis	37.3	24.1	17.6
Juvenile			
M. saxatilis	23.6	4.5	0
Pleuronectes americanus	12.9	10.1	4.2
Paralichthys dentatus	29.5	26.2	26.5
Tautoga onitis	11.8	8.2	6.1
Crustacea			
Larva (molting to larva)			
Homarus americanus	22.1	7.8	3.3
Cancer irroratus	6.7	17.4	23.4
Eurypanopeus depressus	17.7	9.7	8.0
Palaemonetes vulgaris	7.2	6.3	13.4
Dyspanopeus sayi	12.4	15.0	18.1
Larva (molting to postlarva)			
D. sayi	23.6	26.7	–
H. americanus	–	23.1	12.5
Juvenile			
Americamysis bahia	14.1	5.6	29.8

ceans, *Homarus americanus*, *Palaemonetes vulgaris*, *Dyspanopeus sayi*, and *Cancer irroratus* (Fig. 2). Curves for repeated tests are presented for postlarvae and juveniles to illustrate the between-test variance. The results of most repeated tests with crustacean larvae were aggregated, and the mean, SD, and range are indicated for each endpoint. For clarity, the means are not connected. L-PL tests for the crabs are plotted separately. The response curves were essentially linear for most postlarvae and juveniles. Exceptions were one test on postlarvae of *Morone saxatilis* and the tests on *Brevoortia tyrannus* and *H. americanus*. In contrast, the curves for crustacean larvae were slightly sigmoidal towards the LC_{95} and more so between the LC_{15} and LC_{05} endpoints. The patterns for repeated tests were usually similar, even when the LC_{50}s differed appreciably. The small differences between LC_{05} and LC_{95} for the four least-sensitive juvenile fishes (≤ 0.6 mg l^{-1}) produced a very steep curve. The LC_{05}–LC_{95} ranges for the more-sensitive organisms were generally greater. The most sensitive fishes tested were the postlarvae of *M. saxatilis*, whose average difference between LC_{05} and LC_{95} was 1.3 mg l^{-1}, which is more than twice the range for the least-sensitive juvenile fishes. A proportionality is indicated between the LC_{50} and the breadth of the LC_{10}–LC_{90} response (Fig. 4). The narrow response curves when LC_{50} values are low, e.g. ≤ 1.4 mg l^{-1}, are doubtless related to the severity of the low DO stress. A DO of 1.4 mg l^{-1} at 20°C and 30 g kg^{-1} salinity represents < 20% of air saturation.

Estimating onset of lethality

The large uncertainties in the measured lethalities of 5–15%, particularly for sensitive species and life stages, illustrate how difficult it is to describe the onset of lethality empirically. One problem is that only a few individuals react to small stresses. Biological and methodological sources of variability abound (references cited below), and, with larvae, there may be natural changes in sensitivity during development and hence even during a test (Rombough 1988). In contrast, LC_{50}s can usually be estimated acceptably. Mean LC_{10}:LC_{50} ratios calculated from these results can be used to obtain a first estimate of the onset of lethality for other species or life stages where an LC_{50} is available. The mean LC_{10}:LC_{50} ratios observed were 1.32 for larvae, 1.36 for postlarvae, and 1.32 for juveniles, based on mean LC_{50}s and LC_{10}s for each species and life stage tested (Table 2).

Literature comparisons

It is difficult to compare our LC_{50}s with literature values because the values may be affected by endogenous and exogenous factors (Vernberg 1972) and differences in methods, which abound (US EPA 1986; Rombough 1988). These difficulties notwithstanding, the values in the present study for 1- to 4-day LC_{50}s of juveniles fell

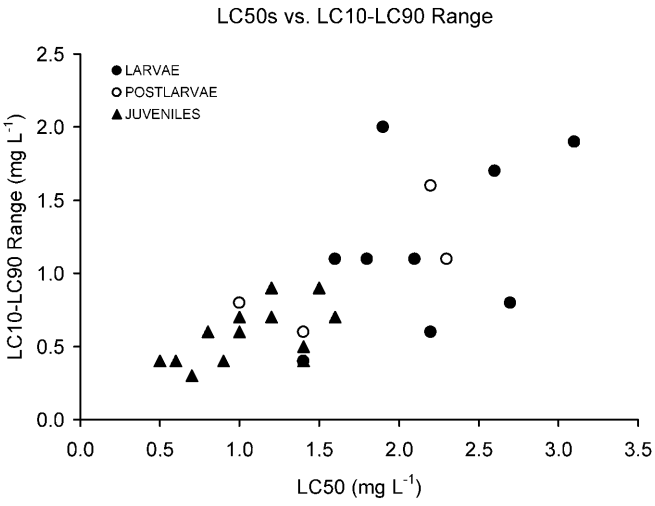


Fig. 4 Relationship between LC_{50} and LC_{10}–LC_{90} range (based on Table 2)

within 0.3 mg l^{-1} of literature values for *Brevoortia tyrannus* (Burton et al. 1980), *Morone saxatilis* (for exposures of 16 and 18 h, Dorfman and Westman 1970), *Homarus americanus* (at 15°C, an intermediate test temperature, McLeese 1956), and *Palaemonetes pugio* (Hutcheson et al. 1985). Another report on *P. pugio* (Stickle et al. 1989; and personal communication), cited a 4-day LC_{50} of 1.0 mg l^{-1} higher. Possibly another stress was present. The similarity of our results for *B. tyrannus* with those of Burton et al. (1980) is noteworthy because the temperature and salinity of the tests differed appreciably (28°C and 6 g kg^{-1} salinity in Burton et al. vs. 19°C and 30 g kg^{-1} salinity in ours). Twelve-hour mean lethal concentrations of 0.4 and 0.6 mg l^{-1} have been published for a fifth species, *Pleuronectes americanus* (Hoff 1967). Those values were lower than the ones we found in the present study, possibly because Hoff's tests were static and ours flow-through; and Hoff's DO was reduced gradually and continuously by the fishes' respiration, while our exposures were acute and kept stable.

Our LC_{50} values for tests lasting 1–4 days are the first to be reported for the larvae and postlarvae tested here. Tests lasting up to 4 h have been conducted with *Cancer irroratus* larvae (Vargo and Sastry 1977). The 4-h LC_{50}s at 20°C ranged from 1.5 to 3.2 mg $l^{-1}$for larval stages 1–5, which is approximately the range we observed. The 4-h LC_{50} for megalops was 3.1 mg l^{-1}, which is 0.9 mg l^{-1} higher than our 4-day value for megalops to crab.

Most LC_{50}s for the three classes and life stages tested here are within the range observed by most previous low DO studies using similar durations and conditions (e.g. under conditions of acclimated temperature, salinity, and pH). This generalization is based on literature for freshwater fish (reviewed and summarized by Doudoroff and Shumway 1970), freshwater fishes and invertebrates (reviewed by US EPA 1986),

early life stages of freshwater and marine fish (reviewed by Rombough 1988), and six marine crustaceans (Stickle et al. 1989; Das and Stickle 1993). A few literature values do appreciably exceed our LC_{50} range, however, sometimes by factors of two to three. Examples for juvenile crustaceans include 4-day LC_{50}s of 2.9–4.4 mg l^{-1} for *Penaeus aztecus* (brown shrimp) and 2.5–4.1 mg l^{-1} for *Callinectes sapidus* (blue crab) reported by Stickle et al. (1989; 4-day data by personal communication), and a 7-day LC_{50} of 2.3 mg l^{-1} for *C. sapidus* (Das and Stickle 1993). Striped mullet (*Mugil cephalus*) larvae at 4.8 mg l^{-1} experienced 75% lethality by 48 h, and at 6.4 mg l^{-1} experienced 63% lethality by 96 h (Sylvester et al. 1975). The methods and exposure conditions for such tests should be evaluated for additional stresses.

An application

The results have been used by the US Environmental Protection Agency to recommend minimum DO values to protect juvenile and adult survival and larval recruitment within the Virginian Province (southern Cape Cod to Cape Hatteras, USA) (US EPA 2000). The data were applied to the case of seasonally persistent reduced DO, such as occurs during summer in central and western Long Island Sound (Welsh et al. 1994) and the Chesapeake Bay mainstem (Officer et al. 1984). The limit for juvenile and adult survival was set at 2.3 mg l^{-1}. The larval data were used with a recruitment model to describe minimum DO conditions necessary to limit recruitment impairment. A recruitment curve generated for four crabs (*Dyspanopeus sayi*, *Eurypanopeus depressus*, *Libinia dubia*, *Cancer irroratus*) indicated the following minimum conditions: 3.3 mg l^{-1} for 7 days, 4.0 mg l^{-1} for 14 days, and 4.5 mg l^{-1} for ≥28 days. Based on larval growth studies, the US EPA (2000) has also recommended a protective value of 4.8 mg l^{-1} in situations where DO is chronically low.

The summer decline of DO in subpycnocline waters of central and western Long Island Sound have been summarized for 1986 through 1992 (Welsh et al. 1994). The temporal pattern was similar among years, with DO declining following summer stratification and often persisting until the thermocline breaks down in the fall. In the Narrows, conditions ≤ 4.5 mg l^{-1} frequently lasted ~65 days; ≤ 4.0 mg l^{-1}, 60 days; and ≤ 3.3 mg l^{-1}, 50 days. The magnitude of DO depletion varied among years. The near-bottom waters of the Central Sound could decline to the <5 but >3 mg l^{-1} range, and the Western Basin and Narrows from <4 to <1 mg l^{-1}. It is evident that the DO limits recommended by the US EPA (2000) for survival of juveniles and adults, growth, and larval recruitment were not met in some bottom waters of the western portion of Long Island Sound during these summers. The limits for growth and larval survival may not have been met during years of sever oxygen depletion in the Central Basin as well.

Conclusions

The present study provides four general conclusions about determining the lethal thresholds of low DO. Firstly, 1-day tests are long enough to reliably estimate the lethal limits for fishes, and postlarval and juvenile crustaceans. The only exception seems to be the larvae of crustaceans, which were more sensitive to exposures that continued into day 4 or involved molting. Their sensitivities may not further increase after this, however.

Secondly, larvae were often the most sensitive life stage to low DO. The most sensitive species were those with pelagic larvae, such as *Homarus americanus*, which live in near-surface waters, while the least sensitive were benthic species, such as juvenile *Tautoga onitus* and *Spisula solidissima*. This confirms Rombough (1988), who noted that the tolerance of ELS of fish species to low DO appears to follow the general levels of DO in the species' normal habitat.

Thirdly, variability increased proportionally with the sensitivity of the organisms tested and at the lower endpoints, e.g. LC_{15} and below. An effort should be made to refine tests with larvae to reduce this variability, given the high sensitivity of this life stage and hence the importance in establishing DO limits or assessing low DO effects. Refined tests would contribute to better estimates of the response curve over all, and are crucial for examining intraspecific differences among early developmental stages, including crab larvae exposed during their the molt to megalops.

Finally, we found the temperatures used (18–29°C) hardly affected the sensitivity to low DO for thermally acclimated organisms, which agrees with the evidence cited by Rombough (1988) for temperatures within the normal range for the a test organism.

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