Short communication

Relative sensitivities of common freshwater fish and invertebrates to acute hypoxia

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Abstract  The acute lethality of low dissolved oxygen (DO) was examined in laboratory studies using several New Zealand freshwater fish and two invertebrates at 15°C. The 48-h LC50 value was used as the endpoint for acute DO sensitivity as, owing to rapid mortality, this was found to best approximate the threshold lethal concentration. Median lethal time to death did not provide a reliable endpoint for comparing sensitivities. Fish LC50 values varied from 0.54 to 2.65 mg litre–1, with inanga whitebait (Galaxias maculatus; 2.65 ± 0.19 mg litre–1, mean ± SEM) being the most sensitive species tested. Common smelt (Retropinna retropinna; 1.83 ± 0.08 mg litre–1) and rainbow trout (Oncorhynchus mykiss; 1.61 ± 0.06 mg litre–1) were similar in their sensitivities, whereas common bully (Gobiomorphus cotidianus; 0.91 ± 0.06 mg litre–1) and shortfin eel elvers (Anguilla australis; 0.54 ± 0.03 mg litre–1) were the most tolerant fish. The shrimp (Paratya curviostris; 0.82 ± 0.09 mg litre–1) and freshwater crayfish (Koura, Paraneophrops planifrons; 0.77 ± 0.06 mg litre–1) were also tolerant to low DO. A subset of experiments to determine the relative sensitivities of larval and juvenile trout and bully indicated no significant differences between these life stages.

Keywords  dissolved oxygen; fish; freshwater; hypoxia; Anguilla australis; Gobiomorphus cotidianus; Retropinna retropinna; Paratya curviostris; Paraneophrops planifrons; Oncorhynchus mykiss; Galaxias maculatus

INTRODUCTION

Despite decades of improvement in the environmental quality of point- and non-point source pollution, low dissolved oxygen (DO) still causes significant impacts on aquatic ecosystems. This is, in part, owing to the worldwide phenomenon of eutrophication in combination with elevated temperature that is strongly influenced by non-point sources of nutrients and lack of riparian zones. The progressive deterioration in eutrophic environments is associated with an increase in hypoxic or anoxic areas of lakes, rivers, and estuaries. In New Zealand, excessive biochemical oxygen demand (BOD) levels are common in small streams, especially those surrounded by cattle pasture (Smith et al. 1993; Taylor & Smith 1997) and daily DO minima have been recorded in the vicinity of 3–4 mg litre–1 in lowland streams in the Waikato Region (Wilcock et al. 1998). At least 10% all New Zealand lakes are considered either eutrophic or hypertrophic and the eutrophic lakes typically have hypoxic or anoxic conditions in the hypolimnion (Smith et al. 1993). In particular, a number of the Rotorua Lakes in the Bay of Plenty Region are known to experience significant episodes of reduced DO over the summer months. Almost complete deoxygenation occurs during this period in Lake Okaro (Scholes 2004) and significant fish kills have recently been recorded in this lake (authors’ unpubl. data).

Chamber et al. (1997) noted that dissolved oxygen fluctuations are a common problem in many aquatic systems, especially those that are subjected to
industrial wastewater discharges. This phenomenon is evident in New Zealand, where reduced DO levels occur in the pulp and paper mill-impacted Tarawera River. It has been reported that in the downstream Tarawera River, DO dropped by a total of up to 5 mg litre\(^{-1}\) over the 20–25 km stretch of river from the pulp and paper mill discharges at Kawerau to the coast (Rutherford et al. 1991). Recent data showed DO concentrations ranging from c. 8–13 mg litre\(^{-1}\) upstream of the pulp and paper mill inputs and 4.5–8 mg litre\(^{-1}\) further downstream (Taylor & Park 2001). Depressed DO levels were consistently recorded below 5 mg litre\(^{-1}\) during the summer months of all years in the early and mid 1990s, trending upwards to 6 mg litre\(^{-1}\) in the late 1990s (Taylor & Park 2001).

Such findings are of significance to fish recruitment, as many of New Zealand’s freshwater fish are diadromous (McDowell 1990) and migrate upstream through lowland rivers and streams with reduced DO concentrations. A recent survey of fish in the Tarawera River failed to find several galaxiid species; inanga (\textit{Galaxias maculatus}), koaro (\textit{Galaxias brevipinnis}) and giant kokopu (\textit{Galaxias argenteus}) (Park 2001). An examination of caged inanga and koaro survival in the Tarawera River found a relationship between DO and koaro mortality when river DO concentrations dropped below 5 mg litre\(^{-1}\) for a considerable portion of the exposure duration (Young 2002). Young (2002) suggested that young migratory fish might be actively avoiding the river as a result of industrial discharges, reduced DO, or a combination of the two.

A previous study has examined the responses of several New Zealand freshwater fish species to low DO (Dean & Richardson 1999). Impetus for Dean & Richardson’s study was that little was known about the sensitivities of New Zealand fish to hypoxia. One of the key findings from their study was that rainbow trout (\textit{Oncorhynchus mykiss}) were the most sensitive of seven fish and one shrimp (\textit{Paratya curvirostris}) species tested. Based on their overall findings, they suggested that adoption of the United States Environmental Protection Agency guidelines (USEPA 1986) for DO in salmonid waters should confer adequate protection upon native New Zealand fish species.

The intention of this study was to complement the work of Dean & Richardson (1999) by re-examining the sensitivity of several New Zealand freshwater fish and invertebrate species to broaden our limited understanding of hypoxia sensitivity by examining a more defined range of DO concentrations using a different toxicity endpoint, the median lethal concentration (LC\(_{50}\)). The study also sought to determine the influence of body size on acute lethality from hypoxia.

**METHODS**

Wild captured animals used in this study were shortfin eel elvers (\textit{Anguilla australis} Richardson, 1848), inanga whitebait (\textit{Galaxias maculatus} Jenyns, 1842)), common smelt (\textit{Retropinna retropinna} Richardson, 1848), common bully (\textit{Gobiomorphus cotidianus} McDowall, 1975), shrimp (\textit{Paratya curvirostris} (Heller, 1862)) and koura (\textit{Paranephrops planifrons} White, 1842). Laboratory-hatched and reared rainbow trout (\textit{Oncorhynchus mykiss} (Richardson, 1836)) were also used. The different life stages for each species were selected for examination largely as a result of timing and available numbers. Inanga whitebait and shortfin eel elvers were specifically selected as they can be collected during annual migration. Smelt were abundant in a range of sizes. However, their sensitivity to handling precluded sorting into distinct size or age groups. As a result, only the larger adults were selected by eye before each assessment. The common bully is also very abundant in certain areas and a range of size classes can be collected. Age can then be approximated from weight and length measurements (Stevens 1982). Rainbow trout are easily hatched in the laboratory, guaranteeing precise age and desired numbers. The life stage of shrimp could not be reliably determined before experimentation and different classes of koura could not be obtained in the required numbers.

Eel elvers were captured in the Rangitaikai River by a commercial trapper (Bill Kerrison) at the base of the Matahina hydro dam. Inanga whitebait and smelt were collected from the Waikato River by seine net. Common bullies and koura were collected from Lake Tarawera; the bullies by seining and koura in minnow traps. Shrimp were captured by backpack electrofishing in the Kaituna River. Rainbow trout were hatched in the laboratory using fertilised eggs obtained from the Department of Conservation Turangi Trout Centre. All captured animals were kept in the laboratory for at least 5 days before experimentation, housed in well aerated tanks supplied with fresh, dechlorinated Rotorua tap water, under a 12:12 light:dark photoperiod. At the completion of lethality bioassays, animals were weighed electronically (± 0.01 g) and measured for fork length (± 0.01 cm).
Using a previously described vacuum degassing and oxygen control system (Landman & van den Heuvel 2003), fish and crustacea were exposed to a range of DO concentrations (e.g., <0.7, 1.0, 1.4, 1.8, 2.4, and >8.5 mg litre\(^{-1}\)) at 15°C for 48 h to determine median lethal concentrations for DO (48-h DO LC\(_{50}\)). The DO control system consisted of two degassing vessels, each with one set of five exposure chambers (aquaria) constructed from PVC. The basic concept of this system involved controlled aeration of degassed effluent water in individual aquaria. Aquaria were 15 litre in capacity and each set of five had an external water jacket to aid in temperature control. Each aquarium was equipped with a COS 4 DO sensor and Liquisys M COM 223 meter (Endress and Hauser, Weil am Rhein, Germany). Small 12 V DC air compressors (Thomas Air Compressors, Hannover, Germany) and model E5CK digital controllers (Omron, Osaka, Japan) were used to provide the controlled aeration as required. The voltage output from the DO metering system was then transferred to the parallel port of a desktop PC via an ADC-11 adapter (Pico Technology, St. Neots, United Kingdom). Data were recorded using PicoLog 5.06.3 for Windows (Pico Technology, St. Neots, United Kingdom).

One aquarium in each set of five aquaria was designated as a fully air-saturated control (continuous aeration) and a separate aquarium in each set designated as essentially devoid of oxygen (no aeration). The remaining three aquaria were set with varying nominal DO concentrations. Clear plastic barriers just under the water surface in each aquarium prevented fish from being able to agitate the water surface during experiments, thus minimizing the amount of oxygen re-dissolving into the water and preventing fish from gulping air or water at the surface. Holes were cut in the barriers for the DO sensors, stand pipes, and hoses. To allow for movement of water and air under the barriers, a 1.5 mm clearance was provided on all sides between the barrier and aquarium walls. Degassed water was supplied to the exposure chambers on a timed basis. Timers were built to control small irrigation solenoid valves for the delivery of water to each chamber. Water was supplied to each aquarium in 30-s bursts at 4-min intervals providing c. 3.5 litre h\(^{-1}\). This corresponded to a 95% replacement time of 10 h.

Data readings for DO were taken at 5–min intervals. The system was allowed to operate over a 48-h period without alteration. The system was capable of controlling DO within 0.05 mg litre\(^{-1}\) of the set point value.

Oxygen concentration series were modified to suit the differing sensitivities of each fish and invertebrate species. Ten animals were exposed to each oxygen concentration in each of the five 15-litre plastic aquaria. Preliminary experiments indicated that mortality occurred very rapidly, usually within 30 min, and did not generally continue after 48 h. For this reason, the 48-h LC\(_{50}\) was considered to be equivalent to the threshold lethal concentration or incipient lethal level. Mortalities were recorded at 0.75, 1.5, 3, 6, 12, 24, and 48-h exposure. Dead animals were removed from aquaria using a small dip net. Mortalities were infrequently observed after 12-h exposure, so animals were rarely disturbed beyond this point. Lethality bioassays were repeated three to six times for each species or life stage, depending on available numbers, to provide a true estimate of bioassay variability for DO LC\(_{50}\) calculations and for statistical comparisons. Summary data for animals (weight and length) and bioassay data (temperature and DO) are presented in Table 1.

The 48-h DO LC\(_{50}\) values were calculated from mortality data using the Spearman-Karber method (Hamilton et al. 1977). The mean measured DO values were used for the LC\(_{50}\) calculations. Where 100% mortality occurred, only the DO values from the start of the experiment to the time of the last recorded mortality were used to calculate the mean DO concentration. Statistical comparisons were made between species using one-way analysis of variance (ANOVA) followed by Tukey’s test. Statistical comparisons of differences among life stages for trout and bully species were conducted using one-way ANOVA. All statistical analyses were performed with STATISTICA v6.1 software. The critical level of statistical significance for all tests was \(\alpha = 0.05\). Data are presented as means ± SEM.

RESULTS

Complete survival was observed in the oxygen saturated control aquaria for all the species tested. Statistical analysis revealed significant differences in the 48-h DO LC\(_{50}\) values for the species examined (one-way ANOVA; \(F = 46.498, P < 0.001, n = 42\)). Between-species differences, as determined by a Tukey’s test revealed three homogenous ranges of lethality (\(P > 0.05\)) across the selected species (Fig. 1). Inanga whitebait was the most DO sensitive of all the species tested (Tukey’s test; \(P < 0.001\)) with a mean DO LC\(_{50}\) value of 2.65 ± 0.19 mg litre\(^{-1}\). Adult smelt and trout parr were not significantly
different from each other (Tukey’s test; $P = 0.620$) with mean DO LC$_{50}$ values of 1.83 ± 0.06 and 1.62 ± 0.06 mg litre$^{-1}$, respectively. Smelt and trout were both more tolerant than inanga whitebait (Tukey’s test; $P < 0.001$), but were more sensitive than the remaining species (Tukey’s test; $P < 0.001$). The remaining species (bully, shrimp, koura, and eel) were not different from each other (Tukey’s test; $P > 0.05$). However, these species were significantly more tolerant of low DO than inanga whitebait, smelt, and trout (Tukey’s test; $P > 0.05$). Of this group, juvenile common bully was the least tolerant with a DO LC$_{50}$ value of 0.91 ± 0.06 mg litre$^{-1}$, whereas shortfin eel elvers were the most tolerant with a value of 0.54 ± 0.03 mg litre$^{-1}$. Corresponding values for shrimp and koura were 0.82 ± 0.09 and 0.77 ± 0.06 mg litre$^{-1}$, respectively.

The time to mortality (LT$_{50}$) could not be successfully used to compare species sensitivities. In many examples, particularly with the more sensitive species at lower DO concentrations, complete mortality had occurred before the first observation at 45 min. Partial mortality usually occurred at only one or two concentrations, so comparisons between species of median time to lethality at the same DO concentration was often impossible. This was further exacerbated by the steep nature of the DO mortality curves, and the range of DO concentrations having to be altered for each species to achieve precise DO LC$_{50}$ estimates.

When comparing the early life stages of rainbow trout and common bully, it was possible to examine both LC$_{50}$ and LT$_{50}$ as endpoints (Table 2). However, the median lethal time endpoint (LT$_{50}$)
was generally far more variable than the results obtained using the median lethal concentration (LC$_{50}$). Although trout were significantly less tolerant of hypoxia than common bully, there were no significant differences in DO sensitivity between the early life stages of trout (one-way ANOVA; $F = 0.028, P = 0.870, n = 12$) or bully (one-way ANOVA; $F = 3.237, P = 0.102, n = 12$).

**DISCUSSION**

This is the first study to examine the sensitivities of New Zealand native freshwater species to low DO using the median lethal concentration as an endpoint. Rainbow trout showed similar sensitivity to smelt, whereas species such as shortfin eel, common bully, and shrimp were more tolerant by comparison. There was no pre-existing information for freshwater crayfish and they also appeared to be hypoxia tolerant. However, an unexpected result was the sensitivity of inanga whitebait to DO. A previous study (Dean & Richardson 1999) found inanga whitebait to be one of the more tolerant species they examined, with 61% mortality at 1 mg litre$^{-1}$ DO. In contrast, our study found trout to be more hypoxia tolerant, with no mortalities occurring at DO concentrations above 2.2 mg litre$^{-1}$, compared with the 13% mortality reported by Dean & Richardson (1999) after 48 h exposure to 3 mg litre$^{-1}$. Variation in the age of the animals used between studies is unlikely to account for these differences since our findings for trout and bully suggests that early life stage may have little relevance to DO sensitivity for fish.

The lack of difference in DO sensitivity between the larval and juvenile stages of trout and bully is interesting. It is generally believed that early life stages, such as developing embryos and larvae, are highly sensitive to numerous toxicants (Weis & Weis 1989). DO is also known to be a limiting factor during early development and growth (Brungs 1971; Carlson & Siefert 1974). Recent studies have shown smaller fish to be more tolerant of low DO (Burleson et al. 2001; Robb & Abrahams 2003) and when subjected to an oxygen gradient, smaller fish will also occupy areas of lower DO than larger fish (Burleson et al. 2001). It is possible that the relatively small size difference between trout and bully life stages selected in our study were insufficient to show any size-related effects on the lethality of acute hypoxia exposure.

Our study demonstrated that inanga whitebait may be more sensitive to hypoxia than previously thought, although the most likely explanation for this discrepancy is in experimental design. Inanga whitebait were denied access to the water surface to minimise the amount of re-oxygenation that occurs when fish agitate the water surface, and also to ensure that fish were exposed to the desired test conditions at all times to provide an estimate of true physiological tolerance. The role of fish surfacing behaviour is to access the more oxygen-rich surface layer to reduce the negative effects of hypoxia (Kramer 1987). Access to the water surface has been shown to reduce mortality in guppy (Poecilia reticulata; Weber & Kramer 1983) and reduce the negative effects on growth rate in mummichog (Fundulus heteroclitus; Stierhoff et al. 2003) owing to hypoxia exposure. Therefore, denying inanga access to the surface water was the most likely cause of difference between studies. Since inanga whitebait lack red blood cells (H. J. Bannon pers. comm. 2003) and appeared considerably more sensitive to hypoxia when denied access to the water surface, our findings suggest that even in mildly hypoxic environments inanga whitebait are obligated to facilitate oxygen uptake through aquatic surface respiration.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Mean (SEM, n) LC$<em>{50}$ and LT$</em>{50}$ values of common bully (Gobiomorphus cotidianus) and rainbow trout (Oncorhynchus mykiss) early life stages for dissolved oxygen (DO).</th>
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</thead>
<tbody>
<tr>
<td>Life stage</td>
<td>LT$_{50}$ (h)</td>
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<tr>
<td></td>
<td>LC$_{50}$ (mg litre$^{-1}$)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td></td>
</tr>
<tr>
<td>Parr</td>
<td>1.62 (0.06, 6)</td>
</tr>
<tr>
<td>Swim-up fry</td>
<td>1.59 (0.16, 6)</td>
</tr>
<tr>
<td>Common bully</td>
<td></td>
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<tr>
<td>Juvenile</td>
<td>0.91 (0.06, 6)</td>
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<tr>
<td>Fry</td>
<td>0.77 (0.04, 6)</td>
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</tbody>
</table>
Although many of the other species tested were reasonably hypoxia tolerant, the adoption of salmonid water guidelines (5 mg litre⁻¹; Davis 1975; USEPA 1986) may still be premature given the sensitivity of inanga whitebait. The effects of low DO may be exacerbated by the additional energetic demands of migration (Davis 1975). This potential barrier to migration should be considered for diadromous fish species, such as inanga. With the exception of the most hypoxia tolerant species examined (shortfin eel), at least partial mortality was observed in all species exposed to DO concentrations between 1 and 3 mg litre⁻¹. Chronic hypoxia exposure is known to reduce growth, survival, and cause reproductive disruption (Brungs 1971; Carlson & Siefert 1974; Brett & Blackburn 1981; Pichavant et al. 2000, 2001). Thus, chronic exposure of sensitive species to moderate DO concentrations (e.g., 3–5 mg litre⁻¹) is likely to result in significant long-term impacts. Furthermore, toxicants are also known to increase the sensitivity of fish to low DO (Downing 1953; Lloyd 1961; Hicks & DeWitt 1971; Graves et al. 1981; Thurston et al. 1981). Although significant cumulative toxicological effects of acute and chronic hypoxia combined with two separate pulp and paper mill effluents were not seen in rainbow trout and common bully (Landman et al. 2004, unpubl. data), this is not to say that other species, such as inanga whitebait, may not respond differently under the same conditions. For instance, Richardson et al. (2001) found differences in the avoidance behaviours in several of the same species tested here (bully, inanga, smelt, and shrimp) when exposed to ammonia and hypoxia, either alone or in combination.

This study sought to determine the true physiological sensitivity in the absence of the adaptive behaviour of surfacing. Though this adaptive behaviour would almost certainly reduce sensitivity to hypoxia, we would suggest that this behaviour is mostly only effective as a short-term mechanism to avoid hypoxia. For example, if fish cannot otherwise avoid hypoxia, surfacing would greatly increase the risk of predation (Wu 2002). Thus, the use of sensitivity in the absence of adaptive behaviour is the more precautionary approach. It is recommended that caution be taken when extrapolating these laboratory data to the natural environment, as fish in the wild would be faced with greater energetic demands owing to exercise and other confounding factors. Future studies examining the effects of chronic and in situ exposures are needed to add further insight into this problem.

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REFERENCES


Brett JR, Blackburn JM 1981. Oxygen requirements for growth of young coho (Oncorhynchus kisutch) and sockeye (O. nerka) salmon at 15°C. Canadian Journal of Fisheries and Aquatic Sciences 38: 399–404.


Thurston RV, Phillips GR, Russo RC 1981. Increased toxicity of ammonia to rainbow trout (Salmo gairdneri) resulting from reduced concentrations of dissolved oxygen. Canadian Journal of Fisheries and Aquatic Sciences 38: 983–988.


