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EFFECTS OF SHORT-TERM OXYGEN DEPLETION ON FISH

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Abstract—Laboratory experiments were undertaken to investigate the influences of exposure duration and frequency on the toxicity of short-term pulses of low dissolved oxygen (DO) to fish. For the investigation of exposure duration, rainbow trout (*Oncorhynchus mykiss* [Walbaum]) and roach (*Rutilus rutilus* L.) were exposed to a range of DO concentrations in single pulses of 1, 6, or 24 h. For the investigation of exposure frequency, brown trout (*Salmo trutta* L.) were exposed to 24-h pulses of DO concentrations of 4.0 and 5.5 mg/L at frequencies of once or twice weekly over a period of 75 d. The results suggest that, for a given duration, there is a narrow threshold concentration range above which mortality does not occur and below which mortality rapidly becomes high. This threshold concentration range increases as exposure duration increases. Roach were able to survive lower DO concentrations than trout. Observations on experimental animals following exposure indicated no significant postexposure effects, even at very low DO levels. For the exposure frequencies used here, DO concentration rather than frequency of exposure was the important factor in terms of effect on fish. No significant effects on growth rate were observed but there were significant differences in hemoglobin levels, hematocrit, and organ weights. These results have important implications for the derivation of environmental quality standards aimed at the control of episodic pollution in rivers.

Keywords—Dissolved oxygen Short-term exposure Toxicity tests Intermittent pollution Environmental quality standards

INTRODUCTION

Dissolved oxygen depletion is a common problem in rivers polluted by the discharge of organic wastes. As a consequence, numerous studies have been carried out to establish how reduced dissolved oxygen (DO) concentrations affect aquatic organisms and to derive appropriate water quality standards. This work as been extensively reviewed [1–3]. Most reported studies have concentrated on continuous exposure to low DO over periods from days up to several weeks. While some polluted rivers suffer prolonged oxygen depletion, particularly during summer months, many others experience only transient DO depletion caused by pollution episodes that may last only a few hours. This has been shown to be the case, e.g., in rivers affected by runoff of farm wastes [4] and those receiving rainfall-generated waste discharges from combined sewer overflows [5]. Although short-term, these transient events may exert a significant influence on fish and other aquatic life.

The study described in this paper was initiated to address the question of how fish respond to short-term episodes of low DO of differing duration and frequency. There has been some previous work on short-term exposure, most notably during the 1950s [6–8]. These earlier studies were primarily concerned with lethal effects, whereas the focus of the present study is defining conditions that will avoid lethality and long-term detrimental effects. The series of experiments reported here was conducted as part of a major research program to investigate the effects of exposure to pollutant pulses of varying magnitude, duration, and frequency. The objective of this program was to apply the ecotoxicological information derived from these experiments to the derivation of environmental quality standards relevant to the control of intermittent pollution in rivers. The results should be seen as preliminary, pending further validation and testing of other species.

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MATERIALS AND METHODS

Test organisms

Three species of fish were used, rainbow trout (*Oncorhynchus mykiss* [Walbaum]), roach (*Rutilus rutilus* L.), and brown trout (*Salmo trutta* L.). The rainbow trout were obtained from Warnford trout farm (West Meon, Hampshire, UK) and when tested were three months old. The roach were obtained from Anglian Water Authority (Norwich Division) Fish Farm (Norwich, Norfolk, UK) and when tested were approximately one year old. The brown trout were obtained from Berkshire Trout Farm (Hungerford, Berkshire, UK). Prior to testing, the fish were held in large tanks with a groundwater flow giving a replacement rate of between 10 and 24 volumes per day. The fish were fed pelleted trout food at 1 to 1.5% wet body weight per day for the trout and 2% for the roach.

Test procedure—Exposure duration

For each test, fish were placed in each tank (25 rainbow trout or 20 roach per tank) and allowed to acclimate for at least 2 h. Six tests were carried out separately, three for each species, with exposure durations of 1, 6, and 24 h. The test vessels were polypropylene tanks with clear plastic lids sealed with a rubber strip and clamped down. Each tank was filled with 150 L of groundwater, giving a 2-cm closed air space between the water surface and lid. Each test had one control tank, and the number of experimental tanks was four for tests with rainbow trout and three for tests with roach, each tank receiving a different DO concentration. The DO concentrations were reduced and maintained at the desired levels by aeration with a mixture of nitrogen, air, and carbon dioxide to achieve as close to square-shape pulses as possible. The control tank

Table 1. Experimental design for exposure frequency experiments

Vessel No.	Target dissolved oxygen (mg/L)	Frequency/week
1 (control)	Air saturation	—
2	4.0	1
3	5.5	1
4	4.0	2
5	5.5	2

received air and carbon dioxide only. At the end of the exposure period, the nitrogen supply was stopped and the air supply increased to bring the DO concentration back to air saturation value. This took approximately 1 h. During the test, the DO concentration in each tank was monitored continuously by DO meters connected to a chart recorder. The tanks were held in a constant ambient air temperature between 12 and 13°C for the rainbow trout tests and between 13 and 15° for the roach tests, with constant low intensity illumination.

The physicochemical variables measured were (with total range over all tests in parentheses) temperature (12.1–14.8°C), pH (7.37–8.44), and total hardness (150–288 mg/L $CaCO_3$). For each test, there was very little variation in these variables between tanks.

For the 1- and 6-h exposure tests, the fish were observed continuously for mortality and sublethal effects. For the 24-h exposure tests, observation was continuous for the first 11 h, no observations were made during the next 5.5 h, and observations were then made hourly to the end of the exposure period. The fish were held in the tanks for a further 4 d for 1- and 6-h exposure tests, 6 d for 24-h exposure tests, and observed at least once per day for any postexposure mortality.

Test procedure—Exposure frequency

The exposure frequency experiment used brown trout. (The use of a different species arose from a request by the project sponsors following the exposure duration test that UK native species be used.) Fish were selected from stock, weighed (mean wet wt 11.2 g), and randomly distributed between five 120-L polythene test vessels, 16 fish per vessel. During the experiment, a groundwater flow of 1 L/min was maintained to each tank, but this was stopped during the low DO exposure periods. The fish were fed at 4% body weight per day and were weighed every two weeks and the feeding rate adjusted accordingly.

The low DO exposures lasted 24 h, and the procedure for reducing DO concentration was the same as for the exposure duration experiments. Each vessel received a different treatment, as shown in Table 1. The test lasted 75 d, with vessels 2 and 3 receiving 10 low DO events and vessels 4 and 5 receiving 20 events.

Water quality was measured throughout experiments. The pH ranged from 7.28 to 8.53 and temperature from 12.1 to 14.3°C. Hardness was measured twice during the test and ranged from 272 to 290 mg $CaCO_3$/L. Only slight differences between test vessels in terms of water quality were observed.

For half the fish in each tank, the measurements made at the end of the experiment were hematocrit (according to the method of Blaxhall & Daisley [9]), hemoglobin (absorbance at 540 nm [9]), and spleen, kidney, and liver weights. Two-way analyses of variance were performed on the data to test for the effects of DO concentration and frequency of exposure. Gills were dissected out and sent to the National Rivers Authority Fish Diseases Laboratory (Brampton, Cambridgeshire, UK) for measurement of surface area and assessment of condition.

The remaining fish were subjected to an acute low DO exposure at 1.5 mg/L. The survival time for each fish was recorded and median period of survival was calculated for each group. Differences between groups were examined using the method of Litchfield [10].

Before the acute exposure, the control fish and those from vessel 4 (worst case treatment) were placed in a WRc Mark III Fish Monitor (WRc, Medmenham, Buckinghamshire, UK) [11] and their ventilatory responses to a low DO pulse monitored. Two runs were carried out, each with four fish from each group in the eight-channel monitor. After a 24-h acclimation period, the ventilation rates were monitored for a 30-min period, DO was reduced to 4 mg/L, and ventilation monitored for a further 30 min.

RESULTS

Exposure duration

Oxygen concentrations during exposure. The mean, minimum, and maximum DO concentrations during the exposure periods are given in Table 2. Dissolved oxygen concentrations

Table 2. Dissolved oxygen concentrations during exposure periods of exposure duration experiment

Exposure period (h)		Dissolved oxygen concentration (mg/L)				
		Vessel 1 (control)	Vessel 2	Vessel 3	Vessel 4	Vessel 5
Rainbow trout						
1	Mean	10.3	3.5	2.5	1.5	0.7
	Range	10.1–10.4	3.1–3.8	2.5–2.6	1.5–1.7	0.5–0.9
6	Mean	10.7	3.2	2.7	1.6	1.2
	Range	10.5–10.9	2.8–3.5	2.4–3.0	1.3–2.1	0.9–1.5
24	Mean	10.4	4.5	3.5	2.5	1.6
	Range	10.1–11.0	4.2–4.9	3.1–3.9	2.0–3.0	1.0–2.2
Roach						
1	Mean	10.5	2.4	1.6	0.4	—
	Range	10.3–10.7	2.3–2.7	1.6–1.8	0.3–0.7	—
6	Mean	10.6	2.5	1.5	0.4	—
	Range	10.5–10.7	2.3–2.8	1.3–1.8	0.2–0.6	—
24	Mean	10.6	2.2	1.3	0.3	—
	Range	10.4–10.9	2.0–2.4	1.1–1.6	0.1–1.5	—

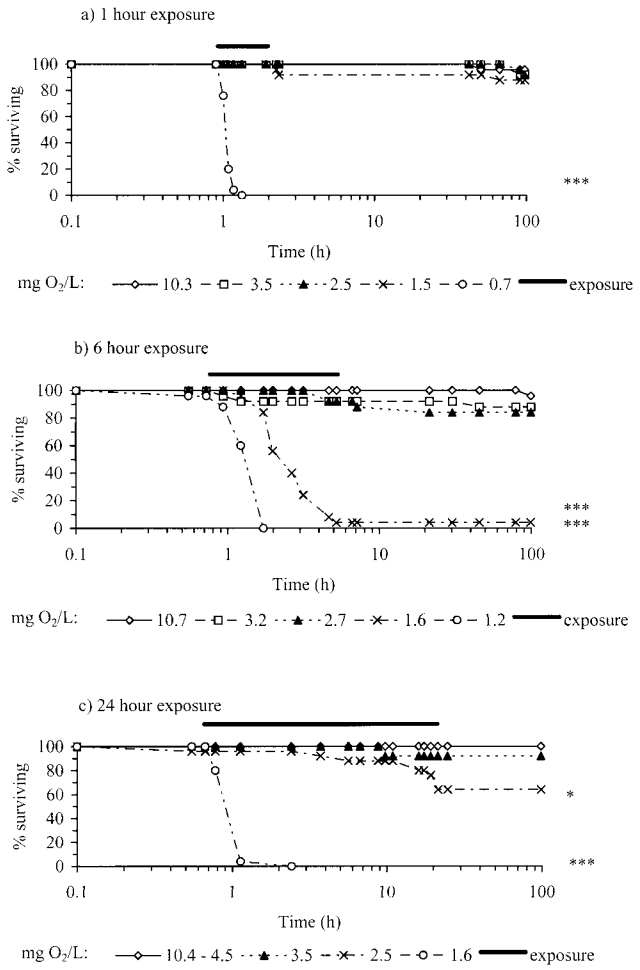

Fig. 1. Mortality of rainbow trout exposed to different concentrations of dissolved oxygen for 1, 6, and 24 h. * = significantly different from control at 5%; *** = significantly different from control at 0.1%. Note that symbols sometimes overlap.

were successfully maintained within a narrow range during exposure, as indicated by the measured ranges shown in Table 2.

Rainbow trout. Mortality curves for rainbow trout are plotted in Figure 1. Mortality was generally restricted to the exposure period; there was no evidence of significant postexposure mortality. The indicated exposure periods in Figure 1 represent the period during which the target concentration was maintained. For the lowest DO concentrations, some mortalities occurred before this period, i.e., during the period of lowering the DO concentration.

For each of the three experiments, a Fisher exact probability test was carried out to identify those exposure regimes where mortality differed significantly from the control. Statistically

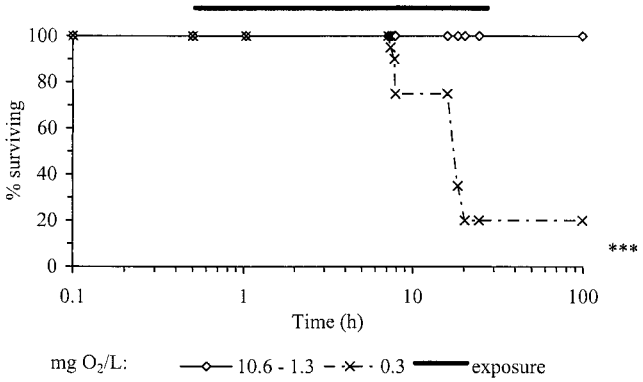

Fig. 2. Mortality of roach exposed to different concentrations of dissolved oxygen for 24 h. *** = significantly different from control at 0.1%.

significant differences were found for the lowest concentration at 1-h exposure and the lowest two concentrations at 6- and 24-h exposure. The results suggest there is a narrow threshold concentration range above which mortality does not occur and below which mortality rapidly becomes high. From Figure 1, it can be seen that the threshold concentration increases as exposure duration increases; mortality was significant at 1.6 mg/L for 6-h exposure but not at 1.5 mg/L for 1-h exposure. Practical constraints precluded the use of replicate vessels in the tests. An indication of repeatability can be gained from comparing the results for different exposure times. Comparison is only possible for 1.6 mg DO/L exposure. There did appear to be some differences in sensitivity; in particular, the onset of mortality was more rapid in the 24-h exposed fish than in the 1- and 6-h exposed fish.

Roach. Mortality of roach was minimal. One fish died during 1-h exposure at 0.4 mg/L and no fish died during the 6-h exposure test. Mortality during the 24-h exposure test, plotted in Figure 2, was restricted to the lowest concentration (mean concentration 0.3 mg/L). The first mortality was seen after 7.5 h, and by 24 h, 16 out of the 20 fish had died.

Exposure frequency

Oxygen concentrations during exposure. Dissolved oxygen concentrations achieved during the 24-h experiment pulses are summarized in Table 3, showing that they were close to the target levels.

Survival. No mortality was observed during the experiment. All fish appeared to be in good external condition at the end of the test.

Growth. The mean weights of fish from each treatment, at days 0 and 75, are shown in Figure 3 and mean lengths are shown in Figure 4. Analyses of variance indicated no signif-

Table 3. Measured dissolved oxygen concentrations during 24-h experimental pulses (exposure frequency experiments)

	Dissolved oxygen concentration (mg/L)				
	Vessel 1 (control)	Vessel 2 (4.0 mg/L, 1/week)	Vessel 3 (5.5 mg/L, 1/week)	Vessel 4 (4.0 mg/L, 2/week)	Vessel 5 (5.5 mg/L, 2/week)
Mean	9.86	4.07	5.32	4.01	5.30
Minimum	8.80	3.40	4.70	3.50	4.70
Maximum	10.70	5.70	5.80	5.80	5.90

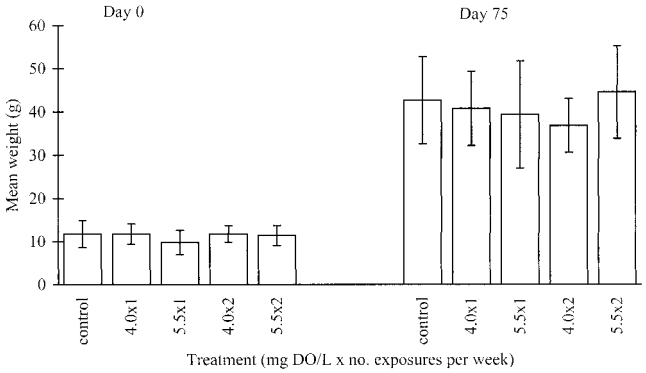

Fig. 3. Mean weights (with standard deviation) of brown trout before and after intermittent exposure to low dissolved oxygen (DO; see text for exposure regimes for each vessel).

icant differences between control and experimental groups in either body weight or length.

Physiological indices. The results of the physiological measurements are shown in Figure 5. One-way analyses of variance indicated significant differences between groups for spleen, liver, and kidney weights (as percentage of body weight) and for hemoglobin but not for hematocrit. The Dunnett's test showed that, for spleen and kidney weights and hemoglobin, some of the experimental groups differed significantly from the control. Inspection of Figure 5 reveals that spleen weight tended to increase as a percentage of body weight in the experimental groups, with the two groups exposed at 4 mg/L being significantly elevated in comparison with the control. In contrast, kidney weight tended to decrease as a percentage of body weight, and here it was the two groups exposed to 5.5 mg/L that showed significant differences from the control. For hemoglobin, there was a tendency to elevation in the experimental groups, all of which were significantly greater than the control except the group exposed to 4 mg/L twice per week.

Two-way analyses of variance were also performed on the experimental data alone (i.e., excluding the controls) to determine the effects of exposure concentration and frequency. These indicated that, for each of the five variables, there was a significant effect of DO concentration but not of frequency of exposure

Gill histopathology. Examination of gill tissues revealed a number of pathological effects, including hyperplasia, hypertrophy, and necrosis, but there was no evidence of any dose–response effects.

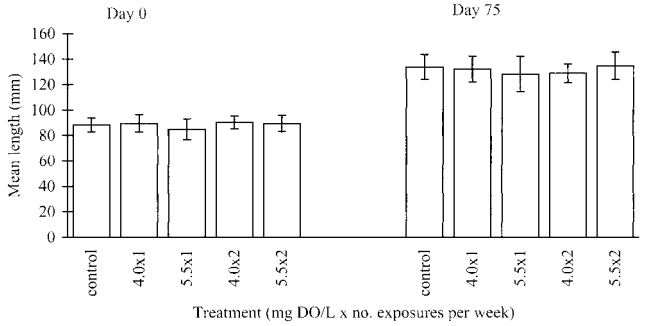

Fig. 4. Mean length (with standard deviation) of brown trout before and after intermittent exposure to low dissolved oxygen (DO; see text for exposure regimes for each vessel).

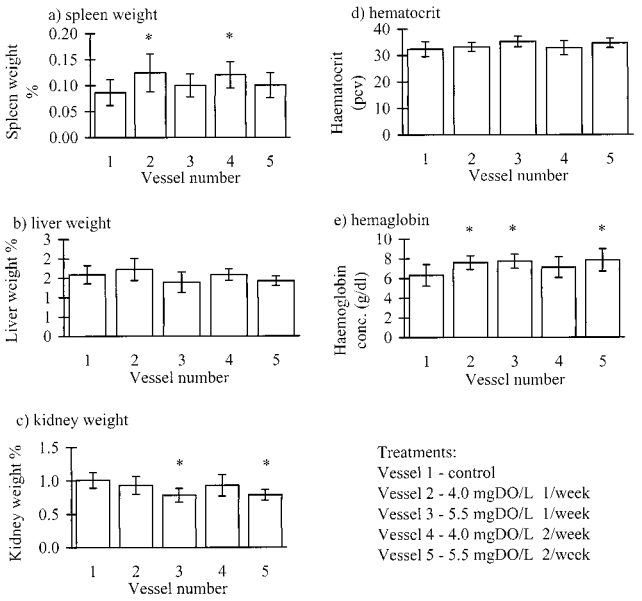

Fig. 5. Organ weights, as percentage of body weight, and blood parameters for brown trout after intermittent exposure to low dissolved oxygen (DO). Values are means with standard deviation. * = significantly different from control at 5%.

Gill ventilation rate response to low DO. Mean gill ventilation rates are shown in Table 4. Both groups (control and worst case exposure) showed almost identical ventilation rates before exposure and identical responses to exposure in the form of an increase in ventilation rate of about 20%. The response was almost immediate. Thus, there was no evidence of any acclimation, in terms of respiratory efficiency, having occurred.

Acute exposure. The median period of survival for each treatment is plotted in Figure 6. There was no evidence that the low DO exposed fish were more resistant; in fact, the control fish showed a slightly longer survival period, though this was only significantly longer ($p < 0.05$) than fish from the 4 mg/L once per week group.

DISCUSSION

There are few published studies on the effects of short-term low DO exposure on fish. Graham [12] and Shepard [6] both investigated the effects of short-term exposure on speckled trout (*Salvelinus fontinalis*). Graham [12] subjected speckled trout to different low DO concentrations at different temperatures and found that lethal low DO concentration was temperature dependent, with fish surviving lower DO concentrations at low temperature. However, only one fish was used for each DO concentration. Shepard [6] performed similar exper-

Table 4. Mean (with 95% confidence limits) ventilation frequencies of fish before and during a 4 mg/L low dissolved oxygen; CI = confidence interval

Fish group	Mean ventilation frequency (±95% CI)		Difference between means
	Pre-exposure ventilation (Hz)	Exposure ventilation (Hz)	
Control (n = 8)[a]	1.66 (±0.17)	2.03 (±0.09)	0.38
4 mg/L 2/week (n = 7)	1.69 (±0.24)	2.06 (±0.27)	0.37

[a] n = number of fish tested.

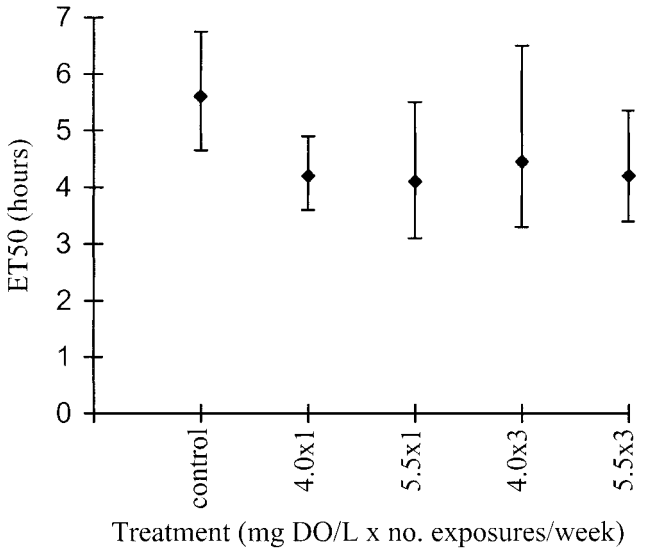

Fig. 6. Median periods of survival with 95% confidence limits for brown trout after acute exposure to a low dissolved oxygen (DO) episode.

iments, but with groups of fish, and found that median survival time was closely correlated with DO concentration and ranged from about 10 min at 0.9 mg/L to 600 min at 1.7 mg/L.

Allan et al. [8] investigated the effects of sewage effluent on rainbow trout. They found that tolerance to low DO decreased as the concentration of either carbon dioxide or ammonia increased. Downing and Merkens [7] exposed eight different fish species (rainbow trout, perch, roach, mirror carp, tench, dace, bleak, and chub) to different DO concentrations and recorded mortality after different time periods. They found rainbow trout to be considerably less tolerant than other species. Over a 3.5-h period, the maximum DO concentration causing 100% mortality and that allowing 100% survival were generally both lower than for a 3.5-d period, though for 3.5 and 7 d, there was generally little difference. The difference between the concentration causing 100% mortality and that allowing 100% survival was generally small, at around 0.5 mg/L. Similarly, Magaud [13] found that rainbow trout showed 100% mortality at 1.7 mg/L over 2.5 h but 0% mortality at 2.3 mg/L. This is supported by the findings in the present study, where high survival and high mortality for rainbow trout were separated by at most 1 mg/L. For roach, the evidence was limited due to lack of mortality, but the indication is for a similar pattern.

These observations suggest there is a critical threshold concentration needed to support essential metabolic processes. The actual threshold concentrations separating survival and mortality in the present study agree well with those found by Downing and Merkens [7], as illustrated in Table 5.

The present study supports the findings of Shepard [6] and Downing and Merkens [7] that the critical threshold concentration depends on exposure duration. For very short-term exposure (minutes to hours), fish can survive low DO concentrations that would be lethal over longer periods.

In all the above reported studies, the experimental approach was to place fish in low DO concentrations and record when they die. Such a method does not allow any assessment of postexposure mortality. The results of the present study, which more closely reproduced a short duration exposure, indicate that there is little or no postexposure effect, even for exposure to very low DO concentration for short periods.

For the investigation of exposure frequency in this study, even the most severe combination of 24-h pulses of 4.0 mg DO/L applied twice weekly had no significant effect on fish growth over the 75-d period. The results of other relevant studies vary widely between tests and between species. The U.S. Environmental Protection Agency [14] carried out an extensive literature review and concluded that production impairment for salmonids was reported to range from slight at 6 and 9 mg DO/L to severe at 4 and 7 mg DO/L for adults and early life stages, respectively. Whitworth [15] found that diel DO fluctuations significantly reduced growth of brook trout. The experimental regime was 14 h at DO concentrations reduced from 11 mg/L to 5.3, 3.6, 3.5, or 2.0 mg/L. At 2.0 mg/L, few survived, but at the other concentrations, growth was severely reduced with no significant difference between groups. However, Alabaster and Lloyd [3] stated that neither reduced DO to 5 mg/L nor wide diel fluctuations about this concentration has much effect on growth of salmonid alevins and there may be only slight growth reduction at 3 mg/L.

Although growth was not significantly impaired as a consequence of the low DO exposures applied in the present study, a number of physiological effects were observed that were attributable more to DO concentration than exposure frequency. However, although some of the effects were statistically

Table 5. Dissolved oxygen concentrations separating high mortality and high survival

		Dissolved oxygen concentration (mg/L)					
		Downing and Merkens [7][a]				Present study	
		Temperature = 10°C		Temperature = 16°C		Temperature = 12–15°C	
Species	Duration	100% mortality	100% survival	100% mortality	100% survival	>95% mortality	>95% survival
Rainbow trout	1 h	—	—	—	—	0.7	1.5
	3.5 h	1.2	1.7	1.5	1.9	—	—
	6	—	—	—	—	1.6	2.7
	24 h	—	—	—	—	1.6	3.5
	3.5 d	1.3	1.9	2.4	3.0	—	—
Roach	1 h	—	—	—	—	<0.4	0.4
	3.5 h	0.2	0.4	0.3	0.6	—	—
	6 h	—	—	—	—	<0.4	0.4
	24 h	—	—	—	—	<0.3	1.3
	3.5 d	0.3	0.6	0.7	0.7	—	—

[a] Dissolved oxygen concentrations calculated from reported percent saturations.

significant, they were relatively small, and their significance for fish health is not clear. The hematocrit and hemoglobin levels found were within the ranges considered normal for farmed trout by Blaxhall and Daisley [9].

A wide range of physiological effects has been reported to be associated with low DO concentrations, including circulatory changes, altered heart rate, reduced blood oxygen saturation, changes in respiratory quotient, and increases in breathing rate and amplitude. Davis [2] calculated a mean incipient threshold for physiological effects in freshwater salmonids of 6 mg DO/L on the basis of a review of reported studies. It is assumed that, below this threshold DO level, fish will be expending excess energy to maintain homeostasis and that some degree of physiological stress is occurring. Alabaster and Lloyd [3] suggested, however, that respiratory and cardiovascular responses to DO changes were not necessarily indicative of impairment of ecologically important functions. This would appear to be the case in the present study for the brown trout exposed to intermittent low DO pulses where, although there appeared to be some concentration-related changes in certain physiological parameters such as hemoglobin levels, there were no significant effects on growth and overall condition.

Acclimation of trout to repeated low DO exposures was investigated by measuring both gill ventilation rates during sublethal DO pulses and mortality during acute lethal exposures following the 75-d, repeat-exposure experimental period. No significant acclimation of fish was observed in these tests, although certain physiological changes such as increased blood hemoglobin levels in experimental fish may indicate some degree of physiological adaptation. Acclimation to low DO has been the subject of many studies, although there has been much debate about the nature and importance of adaptive mechanisms. Shepard [6] demonstrated that acclimated brook trout were able to resist a lethal level of hypoxia longer than control fish. Smith and Heath [16], however, showed that prior acclimation decreased resistance to anoxia in rainbow trout. The severity of hypoxia to which fish are acclimated is likely to be an important factor here.

The findings of this study have important implications for the development of environmental quality standards for intermittent pollution. It is clear from the results of this study that the duration of low DO episodes is a critical factor affecting the survival of fish during and following pollution events. Time-varying standards are therefore required to incorporate this dimension. A preliminary approach to the development of standards that incorporate exposure duration has been proposed by Whitelaw and Solbé [17]. These standards were based on available toxicity data reported in the literature from which short-term 50% lethal concentration values over periods of up to 1,000 minutes could be derived. The drawback of this approach is that the standards were based on DO concentrations that were lethal to fish, giving rise to uncertainties over whether the margin of safety was sufficient to adequately protect fish populations. The results presented here allow the determination of minimum DO concentrations that result in no mortality for a given exposure period. This has provided an improved basis for developing environmental quality standards for intermittent pollution. Standards aimed at avoiding long-term effects have been derived, and these are used as design criteria in the upgrading of storm overflows from sewer systems.

The results of this study present important new information on the sublethal effects of exposure duration and frequency. However, there is a need for further validation of the findings, in particular, assessment of the replicability and repeatability of the results, and comparative assessment of responses of different species would be valuable.

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