DISSOLVED OXYGEN GUIDELINES FOR FRESHWATER HABITATS OF NORTHERN AUSTRALIA

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1. INTRODUCTION

1.1 Background

1.1.1 Genesis of This Project

In many freshwater habitats, low dissolved oxygen (DO) is one of the most important and ubiquitous stressors upon aquatic fauna. However, because of an historical focus upon other chemical parameters such as nutrients, salinity and turbidity, and because of the considerable spatio-temporal variability exhibited by DO, its status has rarely been appropriately assessed in the field. Moreover, due to a lack of experimental data on faunal tolerance to low DO (termed hypoxia) water quality guidelines have traditionally provided few insights into the minimum DO requirements of aquatic ecosystems. This project, funded through the Natural Heritage Trust National Competitive Component, aimed to redress this situation by experimentally testing the hypoxia tolerances of a range of freshwater fish species, developing new effect-based water quality guidelines and devising new methods of monitoring DO and assessing the ecological risks associated with DO deficiencies. The current document provides these outputs.

1.1.2 Testing Faunal Tolerance of Dissolved Oxygen

A review of the world literature on hypoxia testing of freshwater fauna, completed during the early stages of this project, showed that most research has been done more for the purpose of examining ecological interactions than for tolerance testing *per se* (e.g. studying the role of hypoxic habitats as a refuge from predation, the effects of hypoxia on resource and habitat use, interactions with other species and/or on physiological functions). Many of the published studies actually focused on hypoxia-tolerant species and aimed at elucidating the mechanisms by which this tolerance was achieved. Apart from hypoxia-sensitive salmonids, studies designed to test the hypoxia tolerance of hypoxia-sensitive aquatic fauna are rare. Analysis of the existing information on tolerance testing also shows many limitations, for example, the wide variety of laboratory test methods used, including tests done at differing temperatures and with differing acclimation regimes and assessing fish response in different ways (e.g., behavioural, physiological). In addition, there is little experimental data on Australian freshwater fish. Thus it was concluded that in order to develop appropriate experimental-based guidelines it would be necessary to test a range of local species using identical experimental methods. In this case we have chosen short-term acute tests on oxygen consumption and ventilation rate. Fishes were chosen as test subjects as they are considered more sensitive than invertebrates. There are many factors which interact to determine hypoxia responses, but size and temperature are the two most important. Accordingly a range of size classes have been tested for most species. Standard tests have been conducted at 28ºC, though for three key species (barramundi, hardyheads and rainbowfish), tests have also been replicated at 18, 33 and 38ºC. Tests at 18ºC were also performed on a number of other species known to occur in cooler habitats such as upland rainforest streams.

1.1.3 The New DO Guidelines

Brief exposure to acutely hypoxic DO concentrations can cause rapid asphyxiation (i.e. acute lethal effects) in many species. Brief exposure to less severe hypoxia causes a variety of acute sublethal effects, the most obvious and ubiquitous responses being a sudden increase in gill ventilation rate (GVR) and a decline in oxygen uptake rate. Many acute sublethal effects are species dependent but they include a variety of behavioural responses such as avoidance, which can have important ecological implications. Most of the fauna tested to date can survive (though not necessarily prosper) for surprisingly long periods when exposed to DO concentrations that are only slightly above their asphyxiation threshold. It is widely recognised that prolonged or frequent exposure to sublethal hypoxia levels of this sort can gradually undermine the health and physiological fitness of DO-dependent organisms. However, the precise combinations of exposure time and DO concentration responsible for chronic lethal and sublethal effects have not yet been determined. Moreover, because chronic testing is extremely slow and laborious, and there are so many species and scenarios that need to be tested, the existing paucity of chronic data is unlikely to be resolved any time in the foreseeable future.

This project needed to collect enough data in just a few years to be able to devise effect-based DO guidelines, and in order to accomplish this it was necessary to employ rapid acute exposure tests that are not directly indicative of chronic effects. Nevertheless, the changes in GVR, oxygen consumption rate and behaviour observed during these tests provided valuable insights into the risks of adverse chronic effects. On this basis it has been possible to propose trigger values (TV's) for protection against both acute and chronic effects. Since they have been inferred rather than measured, there are obviously uncertainties associated with the chronic TV's (CTV's). However, this is unlikely to lead to serious interpretive errors provided that the TV's are used in the intended manner. Specifically they are designed to be used as a tool for calculating a number of newly developed risk indicator parameters (which are introduced and explained in subsequent sections of this document) – they are not meant to be used to directly assess DO status or hypoxia risks. Breaches of the TV's are not necessarily indicative of a problem but rather of the need for closer investigation and risk assessment. In fact hypoxic conditions occur quite naturally in many aquatic habitats, so breaches of the TV's can be quite common even at some undisturbed sites.

The new parameters differ from conventional water quality indicators in two significant ways: 1) they provide a direct measure of deviations from ideal and therefore of the relative risk of adverse effects, and; 2) each yields a single value indicative of the cumulative risks incurred over the course of a monitoring day (whereas most water quality measurements are indicative of a single instant in time). In most other respects they are the same as any of the other indicators used to monitor the effects of natural stressors, and accordingly they are intended to be dealt with using methods similar to those recommended in the existing Australian Water Quality Guidelines (ANZECC and ARMCANZ 2000). There are not yet sufficient reference data to be able to derive TV's for the new parameters. This is partially because they are so new, but can mainly be attributed to the lack of any established site classification scheme that would allow users to accurately determine whether or not reference sites of are the appropriate type for their applications. A typology of this sort is urgently needed because DO status is so strongly affected by localised variables that sites must be an exceptionally close match in order for one to be a valid reference for the other.

Reference data are currently being collected and these may be included in future revisions of the DO guidelines, but in the interim, investigators will need to collect their own reference data. The main factors that need to be taken into consideration when assessing the suitability of reference data, and/or similarities between sites, are detailed in the appendices.

Although there remain many uncertainties about the hypoxia tolerances of aquatic fauna, these are dwarfed by the uncertainties involved in determining the DO concentrations to which aquatic fauna are exposed in real world situations. DO levels fluctuate enormously, even over relatively small spatial and temporal scales and particular fauna only occupy a small and regularly varying location within any waterbody. Fortunately, the sources of these DO fluctuations and the behaviour of key aquatic species are both sufficiently well understood for it to be feasible to predict when and where it is most appropriate to take measurements in order to obtain ecologically meaningful results. To achieve this, it is imperative that users consult the relevant text in this report before attempting to utilise the guideline values.

1.2 Importance of DO as an Ecological Condition Indicator

Most of the organisms that inhabit natural waters constantly consume dissolved oxygen (DO). In order for life to be sustained this oxygen must be replenished, and in many waters this can be accomplished by simply taking up oxygen from the overlying air – a process called re-aeration. However, re-aeration is only effective if the water is moving and mixing rapidly enough for the DO to reach deeper water before oxygen reserves become depleted. Stream flow is the main potential source of re-aeration and mixing in freshwater systems, but most north Australian rivers stop flowing during or shortly after the wet season, and the few that don't generally flow so sluggishly by the end of the dry season that re-aeration is significantly impaired. Moreover, there are many off-stream aquatic habitats that rarely if ever experience swift flows. A lot of these habitats are highly productive and very warm, so oxygen consumption rates are very high. As a consequence the re-aeration rates required in order to maintain oxygenation are often highest at the sites that are most poorly re-aerated.

Some large exposed waterbodies such as lakes can be partially re-aerated by winds, but most of the freshwater habitats in northern Australia are too small and/or sheltered to allow wind mixing to fully replenish oxygen reserves. Such waterbodies rely heavily on submerged photosynthetic organisms (plants and algae) to produce enough surplus DO during daylight hours to meet the needs of the entire ecosystem during the night. Biological oxygenation of this kind can be surprisingly effective; nevertheless it is still very common to find that DO concentrations have fallen to potentially life-threatening levels by the time the sun rises each day. Such a delicately balanced oxygenation system can collapse if there is even a slight change in just one of the numerous variables that control biological oxygen production (e.g. water clarity, depth, the amount and type of submergent plants, nutrient availability). Accordingly, in this part of the world it is quite common for severe DO deficiency (often termed hypoxia) to develop at least periodically, even in pristine waters.

Most of the common and widespread anthropogenic pressures in northern catchments, such as nutrient enrichment, turbidity change, weed invasion, river regulation, water extraction, structural alteration and loss of riparian vegetation, adversely affect DO availability. Consequently many freshwater ecosystems are now subject to substantially more frequent, severe and prolonged episodes of hypoxia than they were prior to European settlement. Moreover, aquatic organisms die within minutes to hours once DO concentrations fall below critical limits, so even very brief and/or infrequent episodes of acute hypoxia can have severe and persistent consequences for the ecosystem. In fact, for many water bodies, transient episodes of this kind can be the ultimate determinant of ecological outcomes each year.

Consequently DO availability is arguably the single most important ambient water quality issue for freshwater habitats in northern Australia – it is simply not possible to meaningfully assess the condition of a water body without gaining a good understanding of its DO status. Unfortunately DO data can be laborious to collect and difficult to interpret, and as a result this parameter has not historically received the attention it deserves.

By referring to the effect-based trigger values (TV's) and interpretation advice presented in section 3, and applying the new derivative indicator parameters in section 4, investigators should now be able to interpret the ecological meaning of DO data more confidently than most other water quality parameters. However, successful outcomes are still very much contingent on the adoption of appropriate monitoring protocols, so it is imperative that users of the guidelines consult the monitoring advice given in section 2, and if necessary the more detailed guidance in the Appendix.

2. MONITORING REQUIREMENTS

2.1 DO Variations in Natural Waters

DO concentrations can fluctuate wildly over time and space, especially in slow flowing or still waters. Concentrations in the photic zone (i.e. the part of the water column that receives sufficient sunlight for photosynthesis to occur) rise sharply during daylight hours and then gradually fall during the night. The intensity of these cyclical fluctuations, which are often referred to as diel cycling, varies through the water column and often reaches a maximum in pockets of water surrounding submerged plants.

Most tropical waters thermally stratify (meaning that the heat of the sun warms the surface water layer decreasing its density to the point where it no longer mixes with the cooler waters underneath). In some cases stratification remains stable for many months at a time while in others the surface layer cools sufficiently for some mixing to occur overnight (this is called diurnal stratification). The bottom waters in stratified systems are prone to becoming severely hypoxic because they never get to make contact with the air and generally support low levels of photosynthetic DO production, but are subject to substantial respiratory oxygen demand from microbes living in the benthos.

All of these spatio-temporal variability patterns are strongly influenced by fluctuations in the weather, especially cloud cover and wind, and by the inherent biophysical heterogeneity of the waterbodies themselves (i.e. by localised variations in factors such as depth, wind fetch, orientation, and the amount, type and location of aquatic plants).

2.2 Monitoring Objectives

The DO levels in poorly mixed waters can fluctuate so severely over the course of a normal day and/or over spatial scales of considerably less than a metre, that the task of accurately defining the levels of hypoxia exposure in a single waterbody can be a substantial and intellectually-challenging undertaking. Note that this problem has more to do with the fundamental nature of poorly-mixed, hypoxia-prone waters than DO *per se*. Several other key water quality indicators, especially temperature and pH, suffer the same problems in these kinds of waterbodies. Moreover, since these parameters directly influence the toxicity and/or availability of most water contaminants, there are sound grounds to doubt that it is possible to collect any interpretable ambient water quality monitoring data from these kinds of sites without gaining an understanding of these spatio-temporal variability patterns.

It is a major undertaking to assess the DO status of an entire waterbody and so for broad-scale work and site prioritisation purposes, we recommend that these guidelines be used mainly as an aid for assessing fish habitat values and/or the condition of benthic edge habitats. This is because unlike many benthic species, which may be constrained to occupying deeper waters, fish can easily rise in the water column to obtain the DO they require. Accordingly, unless the surface layer is very thin, the DO concentrations available to fish can often be determined by simply carrying out readings within the mixed surface layer of the water column, without the need to carry out detailed depth profiles. However, as discussed in the next section, the precise timing and location of readings is still a critical consideration. DO data collected from the mixed surface water layer can also be used to assess the hypoxia status of benthic edge habitats (i.e. the parts of a water body where the bottom meets the oxygenated surface water layer), provided that these areas are not choked by emergent or floating vegetation. However, the fact that these are likely to be the least hypoxia-stressed benthic habitats in the waterbody must be taken into consideration when interpreting findings.

2.3 Monitoring Tactics and Options

If assessing the risks of acute lethal effects (i.e. fish kills) associated with brief transient episodes of hypoxia, we suggest taking readings in the most highly oxygenated sections of the waterbody, because that is where the fish will go, if and when they need to. The episodes in question normally occur during or soon after a sudden change in the weather, and usually in conjunction with a rain event. The sites at greatest risk are highly productive and poorly flushed so diel cycling is often present even in the aftermath of a flow event, however, in situations where flow rates, water depth, turbidity and/or weather conditions are fluctuating it can be exceptionally difficult to predict the timing of DO variations. Accordingly, DO levels often need to be monitored frequently during events. This is an ideal application for a datalogger provided that the instrument can be deployed in such a manner that the sensor remains in the mixed surface layer even if water levels fluctuate.

When carrying out ambient monitoring and/or assessing the potential for chronic and/or sub-lethal effects, it is important to ensure that DO readings are taken within some of the more hypoxia-prone microhabitats that fish must regularly exploit in order to prosper. The surface layers of open stretches of water are often the most oxygenated, but fish spend much of their time hiding or foraging in and around more sheltered edge habitats and backwaters where DO levels are usually significantly lower. Fish are well attuned to diel variations in the distribution of DO and can adjust their movements accordingly. It is therefore usually valid to focus on monitoring the mixed surface layer of the water column unless that layer is too thin for fish to utilise without exposing themselves to significant risk (of predation or overheating, for example).

There are a few types of large wind-exposed waterbodies and some turbulent perennial streams that are sufficiently well-aerated to ensure that water is almost always very close to equilibrium with the air, and as a consequence these sites seldom exhibit strong diel cycling. These are the only healthy waters where it is possible to assess DO status by taking a single randomly-timed reading, but because they are so well aerated, they are also the least likely to suffer from hypoxia problems and/or to warrant DO monitoring. There are also many wetland habitats in north-eastern Australia that have been so successfully invaded by exotic weeds that the water surface is now entirely covered by mats of vegetation. These sites are so severely and persistently hypoxic that it wouldn’t usually matter what time readings were taken. However, because they are in such obviously poor condition, there would be little to gain from monitoring DO at these sites until the weed infestations are brought under control.

All other waters with the potential to suffer from hypoxia also have the potential to experience significant diel DO fluctuations, and this precludes the use of randomly timed spot measurements. Random spot DO readings are often included in routine monitoring programs on the premise that some information is better than none. This is not a defensible practice, especially when dealing with the kinds of waterbodies where DO monitoring is most needed, because the results obtained are so misleading that they are likely to misdirect management attention. Ostensibly this is a situation where no information is better than misinformation.

Water quality practitioners have historically attempted to ameliorate the problems associated with spot measurements by ensuring that the readings taken at any particular site are always collected at the same time of day. This strategy simply does not work (in the kinds of waters that are in question here at least) unless spot readings are carefully timed to target a known or predicted DO state (such as the daily minimum or maximum).

The minimum daily DO concentration (MDDO) is the key indicator to use for preliminary hypoxia risk assessments. It can be determined most accurately by taking measurements at regular intervals for a full 24 hour period or for at least several hours during the early morning when DO minima normally occur. However, DO cycling patterns are usually predictable enough to be able to obtain acceptable MDDO estimates from targeted spot measurements.

If MDDO values are to be determined by conducting spot readings, sites will need to be monitored very early in the morning, usually within a few hours of sunrise. The precise timing needed at each site is best determined by conducting more detailed monitoring on a few occasions (for example by deploying a data logger) before commencing routine monitoring. Results obtained from other similar sites in the region can also provide an adequate basis for determining the timing of diel fluctuations, but it is important to ensure that the sites really are similar and that flow conditions are identical. The main factors which need to be considered when assessing the between-site similarities are summarized in the appendices.

Correctly timed spot measurements can provide quite reliable MDDO estimates, especially if weather conditions are stable and fine (as they are during most of the north Australian dry season). Readings taken during unstable weather are less reliable but they still usually provide an adequate basis for identifying any sites that might warrant closer attention.

All detailed investigations require monitoring to be sufficiently intensive to be able to quantify the DO variations that occur over at least one full 24 hour period. This can be done either by deploying dataloggers to automatically record DO levels or by conducting prolonged manual surveys. However, in situations where this level of effort is impractical or cannot be justified, it is may be feasible to obtain reasonable estimates by carrying out targeted spot readings at the times of the day when DO concentrations are known to reach the daily minimum and the daily maximum; usually between midday (in swiftly flowing waters) and late afternoon (in stagnant waters). This might be a workable compromise for example, in community-based monitoring programs where the sampling personnel live at or near the site.

2.4 Reference Data Collection

In regional water quality monitoring programs, the sites selected have historically been those least likely to develop hypoxia (e.g., large waterholes, flowing stream reaches), but this is seldom representative of the full diversity of important aquatic habitat types present in the region. If hypoxia-prone habitats are properly represented in monitoring networks then trigger value (TV) breaches should normally become quite common. This is not necessarily indicative of anthropogenic impact because some habitats are inherently hypoxic and many waterbodies experience natural DO sags at least periodically, especially during the build-up to the wet season when first-flush stormwater runoff is present in streams and wetlands. Anthropogenic pressures can substantially increase the duration, frequency and spatial extent of these otherwise natural episodes of hypoxia, but even in cases where impacts are overt, it can still be very difficult to confidently determine how much of the observed effect is unnatural. Theoretically this is most easily done by comparing the data obtained at monitoring sites with data collected at suitable reference sites. In practice, however, it is far from simple to identify and locate suitable reference sites.

DO is very strongly influenced by a wide variety of localised factors such as hydrodynamics, depth, shape, geomorphology, and the amount and type of instream biomass. This means that two sites need to be an exceptionally close physical match before one can be validly used as a reference for the other. All of the key variables involved can change quite dramatically over time, so even if the two sites are a perfect physical match, it is imperative to ensure that the data available for each site are representative of precisely the same combinations of prevailing conditions. It would, for example, make no sense to compare two physically similar sites if the weather had always been fine and windless when data were collected at one site, but cloudy and/or windy when they were collected at the other. Results would also be particularly misleading if one site had been flowing at the time when data were collected and the other had not.

Problems of this kind can be avoided by applying data classifications before carrying out analyses – i.e. by separating data collected under one set of environmental conditions from those collected under another. This is not difficult to accomplish but it is only feasible if prevailing conditions are recorded properly every time a reading is taken. This only needs to entail the collection of qualitative descriptive data and can be accomplished by using simple nominal categories to describe any major differences in key variables. For example field personnel can convey adequate information about wind effects by simply indicating whether the water surface was glassy, smooth, rippled or turbulent, at the time when readings were taken. Routine collection and collation of biophysical background data of this kind is strongly recommended for any DO monitoring program (and in fact any ambient water quality monitoring program).

3. DO GUIDELINE VALUES

3.1 Rationale

The current ANZECC guidelines provide effect-based trigger values (TV's) for toxicants but not stressors. These are designed to protect a designated percentage (e.g. 99%, 95% and 80%) of species and were derived by applying statistical extrapolation models to data obtained from a diverse range of taxonomic groups including various vertebrate and invertebrate groups, plants, and microbes. That approach was not adopted here. Instead this study has focused on identifying and testing the most sensitive potential indicator species that are likely to inhabit various waterbody types, and individual TV's have been proposed for each of these species. This alternative approach was preferred for the following reasons:

- The weight of available evidence (see below) and professional opinion suggests that fish are the most sensitive species that inhabit hypoxia-prone freshwater habitats.
- The freshwater fish species of northern Australia are reasonably well-known and their taxonomic diversity is sufficiently low for it to be feasible to identify and test the most sensitive species – an undertaking that would not be practical for many most other taxonomic groups.
- Many natural bodies of freshwater are inherently prone to hypoxia and do not provide suitable habitat for some of the more sensitive species. The ability to select an ATV suitable for the protection of the most sensitive species that is actually present, and/or which should be present, is an imperative for astute management of such sites. This strategy might be difficult to implement if the sensitive species belonged to a variety of different taxonomic groups (as is often the case when dealing with other water quality parameters) but in this case they are all readily identifiable fish; the presence or absence of which can be established by referring to existing records or implementing rapid surveys.
- The test candidate list for this study includes several species that are socio-economically or ecologically important enough to be the focus of regional management attention. These include icons such as barramundi and mangrove jack, and ecological keystones such as bony bream and rainbowfish.

The desirability of this approach for general ecosystem assessment applications hinges on the validity of the premise that fish are the most hypoxia-sensitive species that inhabit freshwater habitats in northern Australia. Prior to the commencement of this project, the study team believed that this would be a valid assumption, based on field observations carried out during fish kills and the results of ecological surveys carried out in hypoxic habitats. Nevertheless, as a precautionary measure, some preliminary experiments were conducted to determine the relative tolerances of different taxonomic groups.

Our field observations suggested that emergent plant species typically thrive in virtually anoxic waters and there were no obvious signs that submergent plant species are adversely affected by moderate DO deficiencies, although some species, especially *Ceratophyllum* spp., suffered significant loss of biomass during some acute hypoxia episodes. However, these were generally events involving sudden reductions in water clarity, so the effects could potentially have been attributable solely to light deprivation. Subsequent laboratory tests (Pearson *et al.* 2003) demonstrated that, when kept constantly in the dark, the plants steadily lost mass until they had totally disintegrated. It took 3 weeks for the plants to disintegrate under normoxic conditions but only 2 weeks under anoxic conditions, so DO did play some role. However, the viability of the plants did not appear to be otherwise affected by anoxia, as they retained the ability to photosynthesise until they had completely decomposed.

The sensitivity of invertebrates was tested using artificial stream mesocosms populated with macroinvertebrate communities collected from riffle habitats located in undisturbed rainforest streams (Pearson *et al.* 2003). It was reasoned that these organisms should be more sensitive to hypoxia than most related species as they inhabit one of the region's most highly aerated habitats. The results suggest that the DO requirements of the most hypoxia-sensitive macroinvertebrates are roughly equivalent to that of the medium size classes of moderate-sensitivity fish species such as barramundi. The only exception to this was an unusually intolerant mayfly species which exhibited similar sensitivities to that of a small rainbowfish. It is noteworthy though that this species has not been recorded in hypoxia-prone habitats.

Based on their relative sensitivities it should therefore be conservative to apply fish-based TV's to benthic faunal communities. However, it is important to note that fish and invertebrates generally utilise very different micro-habitats with potentially distinctive DO characteristics. The levels of hypoxia to which different kinds of organism are exposed can only be meaningfully assessed if DO measurements are taken at precisely the correct time and location within the waterbody. For example measurements taken at the surface are pertinent to the assessment of free-swimming fish communities but they may be far less relevant to the health of organisms that remain on the bottom.

The guideline values presented in Table 1 of this document comprise chronic/sub-lethal trigger values (CTV's) and acute lethal trigger values (ATV's) for six target/indicator species:

- Banded grunter – along with fly-specked hardyhead, the most sensitive local species tested that inhabits hypoxia-prone habitats
- Fly-specked hardyhead – along with banded grunter, the most sensitive local species tested that inhabits hypoxia-prone habitats
- Rainbowfish – a common, sensitive and widely-distributed ecological keystone species
- Sailfin glassfish – a common, widespread and DO-sensitive species
- Barramundi – the most iconic and socio-economically important species that frequents natural waters that are too hypoxic for the above indicator species
- Gulf saratoga – an icon species that proved to be quite sensitive in laboratory tests

There is also a "default" category which comprises the highest ATV obtained from any species tested to date and a CTV that is considered to be realistic for the majority of species. (As explained later in this document, because of the way that the CTV is designed to be used, an overly conservative trigger value would actually result in overestimation of the benefits provided by high DO values, and therefore underestimation of the potential for adverse effects from hypoxia).

The data upon which the TV's are based were generated by carrying out an intensive testing program expressly designed for the purpose. The methods employed for testing and data analyses, and the rationale behind the TV's have been explained in Butler *et al.* (2007). Only a very brief summary is given here.

The project employed a variety of standardised 3 hour tests to determine the respiratory responses of 17 local fish species at size classes ranging from less than 0.01 grams to 8000 grams. Target species (plus a few others) were tested at temperatures ranging from 18^0C to 38^0C, and all others were tested at 28^0C. Test specimens were acclimated to normoxia prior to testing in order to ensure that they were in their most hypoxia-sensitive state. During the course of each standard test, DO concentrations were steadily reduced from normoxia down to the point where respiratory failure occurred and asphyxiation was imminent. Most individuals were able to be resuscitated once the test was completed. Changes in behaviour, gill ventilation rate (GVR) and/or oxygen consumption rate were measured during each test. In total, 692 oxygen consumption tests and 590 GVR tests were carried out on a total of 1156 test groups (noting that very small fish were sometimes tested in groups of up to 88 individuals at a time).

3.1.1 The Chronic Trigger Value (CTV)

Although only acute exposure responses were tested in this program, the results still provide a defensible basis for inferring the potential for chronic effects. Notably the DO concentrations in hypoxia-prone habitats fluctuate so substantially over the course of a normal day that it would be extremely rare for a fish to be exposed to any one DO concentration for more than several minutes at a time, even if the animal remained stationary. In practice fish need to constantly move in and out of hypoxic microhabitats in order to participate in normal activities such as foraging and hiding, so their exposure patterns can be even more erratic. Accordingly there are sound reasons to expect that chronic effects would normally result from a series of frequent acute exposures rather than prolonged exposure to one hypoxia level.

When DO concentrations are steadily reduced during hypoxia tests, GVR is not significantly influenced by DO concentration until levels fall below a certain point. The CTV is indicative of this point and is derived from the

5% effect concentration for GVR – i.e. on the best available statistical estimate of the DO concentration at which the GVR is elevated above the baseline by an amount equivalent to 5% of the difference between the baseline and the peak GVR.

It seems reasonable to assume that the probability of serious chronic effects developing in local ecosystems while DO concentrations are above the CTV is minor. The consequences of prolonged and/or frequent exposure to DO concentrations less than the CTV have not yet been determined. All of the local aquatic fauna that have been subjected to lethal tests to date appear to be capable of surviving quite prolonged exposure to hypoxia levels that are only barely sub-lethal, however, few of these tests have been conducted over long enough periods to determine if chronic effects develop. In fact long term chronic effects of any kind have only been cursorily examined in a few local species (eg, Flint, unpub. PhD Thesis, James Cook University). It is nevertheless clear from local field surveys and published information available for overseas species that numerous adverse chronic effects can potentially develop if DO concentrations remain well below the CTV for prolonged periods. Potential effects include reduced growth rate, loss of physiological condition, decreased ability to compete with rival species, increased susceptibility to predation, reduced fecundity, breeding failure and increased susceptibility to toxicants.

It has often been tacitly assumed in water quality investigations that the probability of chronic hypoxia developing would be inversely proportional to the average DO concentration. However, at sites that are subject to diel cycling, DO concentrations can remain substantially above the CTV (even becoming supersaturated) for a significant portion of each day. There is no reason to suspect that exposure to these high levels provides any protection against adverse effects from the hypoxia that inevitably develops overnight at these sites. (In fact exposure to high levels is more likely to reduce tolerances to hypoxia. This is discussed further in section 4). These high DO values inflate daily average DO concentration values to the point where they no longer provide realistic indications of hypoxia risk. We recommend using an alternative indicator, the Effective Daily DO concentration or EDDO, to assess the hypoxia stresses that accrue over the course of any given day (see section 4.1). This is basically a censored mean that it is calculated from the DO values in monitoring datasets after substituting all of the higher values with the CTV.

3.1.2 The Acute Trigger Value (ATV)

The ATV is derived from the 50th percentile of the DO concentration at which respiratory failure occurred during GVR tests. It is indicative of the point at which GVR peaked, indicating that test subjects were no longer capable of increasing their ventilation volumes to accommodate further decreases in DO. Because of the ways in which the ATV is intended to be used (explained in subsequent sections), it is important that the values are protective for most individuals but not overly conservative. The relationship between the peak ventilation concentration and the lethal endpoint varies between species and size classes. In some test groups such as large barramundi for example, GVR declines very gradually after the peak is reached, so they can survive at considerably lower DO concentrations than smaller barramundi. Small individuals of the same species do not have the same kind of stamina and begin to asphyxiate much sooner after the peak is reached. The ATV was derived by comparing the statistical distribution of the peak ventilation concentrations to that of the acute lethal concentrations obtained from respirometer tests. This was done separately for each test group (i.e. for each combination of species, size class and temperature). The 50th percentile of the peak ventilation concentrations proved to be the minimum concentration that exceeded the maximum lethal concentration for all test groups, and provided the best compromise for the intended application.

Note that this means that the ATV's for small sensitive fish are generally quite similar to larger more tolerant fish and do not strongly reflect the significant differences in their acute lethal responses. This actually makes the ATV's more realistic for real world applications, because in the wild very small fish are almost always able to obtain DO from microhabitats that are not normally detected in monitoring programs so the concentrations to which they are exposed are normally underestimated. Larger fish on the other hand are unable to exploit microhabitats and are therefore exposed to the lower concentrations being measured in the water column.

Fish in the wild can survive for significant periods in waters with DO concentrations that are below their physiological limits by employing a variety of survival tactics (such as ASR[a] and/or air gulping), and there are indications that some species may actively avoid hypoxic water long before DO concentrations fall to hazardous levels (Pearson *et al.* 2003). The fish in our tests were unable to do any of these things, so the lethal limits determined in the laboratory are indicative, not of the DO concentrations that would normally be expected to cause asphyxiation in most real world situations, but rather of the concentrations at which fish will be forced to employ a variety of high risk and potentially unsustainable survival strategies.

It is also important to realise that in the field DO concentrations must normally be determined by taking measurements at least several centimeters below the water surface. However, there is always a thin oxygen-enriched surface layer present whenever water is in direct contact with air. When the water column becomes hypoxic this so-called boundary layer can become very thin reaching a minimum thickness when the water column is very still and calm, and when DO consumption rates are high – i.e. under the kinds of conditions that often allow hypoxia to develop in the first place. Under such circumstances the layer can be too thin to detect with conventional instruments, yet it can still be successfully exploited by many aquatic organisms. In other words the DO readings that will normally be used to assess compliance with the TV's will almost always under-estimate the DO concentrations available to fish, and this makes the TV values quite conservative.

Large fish, especially those with large mouths, cannot perform ASR as efficiently as others. However, some still try to use it in emergency situations; adult barramundi for example lie on their side at the surface in a final attempt to gain a little extra oxygen from the surface layer. Other species which are more strongly predisposed to remain near the bottom adopt different survival strategies. Catfish for instance have often been observed lying on the edges of hypoxic water bodies, in water so shallow that their dorsal fins protrude into the air. This allows them to exploit some of the extra DO available near the surface without leaving the bottom. When conditions become very stressful they periodically rise to the surface and gulp air. Many macroinvertebrates also rise to the surface in search of oxygen when DO levels fall below critical limits. They are often observed clinging to the leaves of emergent/floating vegetation and/or drifting near the surface. Freshwater shrimps actually begin to leap out of the water when conditions become severe, and as a result many individuals die of dehydration and/or exposure rather than asphyxiation.

Ostensibly, other factors being equal, the risks incurred while carrying out ASR and other related survival tactics increase exponentially with increasing body size. When conditions become critical very large fish such as adult barramundi have very few options at their disposal – they must go to the surface where they usually end up lying on their sides, exposing a very large body surface to the heat of the sun and making them highly visible to predators such as crocodiles and sea eagles. They are too large to find shelter in open waters or to make use of the many tiny oxygenated micro-habitats that exist in most local fish habitats.

This is a key factor to consider when considering the relative sensitivities of different species and size classes – since they are less able to exploit alternative oxygen sources without exposing themselves to mortal risk, the larger individuals of some fish species need to be able to tolerate lower DO levels than smaller ones.

The ATV's are therefore notionally indicative of the DO concentrations below which fish will be forced to adopt high risk survival tactics. There are a few situations in the real world where asphyxiation could conceivably occur at these DO levels – for example in waters where animals are unable to reach the surface due to the presence of impenetrable mats of weeds (e.g., water hyacinth) – but in most cases animals will not actually asphyxiate until DO concentrations have fallen to significantly lower levels. Nevertheless mortality rates can still be elevated due to indirect causes such as overheating, sunburn, predation, reduced capacity to tolerate other stresses and/or excessive uptake of toxins (the latter being a consequence of passing larger volumes of water across the gills).

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[a] ASR – aquatic surface respiration is the process of selectively breathing in water from the surface boundary layer. This still entails normal gill breathing and should not be confused with air gulping.

The probability that fish will either suffer collateral damage of this sort or eventually asphyxiate will increase in direct proportion to the duration and severity of each acute hypoxia episode. Based on this premise we have recommended the use of a parameter called the Acute DO Deficit (ADOD) which calculates the total cumulative oxygen deficit incurred during any periods when DO levels are below the laboratory determined lethal limits. This parameter, which is explained fully in section 4, provides an objective and ecologically meaningful way of comparing the relative risks associated with different hypoxia episodes. Since diel cycling is a natural feature of most wetland types, it is likely that some healthy wetlands will report significant ADOD values. In these cases the ATV is usually only breached during the night and/or very early morning when the risks of collateral damage are at a minimum. These risks increase substantially if conditions remain severely hypoxic during the heat of the day. As an aid for identifying these higher risk situations we recommend the use of an additional indicator, the Diurnal Acute DO Deficit or DADOD. This is similar to the ADOD except that it is calculated using only data collected during daylight hours.

ADOD and DADOD values should not be used to compare between sites with strikingly different long term hypoxia exposure patterns; biota inhabiting sites that only experience brief infrequent DO sags (for example in association with storm events) do not have the opportunity to acclimate and are usually more hypoxia-sensitive than organisms that live in chronically hypoxic waters.

3.2 Using the Guidelines

Investigators should generally use the default TV’s in Table 1 unless there is convincing evidence to suggest that the water body is naturally incapable (for whatever reason) of supporting the most sensitive species. If this proves to be the case it would be sensible to use the ATV of the most sensitive of the target species that are known to naturally inhabit the waterbody. Note that because the CTV is to be used to calculate a new parameter – the effective daily DO concentrations (explained in subsequent sections) it is important that the CTV as realistic as possible, hence the default CTV has been intentionally set at a lower value than that of banded grunter and glassfish, each of which has a significantly higher CTV to ATV ratio than other species. In situations where the most sensitive species that naturally inhabits a site has a lower CTV than the default, it is appropriate to employ the lower value. However, CTV’s higher than the default value should only be used in specialised studies that are focusing on just the one particular species. This is because CTV’s are used to calculate the EDDO, and unrealistically high EDDO values effectively result in overestimation of the benefits that less sensitive fish obtain from high DO concentrations (refer to section 4.1 for a detailed explanation of the EDDO).

TV’s should normally be selected from Table 1. Indicative guideline values for a few other species are shown in Table 2, but these are mainly intended just to provide qualitative indications of the relative sensitivities of these species. The relative risk rankings shown in Table 3 have also been provided for this purpose. These rankings are based on professional judgement and are based on consideration of all of the observations available from 28°C acute exposure tests, including GVR responses, acute asphyxiation concentration, behavioural responses and the dynamics of oxygen uptake measured in respirometers.

A suggested framework for applying the guidelines is shown Figure 1.

Only minimum daily DO (MDDO) values should be used to assess compliance with the TV’s. For accurate work the MDDO should be determined by monitoring DO for at least 24 hours each time readings are taken. However, for preliminary screening purposes it may be adequate to use appropriately timed spot measurements, as discussed in section 2.

More detailed work would be required to properly assess hypoxia risks at sites that breach a TV. However, breaches could be quite common if dealing with habitats that are naturally hypoxia-prone, so it is unlikely to be advisable to scale up investigations every time breaches are encountered. This is particularly true of CTV breaches which are likely to occur on most days in many types of wetland habitats. The frequency and severity of the breaches observed at each site provide a basis for determining which sites are most at risk from hypoxia, but this is only one of the many factors that need to be taken into consideration when determining if detailed investigations should proceed. The relative ecological and socio-economic value of the sites in question, their relevance to existing or planned management programs, and the availability of monitoring resources must also be taken into account.

In order to carry out detailed investigations it is necessary to obtain DO data indicative of the diel variability at each site. It may still be possible to obtain some coarse estimates by using targeted spot measurements, although in this case it will be necessary to estimate both the MDDO and the maximum daily DO. However, this approach is far from optimal and should only be used as a last resort. In most cases it will be necessary to monitor at regular intervals for at least one full 24 hour period each time a site is assessed, and this would normally require the use of datalogging instruments.

Once these data are available, the TV’s can be used to calculate values for the new risk indicator parameters mentioned in the preceding section. Specifically, if the CTV is breached then the CTV can be used to compute EDDO values as outlined in section 4.1.2, and if the ATV is breached the ATV can be used to calculate ADOD and DADOD values as described in section 4.2.2.

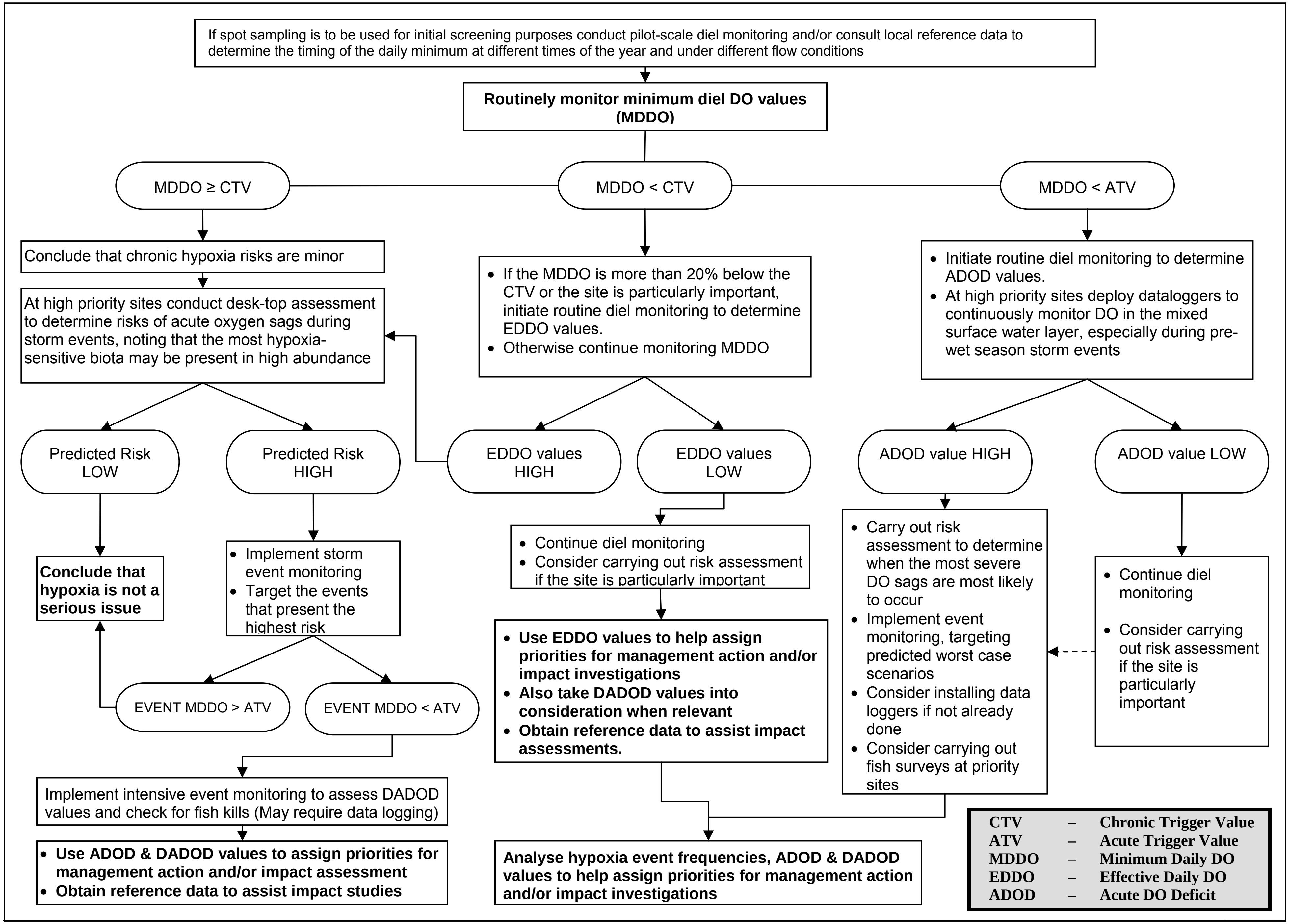
3.3 Applying Laboratory-Derived Guideline Values in the Real World

It is important that users of these guidelines do not have unrealistic expectations – the fact that fish can often be found surviving in waters where DO concentrations are well below trigger values does not necessarily indicate that the guidelines are overly conservative in the longer term; it might simply mean that the timing of events was fortuitous, and that on those particular occasions the species and size classes present at the time were able to successfully employ one of the many emergency survival tactics at their disposal without suffering excessive collateral damage in the process. It could also indicate that DO readings were taken at the wrong place and are not truly indicative of the levels to which fish have been exposed, and this could be the case even if readings were taken at points in the water column where fish were visible, as that is not necessarily where they were a short time ago – for example predators or changing environmental conditions could have recently forced the fish in question to utilise less desirable microhabitats.

Moreover, experience shows that many fish kills may go undetected and that the presumption that hypoxia-sensitive fish have survived may be incorrect. Fish that asphyxiate in fresh water almost always initially sink to the bottom (this does not usually happen in salt water). They may eventually bloat and return to the surface but this does not happen in all species and may take considerably longer than 24 to 48 hours to occur (Pearson *et al.* 2003). Hence the fact that dead fish did not appear at the surface is not a reliable indicator of survival. Nor is it possible to reach any accurate conclusions without proving that hypoxia-sensitive fish were actually present at the time of the event – there are many wetland habitats that rarely experience fish kills simply because sensitive species do not occur there, or have already been alienated or killed during previous events. It is important to recognise that severe hypoxia allows tolerant species to escape predation and competition, so they often thrive under low DO conditions. Some such species (for example tarpon) breathe air and often dart to the surface in order to do so. This overt display of fish movement is often mistaken for feeding activity and misconstrued as being indicative of healthy conditions when in it fact it simply indicates that conditions are unsuitable for DO-dependent fish.

There are undoubtedly hypoxia-prone habitats where fish can acclimate and increase their capacity to survive exposure to low DO concentrations. In situations where managers are considering taking any form of action at such sites, it might be desirable to develop site-specific trigger values. However, this should not be contemplated unless all of the above-mentioned sources of uncertainty have been addressed and/or until the default trigger values have been tested on numerous occasions, under different sets of environmental conditions at the site in question.

Figure 1: Recommended framework for applying these DO guidelines



If spot sampling is to be used for initial screening purposes conduct pilot-scale diel monitoring and/or consult local reference data to determine the timing of the daily minimum at different times of the year and under different flow conditions

Routinely monitor minimum diel DO values (MDDO)

Conclude that chronic hypoxia risks are minor

At high priority sites conduct desk-top assessment to determine risks of acute oxygen sags during storm events, noting that the most hypoxia-sensitive biota may be present in high abundance

- If the MDDO is more than 20% below the CTV or the site is particularly important, initiate routine diel monitoring to determine EDDO values.
- Otherwise continue monitoring MDDO

- Initiate routine diel monitoring to determine ADOD values.
- At high priority sites deploy dataloggers to continuously monitor DO in the mixed surface water layer, especially during pre-wet season storm events

Conclude that hypoxia is not a serious issue

- Implement storm event monitoring
- Target the events that present the highest risk

- Continue diel monitoring
- Consider carrying out risk assessment if the site is particularly important

- **Use EDDO values to help assign priorities for management action and/or impact investigations**
- **Also take DADOD values into consideration when relevant**
- **Obtain reference data to assist impact assessments.**

- Carry out risk assessment to determine when the most severe DO sags are most likely to occur
- Implement event monitoring, targeting predicted worst case scenarios
- Consider installing data loggers if not already done
- Consider carrying out fish surveys at priority sites

- Continue diel monitoring
- Consider carrying out risk assessment if the site is particularly important

Implement intensive event monitoring to assess DADOD values and check for fish kills (May require data logging)

- **Use ADOD & DADOD values to assign priorities for management action and/or impact assessment**
- **Obtain reference data to assist impact studies**

Analyse hypoxia event frequencies, ADOD & DADOD values to help assign priorities for management action and/or impact investigations

CTV	–	**Chronic Trigger Value**
ATV	–	**Acute Trigger Value**
MDDO	–	**Minimum Daily DO**
EDDO	–	**Effective Daily DO**
ADOD	–	**Acute DO Deficit**

Table 1: DO guidelines for key target/indicator species (all values in % saturation)

Indicator Species	**Water (2) Temperature °C**	**Acute Trigger Value (ATV)**	**Chronic Trigger Value (CTV)**
Default	**23 to 33**	**30**	**75**
Banded Grunter *(Amniataba percoides)*	28	30	85 (8)
Fly-Specked Hardyhead *(Craterocephalus stercusmuscarum)*	18 to 33	30	67
Eastern Rainbowfish *(Melanotaenia splendida splendida)*	18 to 33	27	69
Sailfin Glassfish *(Ambassis agrammus)*	28	24	78 (8)
Gulf Saratoga *(Scleropages jardinii)*	28	23	73
Barramundi *(Lates calcarifer)*	23 to 33 (38)[3]	16	62.5

Notes:

1. These guidelines should not be used to assess the significance of spot DO readings (see note 5) unless the measurement has been carefully timed to coincide with the daily DO concentration minimum or was taken at a site where there was no significant diel cycling. MDDO results can be directly compared to ATV's. If a trigger value is breached, then further investigation may be warranted in accordance with the flow chart in Figure 1.
2. Fish become less tolerant of hypoxia if temperatures are outside of the ranges indicated in the table. Species with a single temperature value have only been tested at that temperature.
3. Small barramundi (less than 160g) become significantly less tolerant of hypoxia if forced to endure temperatures above 33°C for more than several hours at a time. Accordingly an ATV of 25 is recommended for the protection of small barramundi living in waters that are consistently warmer than 33°C. Larger barramundi are substantially more tolerant than small individuals and should still be adequately protected by the default ATV.
4. Available evidence suggests that the most sensitive fish species are less tolerant of hypoxia than any other aquatic species, so the default guideline values should more than adequately protect biodiversity and overall ecosystem health even though they are based mainly on fish data. However, the guideline values must be applied judiciously because not all natural waters are meant to be capable of supporting all hypoxia-sensitive species.
5. Benthic fauna occupy a different part of the water column than fish, so DO readings that are taken in the correct places to assess risks to fish (usually near the surface) will almost never be an appropriate place to assess macroinvertebrates (which live mostly on the bottom). It may be valid to use surface DO measurements to assess the hypoxia status of edge habitats where the bottom lies within the oxygenated surface layer of the water column, but the fact that these are likely to be the least hypoxia-stressed benthic habitats in the waterbody must be taken into consideration when interpreting findings.
6. DO values can vary enormously throughout a water body and over the course of a normal day, so it can be very difficult to collect meaningful DO measurements. In fact the errors and uncertainties resulting from inadequate monitoring procedures far outweigh uncertainties about the DO requirements of aquatic fauna. As a rule of thumb randomly timed spot readings are meaningless and more often than not completely misleading. Users should carefully read the monitoring advice in the explanatory text before attempting to make use of the guideline values provided in the tables.
7. Investigators dealing with naturally hypoxic habitats may encounter waters that are not normally inhabited by the key indicator species. In this situation it may be advisable to use the TV's for one of the more tolerant species in Table 1. The indicative TV's for other species and relative sensitivities listed in Tables 2 and 3 have been provided to assist selections.
8. The default CTV has been intentionally set at a lower value than that of Banded Grunter and Glassfish. CTV's higher than the default value should only be used in specialised studies that are focusing on just one particular species. However, in situations where the most sensitive species that naturally inhabits a site has a lower CTV than the default, it is appropriate to employ the lower value. This is because CTV's are used to calculate the EDDO, and unrealistically high EDDO values effectively result in overestimation of the benefits that less sensitive fish obtain from high DO concentrations. (Refer to Section 4.1 for a detailed explanation of the EDDO).
9. Data derived from testing procedures described in Butler *et al.* (2007)

Table 2: Indicative TV's for species which have not yet been tested thoroughly enough to recommend a guideline value. They are low confidence estimates which should not be used in quantitative applications. All tests were conducted at 28°C. See Butler *et al.* (2007) for details of testing.

Indicator Species	**Approximate ATV % saturation**	**Approximate CTV % saturation**
Blue Eye (*Pseudomugil signifer*)	27	73
Seven Spot Archerfish (*Toxotes chatareus*)	23	70
Jungle Perch (*Kuhlia rupestris*)	23	72.5

Table 3: Relative sensitivities of different test groups based on combined consideration of GVR response, acute asphyxiation concentration, behavioural responses and the dynamics of oxygen uptake rate during acute exposure tests conducted at 28°C. Shaded rows indicate that values lack confidence due to limited replication and/or size class availability. See Butler *et al.* (2007) for details of testing.

Species	**Size (g)**	**Relative Sensitivity Ranking**
Banded grunter	0.07 - 0.4	1
Fly-specked hardyhead	0 - 0.5	1
Fly-specked hardyhead	0.5 - 1.3	1
Bony bream	0.3 - 17.0	2
Banded grunter	0.4 - 0.6	2
Jungle perch	0.16 - 0.24	3
Mangrove jack	0.3 - 18	3
Eastern rainbowfish	0.01 - 0.05	3
Bony bream	34 -89	3
Eastern rainbowfish	0.06 - 0.3	3
Mouth almighty	0.3 - 22	3
Banded grunter	2.5 - 40	4
Sailfin glassfish	0.07 - 0.5	4
Archerfish	0.25 - 25	4
Eastern rainbowfish	>2	4
Barramundi	0.12 - 0.25	4
Sailfin glassfish	0.9 - 3.9	4
Eastern rainbowfish	0.3 - 2	4
Empire gudgeon	9.0 - 11.0	5
Jungle perch	3 - 28	5
Sooty grunter	75 - 350	5
Gulf saratoga	161 - 500	5
Barramundi	0.25 - 4.0	5
Sailfin glassfish	0.5 - 0.9	6
Gulf saratoga	4.5 - 32	6
Barramundi	4.0 - 160	6
Black catfish	0.06 - 1.5	6
Empire gudgeon	0.008	7
Freshwater Prawn	0.15 - 4.5	7
Barramundi	160 - 900	7
Black catfish (juvenile)	1.5 - 25	7
Barramundi	900 -	8
Tilapia	75 - 350	8

4. NEW DO INDICATOR PARAMETERS

4.1 The Effective Diel Dissolved Oxygen Concentration (EDDO)

4.1.1 Rationale

EDDO values are intended to provide an indication of the average respiratory deficit that accumulates over the course of a day due to normal DO fluctuations. Hypoxia tolerance tests indicate that respiratory responses do not correlate with DO levels until concentrations fall below the CTV. There is no evidence that the hypoxia tolerances of fish decrease if there is surplus oxygen available at certain times of the day. High DO levels may actually cause some adverse effects, but this is yet to be fully investigated. This first version of the guidelines focuses on hypoxia because that is undeniably the most common and widespread source of adverse DO-related effects in northern aquatic ecosystems. It should nevertheless be noted that there is potential for acute physiological problems such as embolisms to develop if an animal's blood becomes supersaturated, and that regular exposure to normoxia may potentially prevent fish from fully acclimating to hypoxic conditions

Once concentrations fall below the CTV, fish must either allow their blood to become hypoxic (thereby reducing their stamina and exposing themselves to risk of adverse physiological effects such as acidosis) or expend extra energy in order to regulate their internal DO. Most of the sensitive species tested to date attempt to regulate and they do this by increasing their gill ventilation rate (GVR) – the piscatorial equivalent of panting. In the process they must expend energy that would otherwise be used to grow body tissue and/or to participate in high energy pursuits such as breeding or hunting. The energy budgets of local fish species have not yet been studied in detail, but it is well established that animals use progressively more energy to respire as their breathing becomes faster and/or harder, and that this can significantly deplete their energy reserves.

The gill ventilation responses of local fish species have now been thoroughly studied. The results show that once the CTV has been passed, fish increase their GVR at an almost linear rate in direct response to subsequent decreases in DO concentration, and it is reasonable to assume that energy losses accrue at a similar rate. These increases are maintained until DO levels closely approach the asphyxiation threshold, at which point GVR begins to fall. GVR may increase by more than ten-fold during this process, meaning that very small fish (which have very high metabolism) may need to beat their gills many times per second**.**

The EDDO is primarily designed as a tool to assess the relative potential for chronic hypoxia effects to develop over the course of a day due to diel DO cycling. Cycling may lead to the development of potentially lethal hypoxia levels. However, this occurs mainly overnight when fish are able to either safely avoid exposure by rising to the surface under cover of darkness or reduce their respiratory requirements by going to sleep – natural behaviours that undoubtedly allow fish to survive the daily oxygen sags that occur in many pristine wetlands. Hence the EDDO does not take the potential for acute effects into consideration – these are dealt with separately using the additional parameters discussed in later sections of this report.

Basically the EDDO is premised on the following assumptions:

- exposure to DO concentrations higher than the CTV value does not reduce the risk of adverse effects from subsequent exposure to hypoxia;
- the DO deficit experienced by fish depends on how far below the CTV the ambient DO concentration is, and;
- when DO levels are below the CTV, the risk of a serious energy deficit developing will be inversely proportional to the DO concentration.

4.1.2 Calculating EDDO Values

The EDDO is essentially just a weighted mean DO concentration which is calculated after first substituting all DO values greater than the CTV with the CTV value. The transformed DO data are referred to here as Effective DO (EDO) concentrations. This could be done manually by simply finding and replacing all values greater than the CTV, but it is best accomplished automatically in spreadsheets either by using a formula that contains an IF statement or by simply using the following mathematical equation:

$$\mathrm{EDO} = \mathrm{CTV} - \left[\frac{\left|(\mathrm{CTV} - \mathrm{DO})\right| + (\mathrm{CTV} - \mathrm{DO})}{2}\right]$$

Where $\left|(\mathrm{CTV} - \mathrm{DO})\right|$ denotes the absolute value of (CTV minus DO).

The EDDO is then simply calculated by taking the mean of all the instantaneous EDO values recorded that day. Provided that the intervals between readings are constant and a full 24 hour period is represented, this yields a single value indicative of the average levels of chronic hypoxia stress experienced by fish over the course of the whole day. This is a far more meaningful statistic than an average DO value, especially in cases where DO rises to levels well above the CTV for a significant part of the day (as commonly happens).

4.2 The Acute Dissolved Oxygen Deficit (ADOD)

4.2.1 Rationale

There is a risk of acute lethal effects resulting either directly or indirectly from exposure to hypoxia while ever DO concentrations within the water column are below the ATV's in Table 1, especially if this occurs for significant periods during daylight hours. The precise effects of all the different possible combinations of DO concentration and exposure time that can be encountered in the real world have not yet been determined in the laboratory, but even if such data were available it would still be very difficult to confidently predict what will happen during any particular hypoxia episode because, as mentioned earlier, outcomes are governed by numerous environmental variables, vary significantly between aquatic habitat types and depend on which species and size classes are present.

The recommended approach for measuring acute daily oxygen debt is to employ two related indicators:

- the acute DO deficit (ADOD), and;
- the diurnal acute DO deficit (DADOD).

Each of these provide a measure of acute daily DO deficit accumulation, but ADOD values encompass a full 24 hour period, while the DADOD covers only the substantially more hazardous daylight hours.

4.2.2 Calculating ADOD and DADOD Values

The ADOD is calculated by first determining the average deficit experienced during any periods of the day that DO concentrations were below the ATV (from Table 1), and then multiplying that value by the number of hours that the ATV was breached . Note that this can only be accomplished if the available data adequately cover the entire period of the day when DO was at or below the ATV.

The first step is to calculate the DO deficit (DOD) represented by each individual DO value in the 24 hour dataset, by applying the following data transformation:

$$\mathrm{ADOD} = \frac{\left|(\mathrm{ATV} - \mathrm{DO})\right| + (\mathrm{ATV} - \mathrm{DO})}{2}$$

Where: ATV is the Acute Trigger Value for the selected indicator species, and;
$\left|(\mathrm{ATV} - \mathrm{DO})\right|$ denotes the absolute value of (ATV minus DO).

Note that DO values above the ATV simply yield DOD value of zero so the transform can be applied to all DO data. However, only non-zero DOD values are used to calculate the ADOD.

The next step is to calculate the arithmetic mean of all non-zero DOD values (for that day) and then multiply the answer by the number of hours (for that day) that non-zero DOD values were recorded. This yields a single ADOD value indicative of the total cumulative exposure to acute lethal risk for the day.

The DADOD is calculated the same way except that it is only applied to data collected during daylight hours.

These two indicators should be used in conjunction with one another to help identify which sites are most at risk of fish kills and/or determine which sets of conditions present the greatest threat. In each case a higher value indicates a higher fish kill risk but an elevated DADOD is indicative of substantially higher risks than an equivalent ADOD value. This information can then be used to allocate priorities for management attention and/or more detailed investigations such as fish surveys or fish kill monitoring programs.

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APPENDIX

A.1-A.10 EXAMPLES OF SITE PROFILES

A.11 HYPOXIA RISK ASSESSMENT FACTORS

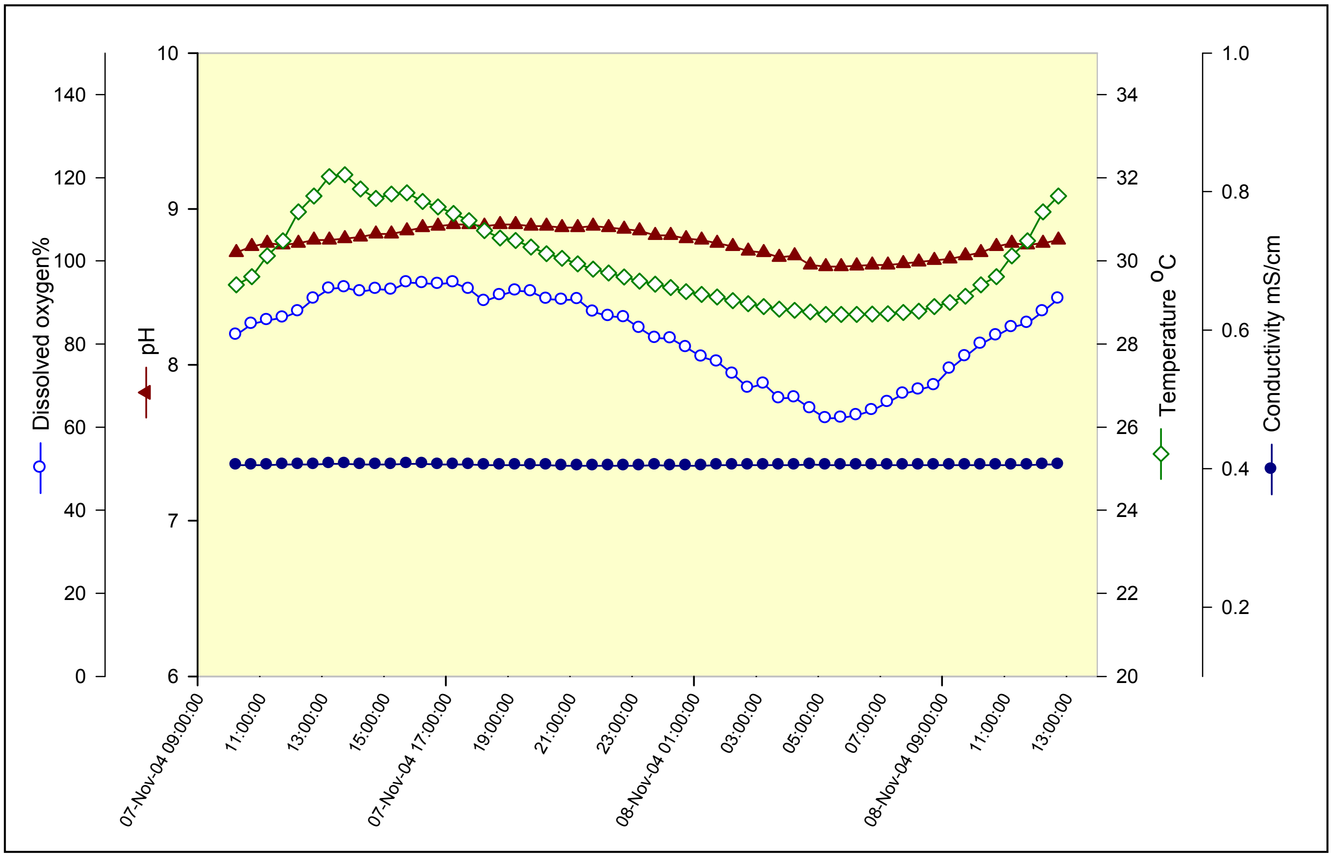
A.12 DISSOLVED OXYGEN MONITORING METHODS

A. EXAMPLES OF SITE PROFILES

All examples are from the Burdekin River catchment, NE Queensland and are taken from Loong *et al.* (2004), except Inkerman Lagoon which is taken from Faithful (2002).

A.1 Big Bend – Burdekin River (19°50’52’’S 146°07’59’’E)

Figure A.1.1 Diel cycling (over one 24 hour period) 30cm below the water surface at Zone1.



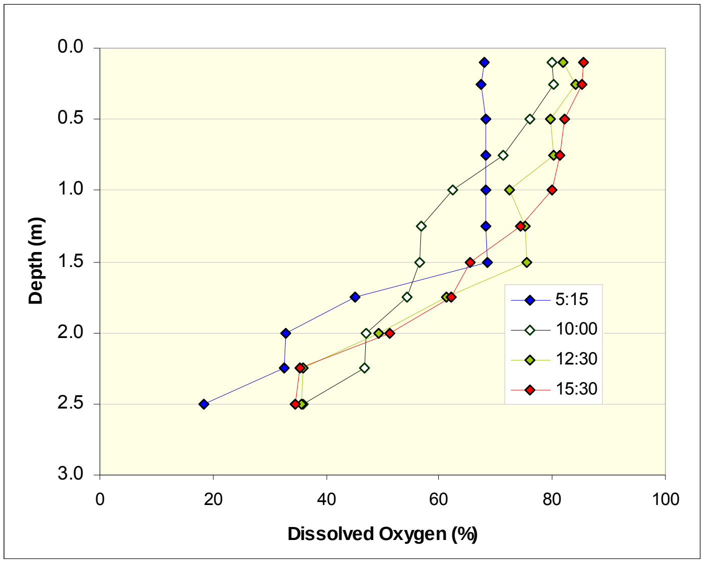
A.1.1 Diel Cycling 30 cm below the water surface at Zone 1

This subdued diel cycling pattern is typical of many large seasonally turbid waterholes located within perennially flowing reaches of large sandy rivers like the Burdekin. Due to constant flow, reasonable wind exposure and moderately high surface area to volume ratios, these waterbodies are sufficiently well re-aerated to prevent severe hypoxia from developing unless instream productivity levels become very high. In this example productivity was only moderate and was predominately heterotrophic (i.e. based on consumption of organic matter and oxygen). As will be shown in the next figure, the water column was also deep enough to substantially isolate the near surface layer from the benthos under existing mixing conditions (i.e. the observed DO cycling primarily reflects the effects of limnetic rather than benthic productivity).

The key characteristics of Figure 1 are as follows:

- By northern Australian standards this is a well-oxygenated waterbody, even though DO levels never reach saturation. (Daily maxima often range between 60% and 90 % at sites such as this, and as a general rule DO values are inversely proportional to turbidity – i.e. other factors remaining equal, DO maxima and average DO concentrations usually increase when turbidity decreases).
- The daily DO maximum is reached quite rapidly after sunrise (within six hours in this case), but the peak is flat and broad compared to other more productive sites, with DO levels remaining relatively constant through the afternoon and until well after sunset. There is actually a very subtle decline in DO levels at sunset (due to the cessation of photosynthesis within the water column), however, concentrations rise again very early in the night and remain elevated for a few hours before declining continuously through till dawn. This second peak is very commonly observed whenever DO sensors are deployed at depths of 25 to 50 cm below the water surface (as will be seen in subsequent examples). It occurs because the highly oxygenated near-surface water layer, which becomes very warm and therefore buoyant during the heat of the day, cools very rapidly after the sun sets and mixes with the water surrounding the sensor. The temperature plot provides an indication of what the DO curve would have looked like in the absence of this effect. Note that the temperature maximum occurred at around midday and coincides with the daily peak in the intensity of solar radiation. This is characteristic of reasonably well-mixed flowing water – in stagnant waters daily maxima may not occur until mid to late afternoon.
- DO concentrations reach a daily minimum around dawn. This timing is typically observed in the near-surface layer of open unshaded waterbodies that are sufficiently transparent to allow phytoplankton to exploit the low light levels available immediately after sunrise.
- Electrical conductivity (i.e. salinity) levels are remarkably constant. This indicates that flows were stable and that there were no inflows of stormwater during the monitoring event.

Figure A.1.2 Diel variations in vertical profiles at Zone 1.



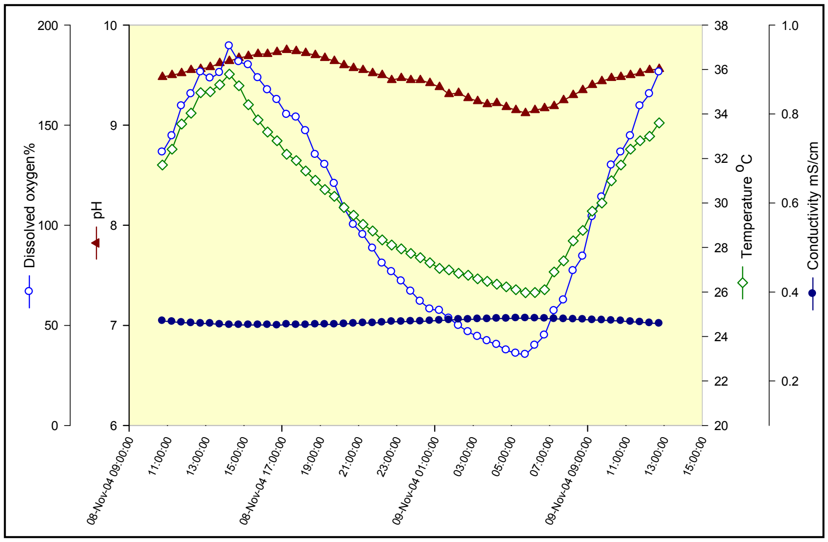
With the exception of some swiftly flowing turbulent streams in the Wet Tropics, the baseflows in northern rivers late in the dry season are rarely adequate to induce deep mixing. Generally, as in this example, there is inadequate turbulence to prevent the formation of strong vertical gradients in DO concentration and water temperature (not shown here), especially during daylight hours.

In this case, the top 1.5 m of the water column cooled sufficiently to mix by early morning (as evidenced by the absence of DO and temperature gradients at that time), but gradients were present throughout the entire water column during daylight hours. Waters deeper than 2 m did not mix at any stage, and maintained DO concentrations below 50% all day.

Apart from some large waterholes that benefit from significant wind mixing, few lowland river reaches in this region become more thoroughly mixed than this during the late dry season. Many riverine waterholes in this region become fully mixed for a short time during the coolest days of winter, but most of the time temperatures are sufficient to prevent mixing during daylight hours.

A.2 Sellheim – Burdekin River (20º00’26’’S 146º26’12’’E)

Figure A.2.1 Diel Cycling (over one 24 hour period) 30 cm below the water surface



This waterhole is located in the same arm of the Burdekin River as the previous site, however, it is different in three important respects – it is shallower, contains slightly clearer water, and supports significant assemblages of submergent plants (mainly *Myriophyllum*) and benthic algae. As can be seen in Figure A.2.1 this results in substantially enhanced diel cycling with DO values fluctuating between 30 % and 180 %. Sites like this gradually accumulate organic detritus and as a consequence respiratory oxygen consumption rates often increase towards the end of the dry season. Hence average daily DO levels decline and minimum daily values as low as 10 % to 15 % are not uncommon.

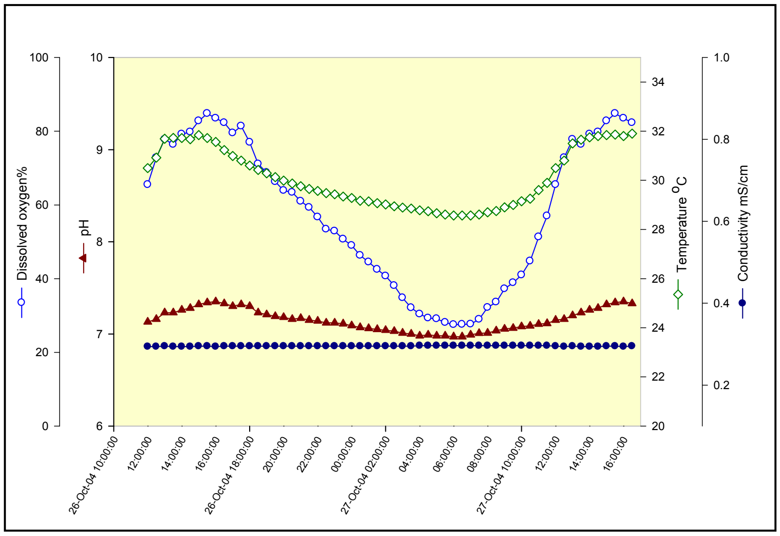
Note that the elevated pH values in this figure (9.3 to 9.7) are diagnostic of intensive submergent macrophyte productivity.

A.3 Rocky Waterhole - Majors Creek, Haughton River Catchment (19º40’09’’S 147º01’48’’E)

This site is reasonably representative of one type of instream waterhole that commonly occurs in intermittent rivers and tributary streams throughout most of the wet-dry tropics. These waterbodies are reasonably well flushed by strong storm flows each wet season, but they generally stop flowing early in the dry and remain stagnant until the next major wet season storm event. Broadly speaking waterbodies of this sort can be classified into different categories based on their water clarity characteristics. Most of these sites carry turbid stormwater during the wet season, but in the aftermath of storm events, some receive sufficient quantities of clear baseflow to substantially displace and/or dilute turbid stormwaters before they stop flowing and consequently they can contain fairly clear water for most of the year. Other sites (usually those with very low gradient catchments and/or highly infrequent episodic rainfall patterns) stop flowing before stormwaters have passed through. Accordingly they can remain highly turbid most of the time. The waterhole shown in this example is of the seasonally clear type, although it should be noted that this classification can be subject to significant interannual variability because during droughts many such sites may receive insufficient baseflow to be able to run clear.

The diel cycling pattern shown in Figure A.3.1 is quite typical of a “healthy” waterhole of this kind. In terms of water clarity, substrate type and productivity level, this site is fairly similar to the one shown in the previous example – the main difference here is that re-aeration rates are substantially lower due to the absence of flow, smaller surface area and reduced wind exposure at this site. Consequently the diel DO range is significantly greater, mean DO levels are reduced and minimum daily DO values are lower.

Figure A.3.1 Diel Cycling (over one 24 hour period) 30 cm below the water surface

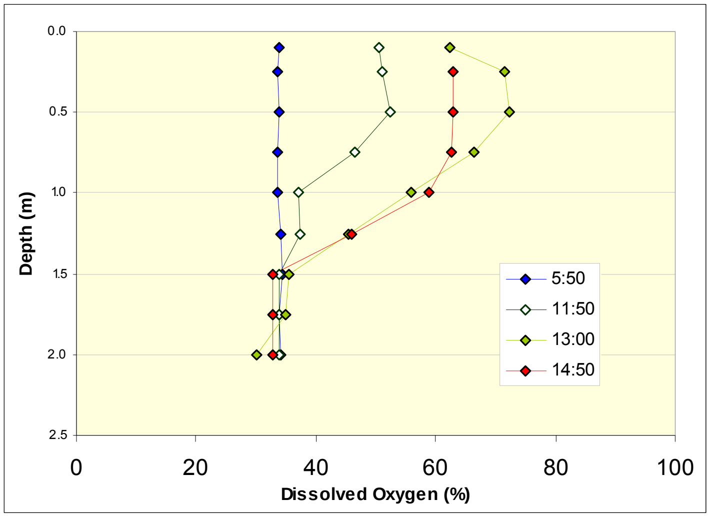


Note that due to the very low levels of surface mixing during the heat of the day the daily maxima does not occur until about 15:30 – three and a half hours after the noon peak in solar radiation (which is the time that the DO peak would normally occur in a well-mixed surface layer). As was the case in the previous example there was a secondary peak soon after sunrise indicative of rapid cooling of the overlying surface water and consequent mixing. (This secondary peak may be observed in deeper parts of the water column but it arrives later in the night and is generally less pronounced.)

For monitoring purposes it is pertinent to note that the DO minimum at this site occurred about one and a half hours after dawn. This slight delay in the commencement of DO production is characteristic of situations where riparian vegetation is sufficiently dense to attenuate sunlight during the early hours of the morning. Note however, that this normally only happens in fairly narrow stream channels that are orientated perpendicular to the angle of the sun – streams that flow east-west generally receive direct sunlight as soon as the sun rises.

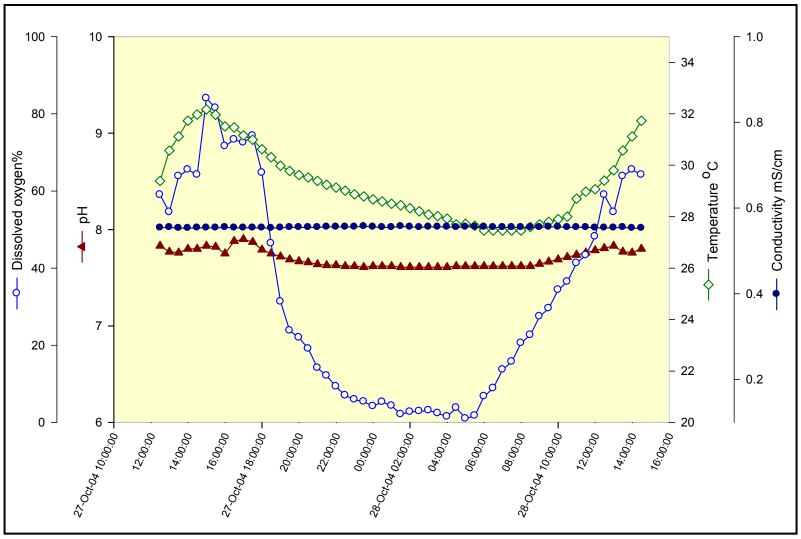
This site exhibited fairly strong diurnal stratification with a pronounced oxycline forming within the top 1.5 m of the water column during daylight hours (Figure A.3.2). This surface layer cooled over night and by early morning the water body was fully mixed. Under these conditions DO concentrations of less than 35% were registered throughout the entire water column.

Figure A.3.2 Diel variations in vertical DO profiles.



A.4 Croc Hole – Reid River, Haughton River Catchment (19º44’22’’S 146º46’06’’E)

Figure A.4.1 **Diel Cycling (over one 24 hour period) 30 cm below the water surface at Zone 1**

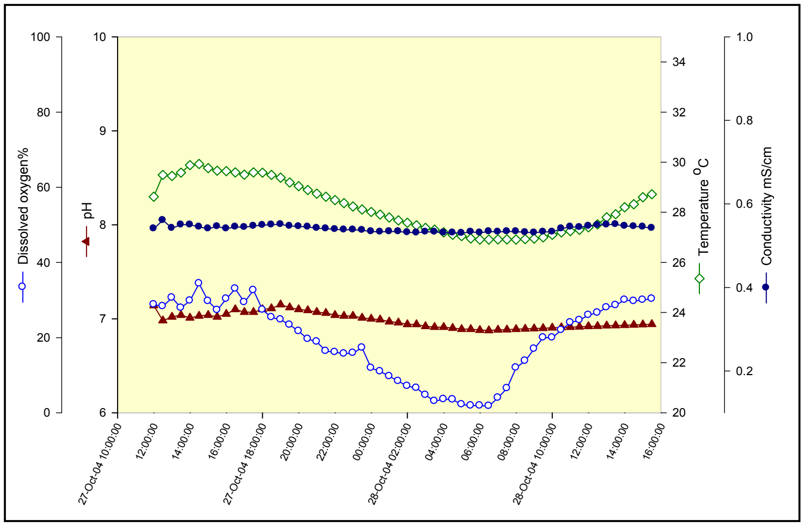


This waterhole is quite similar to the Majors Creek example except that it is a little more turbid and receives considerably higher litter fall from overhanging riparian vegetation, and as a result the sandy substratum is covered by a comparatively thick layer of organic detritus and fine sediment. This makes the site considerably more heterotrophic and leads to high oxygen consumption rates, as evidenced by the very low overnight DO levels shown in Figure A.4.1. This plot displays data recorded in an open fairly exposed section of the waterbody, designated here as zone 1. This zone was located well away from the banks and consequently, even though the site is relatively shady and the water was moderately turbid, the daily DO minimum still occurred at dawn. (For monitoring purposes note also that the daily peak occurred three and a half hours after noon, and that the secondary peak was again present.)

The data shown in Figure A.4.2 were collected from a second monitoring station located within a very shady section of the waterbody. Here photosynthetic DO production was significantly impaired due to lack of light, and as a result concentrations rarely exceeded 30 % at any stage during the day. pH values (mean of 7.0) were also significantly lower than they were in zone 1 (mean of 7.6). This is also typically indicative of suppressed photosynthesis.

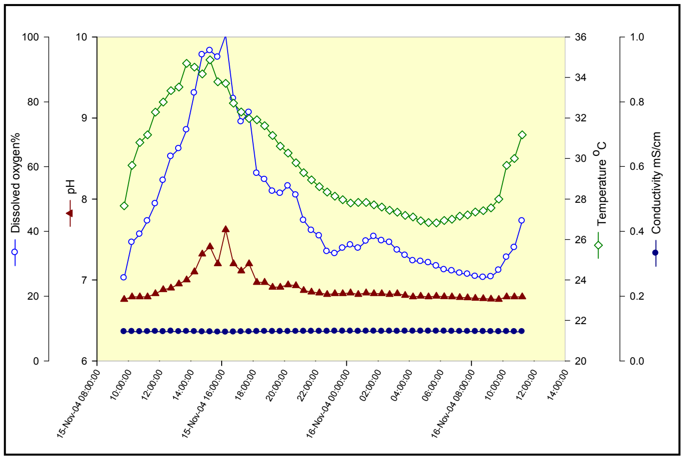
In this shadier zone the daily DO minimum occurred about an hour later than it did in zone one. The plot was also somewhat noisier than previous examples especially during the first afternoon. This is the result of passing clouds and is a common feature of light limited waters. (Most productive waters react to cloud cover but the responses are most obvious when conditions are light-limited).

Figure A.4.2 **Diel Cycling (over one 24 hour period) 30 cm below the water surface at Zone 2**



A.5 Twin Hills on Mistake Creek (Belyando-Suttor catchment) (21º57’43’’S 146º56’25’’E)

Figure A.5.1 Diel Cycling (over one 24 hour period) 30 cm below the water surface



Like many of rivers in Belyando-Suttor catchment this stream site is persistently turbid and supports significant levels of heterotrophic productivity and hence, high oxygen consumption rates. Conditions are light limited throughout most of the water column, nevertheless, there is significant photosynthetic production in the near-surface layer and this is a crucial source of oxygenation.

As can be seen in Figure A.5.1, the typical double DO peak was again observed at 30 cm depth. The “secondary” late afternoon peak reached a concentration of almost 100% suggesting that DO levels in the very near surface layer would have been supersaturated earlier in the afternoon. The partial peaks and inflections observed during the night are indicative of complex diurnal mixing patterns but these effects will not be discussed in detail in this example.

It is particularly noteworthy that the DO minimum does not occur until about three and a half hours after sunrise, even though the waterbody receives relatively little shade (compared to previous examples). This time delay is fairly typical of turbid and/or strongly coloured waters, some of which experience minima as late as 10:00 in the morning (especially if conditions are overcast or shady).

A.6 Horseshoe Lagoon – Burdekin Floodplain, within irrigation scheme (19º32’50’’S 147º07’13’’E)

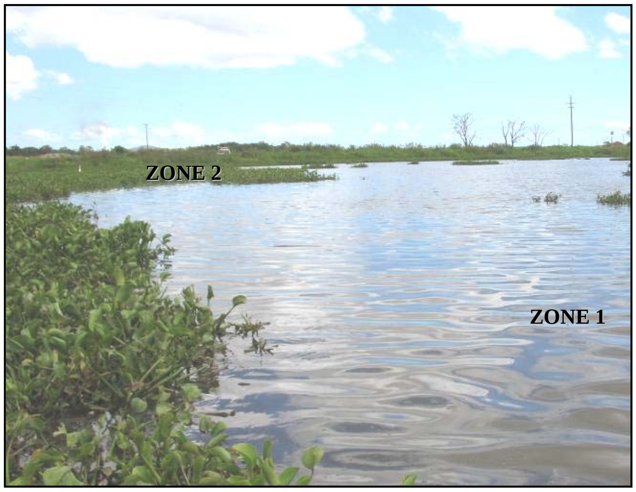
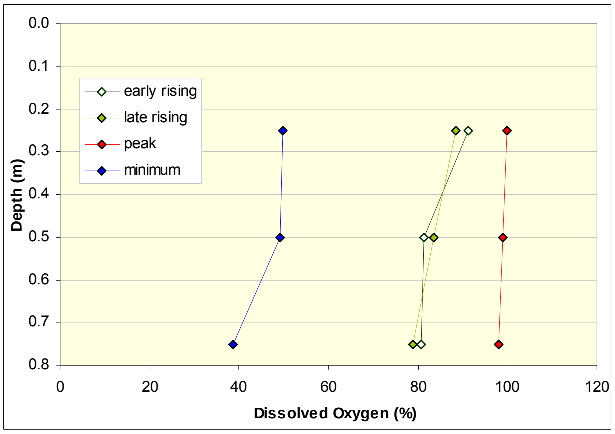


Figure A.6.1 Diel variations in vertical DO profiles in the open water zone.



Horseshoe lagoon is a naturally permanent waterbody that was once a surface expression of the local watertable. During the dry season it usually contained clear but strongly coloured, nutrient-limited water and was only moderately productive. However, the lagoon is now heavily supplemented by inflows of chronically turbid irrigation supply water from the Burdekin Falls Dam (Burrows and Butler 2007). The nutrient concentrations in the irrigation water are only moderate but constant inflows have led to a substantial increase in nutrient loading. Productivity within the water column is limited due to chronic turbidity and this has created conditions favourable to the growth of floating and emergent plant species (which obtain light from above the water surface), and especially introduced weed species such as paragrass, hymanachne and water hyacinth. Most of the wetlands on the left bank of the Burdekin River’s coastal floodplain receive some supplementation and now suffer from chronic weed problems. Water hyacinth infestations are a particular problem in poorly flushed wetlands because the plants are not washed away and can accumulate very efficiently, often covering the entire water surface. Once this happens the underlying water column becomes severely hypoxic (DO concentrations rarely if ever exceeding 8%) and sometimes completely anoxic (Perna and Burrows 2005).

The severity of this effect is unique to water hyacinth. Native species of floating plants can also cover the water surface in some waterbodies but they seldom have such a drastic effect on the oxygen status of underlying waters (discussed in a later example). This is mainly because native floating plants are smaller, meaning that they do not fully shade the water column, contribute far lower quantities of organic matter, inhibit re-aeration much less efficiently and are more easily flushed away. To date management attention has focused on physically removing established hyacinth mats and employing herbicides to prevent excessive re-invasion (see Perna 2003a, 2003b, Veitch *et al.* 2007a, 2007b). The question of how much hyacinth re-growth should be tolerated is quite complex and has not yet been fully answered, however, it is apparent that tolerances will vary substantially between sites. This example provides only a qualitative indication of the kinds of effects that a partial coverage by hyacinth mats can have on DO concentrations.

This is a relatively shallow waterbody with high surface area to volume ratio and a large wind fetch. Accordingly as can be seen in Figure A.6.1, the water is quite well-mixed and the cycling results shown below are reasonably indicative of what happens through the entire water column. These figures speak for themselves with significant suppression of DO in Zone 2.

Figure A.6.2 Diel Cycling zone 1

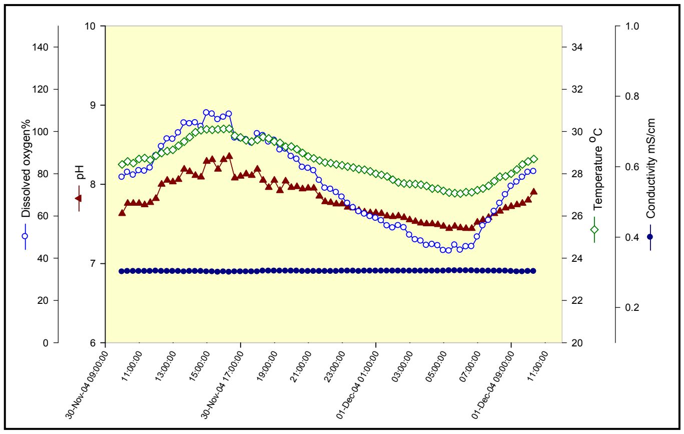
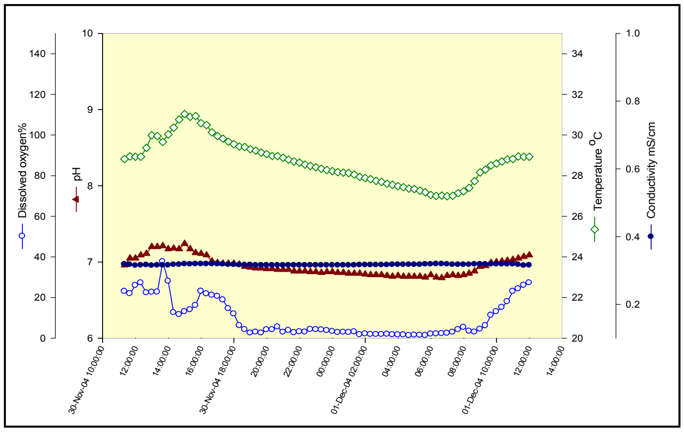


Figure A.6.3 Diel Cycling in zone 2



A.7 Kelly’s Lagoon – Burdekin Floodplain, within irrigation scheme (19º36’06’’S 147º19’56’’E)

Kellys Lagoon is on the Burdekin floodplain and surrounded by intensive agriculture (sugar cane) but unlike most lagoons there, it does not normally receive supplemental flows of turbid water from the irrigation supply. These figures compare three sections of Kelly’s lagoon: 1) a central open water zone that was too deep (>2m) to support submergent macrophyte growth under prevailing water clarity conditions; 2) a 2 m deep mostly open water zone with dense submergent macrophyte assemblages, and 3) a 2 m deep zone that was similar to 2 except that the water surface was fully covered by Azolla, a native floating plant. The results show that a dense coverage of Azolla barely affected photosynthesis rates in the underlying water column, but for this reason the combined surface and subsurface biomass levels were extremely high. Consequently oxygen demand was very high and DO levels fell very rapidly after sunrise, and remained very low for most of the night.

The extreme variations in pH within the productive zones are typical of such sites. They promote suspended sediment flocculation so water clarity is usually somewhat higher in these sections and this enhances photosynthesis rates.

Figure A.7.1 Diel Cycling at 30 cm in zone 1

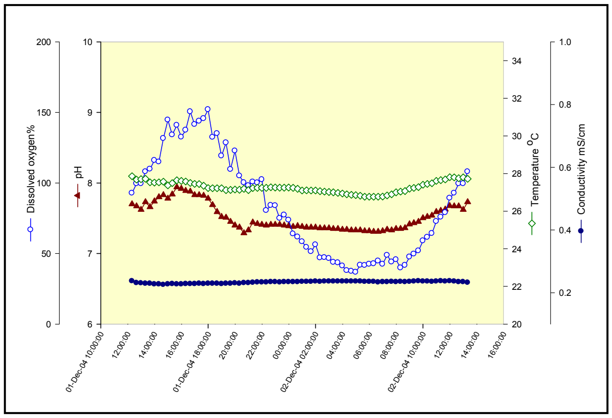


Figure A.7.2 Diel Cycling at 30 cm in zone 2

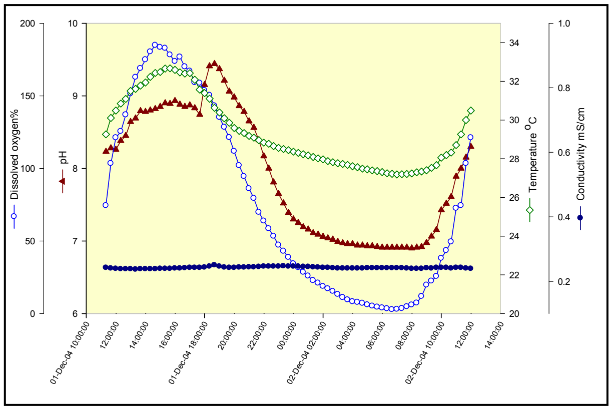
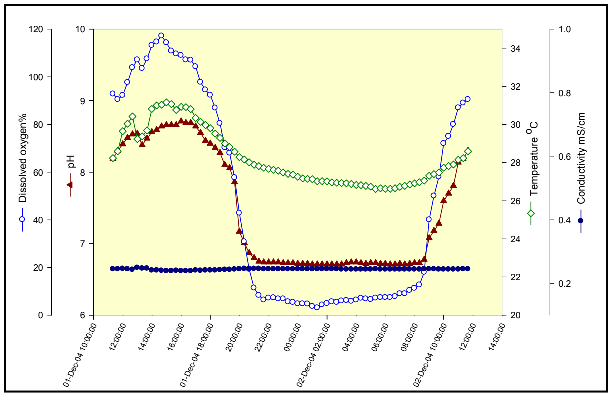


Figure A.7.3 Diel Cycling at 30 cm in zone 3



A.8 Inkerman Lagoon – Burdekin Floodplain, outside of irrigation scheme (19º45’33’’S 147º27’01’’E)

Inkerman lagoon is one of the very few relatively intact deepwater habitats remaining on the left bank of the Burdekin River floodplain and it is often considered by local scientists to be in near-natural condition even though it lies within a grazing property and can be directly accessed by livestock. The emergent plants visible in the background of this photograph are Lotus Lilies – Water Hyacinth has never successfully invaded this site to date. This is generally an aesthetically pleasing waterbody that supports moderate assemblages of native vegetation, although it can be entirely taken over by Lotus Lilies during prolonged dry spells.

Despite these positive features, the site is highly hypoxia prone and this is apparently due to its inherent limnological character. The water is naturally quite strongly coloured and this limits DO production to the very near-surface layer of the water column (Figure A.8.1). The site is also quite deep and narrow giving it a very low surface to volume ratio and limiting wind mixing. Consequently thermal stratification is very strong and stable, surface mixing being confined to the top 30 cm of the water column during the day and the top metre or so during the night. As can be seen in Figure A.8.1, the ultimate outcome is that more than 80% of the water in the lagoon is severely hypoxic all of the time. Moreover, under normal day to day conditions during warm months, the maximum DO concentration that can be found anywhere within the waterbody in the early morning is only about 12%.

Tolerant DO-dependent fish species are able to successfully exploit the limited reserves of oxygen available in the thin surface layer at sites such as this; however, the situation is so delicately balanced that their survival can be threatened by quite minor disturbances. Specifically, because the bottom water layer is so large and hypoxic, any sudden intermixing can cause a catastrophic decline in surface DO levels. Sudden destratification of this kind, often referred to as a turnover, can be brought on by numerous natural factors such as wind storms, cold snaps (which cool the surface layer causing it to sink) or inflows of cool stormwater (which flow under the warmer receiving waters forcing them to the surface).

Many narrow deeply incised waterbodies in this region, including many weirs, become strongly stratified and are vulnerable to problems of this kind. The fact that in this case conditions in the surface layer regularly become hypoxic actually reduces the risk of acute fish fatalities occurring in the event of a turnover, because resident fish have the opportunity to acclimate (or in some instances move to another waterbody). The highest risk situations in this regard are found in weir pools with well-oxygenated surface waters and anoxic bottom waters because these often support large unacclimated populations of hypoxia-sensitive species.

Figure A.8.1 Diel variations in vertical DO profiles

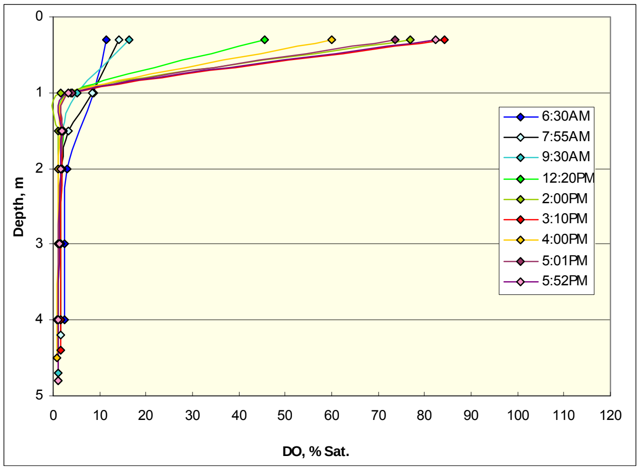
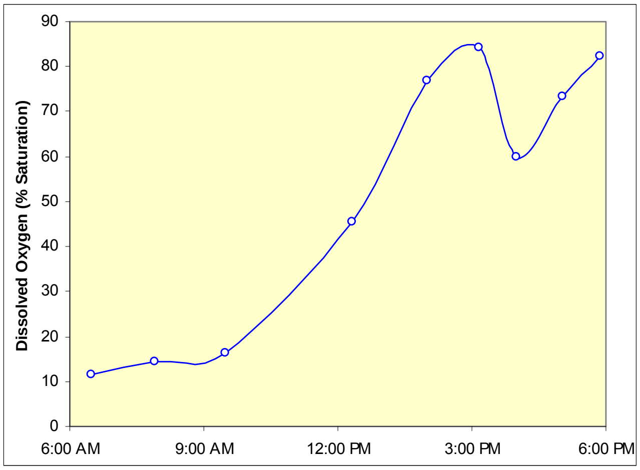


Figure A.8.2 Diel Cycling at 30 cm



It is noteworthy that although many wetlands on the adjacent floodplain now suffer from hypoxia problems due to weed invasions and other anthropogenic disturbances, most of these are shallower and/or better mixed than Inkerman Lagoon and are not nearly as naturally prone to hypoxia. Accordingly, even though Inkerman Lagoon is in fairly intact condition, it is not capable of providing the same kinds of safe aquatic habitat that were once available in other nearby wetlands.

A.9 Lake Toomba – Lolworth Creek, upper Burdekin Catchment (20º00’28’’S 145º35’24’’E)

Figure A.9.1 Diel Cycling at 30 cm depth

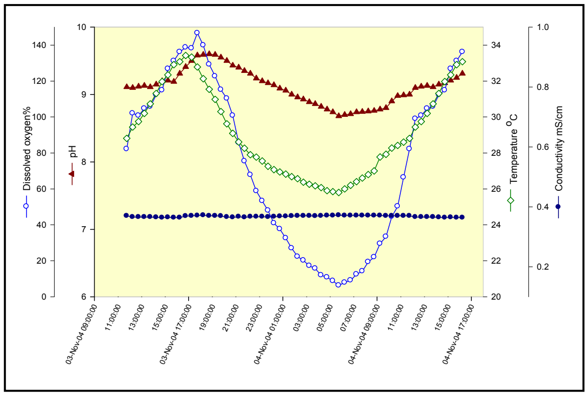
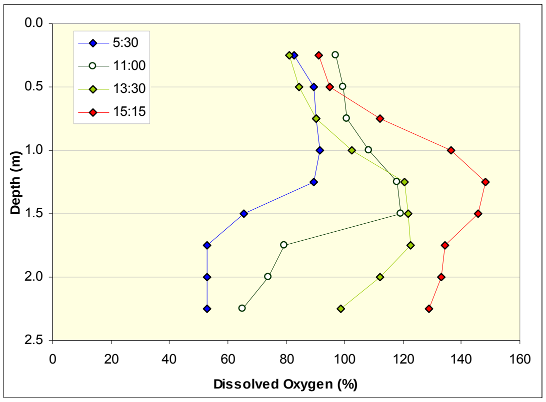


Figure A.9.2 DO profiles



Lake Toomba is a large relatively undisturbed shallow lake which typically contains clear water and supports dense assemblages of submergent vegetation. The intensity of diel cycling varies with the density of macrophyte stands, the plot in Figure A.9.1 being fairly representative of the large shallow sections that resemble the above image. Thought not so visually evident, there are also macrophyte beds throughout the deeper zones of the lake and these have a strong influence on DO distributions through the water column, with concentrations reaching a very high peak during the afternoon at a depth of 1 to 1.5 m, and the most intensive cycling occurring at a depth of 2.3m, as shown in Figure A.9.2. This effect is observed quite commonly in clear submergent plant-dominated waters.

This is one of the few situations where DO profiles do not directly reflect temperature stratification patterns, the lake being considerably more strongly stratified than the DO data would otherwise suggest, as shown in Figure A.9.3. The data shown here were collected under normal weather conditions, but since this site is so shallow and exposed, it is highly likely that the water column destratifies whenever it becomes windy. The spatio-temporal variations in pH shown in Figure A.9.4 are also notable, with values ranging from 7.4 to 7.9 m at a depth of 2.3 m, and from 9.5 to 10.2 at a depth of 1.0 m.

Figure A.9.4 pH profiles

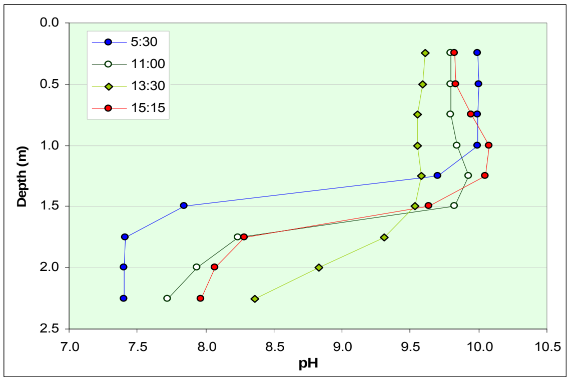
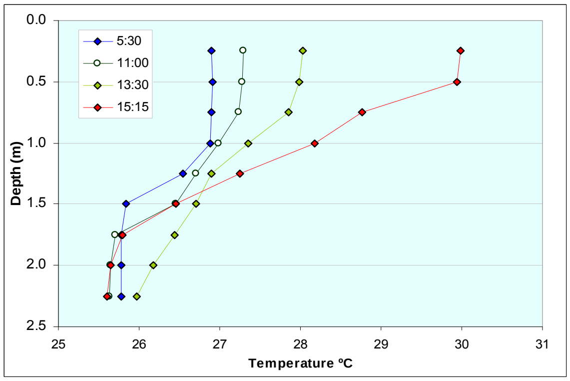
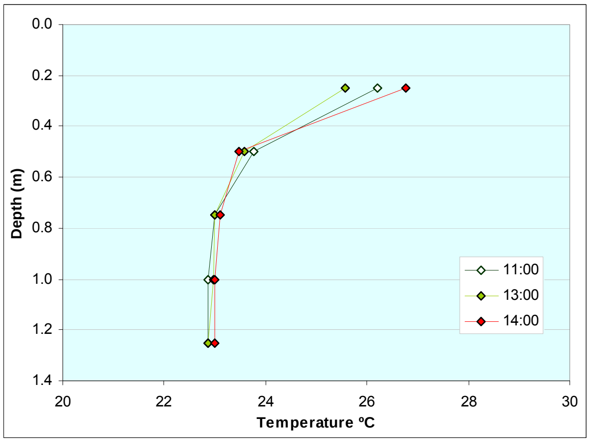


Figure A.9.3 Temperature profiles



A.10 Donald's Dream Waterhole - Pelican Creek, Bowen River Catchment (20°36'48''S 147°43'54''E)

Figure A.10.1: Thermal stratification during the hours leading up to the event



This example illustrates the remarkable differences in the ways that different parts of the same watercourse can respond to disturbances, in this case a pre-flush storm event – i.e. a small, late dry season rain event that generated sufficient runoff to raise water levels and wash contaminants into the stream without inducing an sustained flow capable of flushing the storm water downstream. It also demonstrates the value of collecting data for parameters other than DO.

Figure A.10.1 shows that this site was thermally stratified during the hours leading up to the flow event, even though conditions were overcast and light rain was falling. DO (not shown here) exhibited similar stratification and was largely confined to the top 60 cm of the water column.

The fluctuations in plots of electrical conductivity (solid dark blue) and pH (brown) in Figure A.10.2 provide a very precise indication of the timing of the flow event. The arrival of stormflow is marked by a sudden dramatic fall in conductivity and an accompanying rise in pH. Experience tells us that, had this been a larger scale or post-flush event, conductivity levels would have fallen to slightly lower levels and pH, rather than rising, would have fallen to a value of less than 6.0 indicating a predominance of fresh rainwater runoff. However, the water carried into the monitoring site during this pre-flush event originated mainly from productive waterholes situated in upstream reaches, and this resulted in elevated pH values. The end of the event is marked by the return of pre-event pH and conductivity levels.

Figure A.10.2 Water quality fluctuations in the near surface layer of zone 1 during a pre-flush storm event

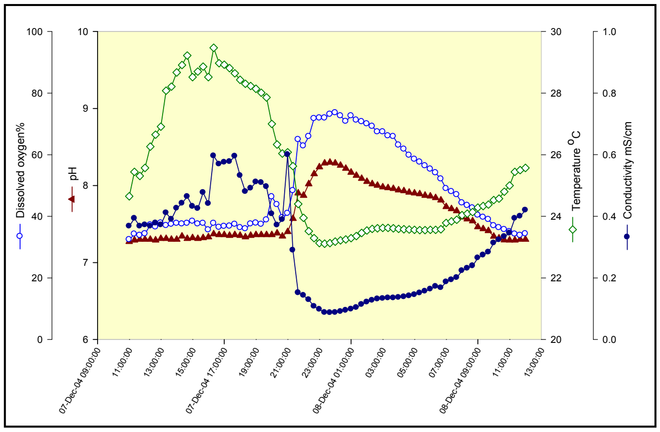
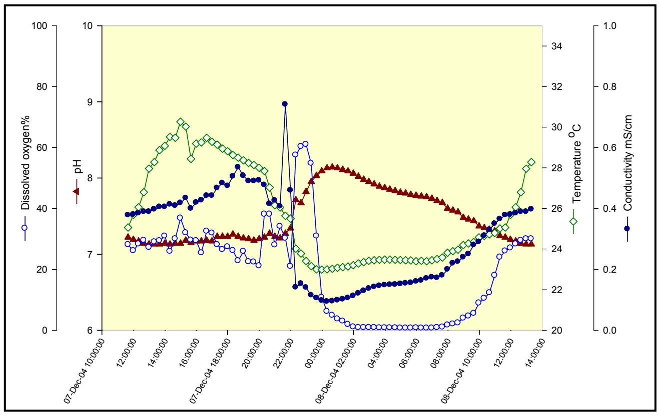


Figure A.10.3 Water quality fluctuations in the near surface layer of zone 2 during a pre-flush storm event



Zone 1 (A.10.2) was located midstream at the point of maximum flow while Zone 2 (A.10.3) was located in a shallow backwater that was judged to be representative of the kinds of habitats that fish and other mobile aquatic fauna utilise as refugia to avoid high flows during events. Both sites were quite hypoxic leading up to the event due to the combined effects of turbid water and cloudy conditions. Zone 1 clearly benefited from the increased re-aeration provided by swift stormflow, DO concentrations rising at the first sign of flow and remaining elevated until flows subsided. Zone 2 exhibited a very brief increase in DO when fresh stormwaters first entered the backwater habitat but this oxygen was rapidly consumed and in the absence of elevated re-aeration, DO levels fell to virtually zero and remained there until conditions returned to normal. The high DO consumption in this zone can mainly be attributed to microbial respiration within the sediments of the edge habitats that were temporarily inundated during peak flow.

A.11 HYPOXIA RISK ASSESSMENT FACTORS

The effect-based trigger values and new monitoring parameters presented in the DO guidelines are designed to aid the assessment of hypoxia risks at sites for which monitoring data are available. However, for this to be an effective approach, it is imperative to ensure that DO data are collected at precisely the right time and place to be able to detect existing or emerging hypoxia problems. DO concentrations can vary so much over time and space, even within a single body of water, that this can be an intellectually challenging task. The purpose of this section is to explain the key factors that drive these DO variations and ultimately determine when and where hypoxia risks are most likely to develop. Most of these factors have already been alluded to in the explanatory notes included with the examples in Appendix A.1-A.10, but here they are discussed in a broader context.

The following sections identify numerous different types of waterbodies and/or hydrogeomorphic units (i.e. physically distinctive parts of waterbodies) that possess contrasting DO characteristics and hypoxia risks. It should be noted that each of these types also represent a different kind of natural habitat potentially supporting different biological communities and, in many cases, distinctive ecological processes. This factor needs to be taken into consideration when determining overall risks in situations where the individual parts of a waterbody or drainage system exhibit very different hypoxia vulnerabilities.

A.11.1 Time of Year

As a general rule hypoxia risks increase substantially, and sometimes dramatically during prolonged dry spells. Throughout northern Australia most catchments experience a drought during the dry season and in the dry tropics interannual droughts are very common. Flow, standing water volume, water residence time, water surface area, mixing and re-aeration all generally reach a minimum towards the end of the annual dry spell, while temperatures, respiration rates, biomass concentrations, benthic accretions and stratification all reach a maximum. Under these conditions natural oxygenation systems are often so delicately balanced that even minor perturbations can cause a collapse, leading to the development of acute hypoxia. Since severe hypoxia can cause almost immediate asphyxiation in many animal species, a single brief episode of this kind can cause extensive damage to the ecosystem, and in isolated waterbodies the effects can persist until the next flow event – which during droughts, could be years away.

It is impossible to evaluate the health of aquatic ecosystems in this region without confirming that drought refugia are fulfilling their required function, and this can only be done by testing their capacity to cope with the very stressful conditions that develop during the pre-wet season period, especially during dry years. This can seldom be accomplished without monitoring during the critical hot dry times of the year, and the results of routine long-term/seasonal monitoring programs that are notionally representative of average seasonal conditions must be interpreted cautiously, because the long-term fate of many species and biological communities can be almost entirely dependent on just one or two transient events. In fact the worst hypoxia-related fish kills occur in waterbodies that maintain very good conditions for most of the dry spell, only to suffer catastrophic failures before wet season flows are re-established. (This is because sites of this kind attract sensitive species and often support large populations, but provide limited opportunity for them to acclimate to hypoxic conditions).

There are many natural and anthropogenic pressures with the potential to cause acute hypoxia episodes in drought refugia. Any inputs of contaminants that occur while waterbodies are in their vulnerable pre-wet season state can cause problems, especially in the very large number of waterbodies where flow is negligible or absent. This is the time of the year when effluents of any kind will cause acute problems, if they are ever going to. In fact any sudden input of water, even if it is of very good quality, can potentially cause major problems if the input volume is large enough to significantly increase water depth, but inadequate to provide strong persistent flows. (The reasons for this are explained in subsequent sections).

At this time of the year, and until sufficient rain arrives to restore baseflows in regional rivers, most natural waterbodies in this region receive few if any inputs of water. However, the bottom sediments within a waterbody can be a significant source of contaminant inputs to the water column, and hypoxia problems can ensue if these are disturbed and/or resuspended. Potential sources of episodic sediment perturbations include natural factors such as wind storms and congregations of waterbirds, and a wide variety of human activities such as motor boat usage and associated recreational activity, harvesting of aquatic weeds, and the construction and maintenance of water infrastructure, bridges and culverts. However, at a regional scale throughout northern Australia, the overwhelming majority of problems of this kind are caused by domestic and feral livestock wading and/or wallowing in waterholes.

Direct non-fluvial inputs of contaminants from highly localised riparian and instream sources can also be quite a significant source of episodic water quality problems in small stagnant waterbodies. Potential sources include, but are by no means limited to, dense colonies of bats, birds and other wildlife (especially those that roost in riparian trees), excessive leaf litter, flowers and/or fruit from riparian trees (especially introduced species and artificially planted stands), spillage of fertilisers and other chemicals, illegal disposal of biodegradable materials and chemicals, decaying animal carcasses (including dead fish from previous fish kills) and direct herbicide applications to large dense stands of aquatic weeds. However, the direct introduction of organic matter and nutrients in the form of excrement from feral and domestic livestock is by far the most common widespread source of hypoxia problems.

Unseasonable rain events and the isolated storms that occur at the beginning of the wet (which in some years doesn’t actually start until very late in the wet season) seldom relieve the stresses built up during the dry. In fact in many cases they exacerbate existing problems by washing contaminants from the riparian zone and nearby catchment, into adjacent waterbodies without generating enough flow to re-aerate the water or flush away contaminants (see A.10 for an example). Such events can also cause sudden increases in water depth and turbidity, and as will be discussed in later sections of this report, changes of this kind can also bring about episodes of acute hypoxia. Rain events of this kind will be referred to in this document as pre-flush storm events.

The problems discussed above are most likely to occur during the pre-wet season but they can occur at any time during the dry. In particular, inputs of oxygen demanding substances can cause hypoxia problems at any time, especially if the receiving waterbody is stagnant and/or small.

There are certain types of waterbodies that are also vulnerable to hypoxia problems at other times of the year. For example, deep thermally stratified waters, including some natural lagoons (see A.8 for example) and most weir pools, contain a thin surface layer of warm oxygenated water floating on top of a large volume of virtually anoxic bottom water (stratification is discussed in more detail in a subsequent section). Severe, though sometimes brief, oxygen sags can occur if waterbodies of this kind suddenly become mixed. This phenomenon, called a turnover, can happen if the surface layer cools and/or if there is a sudden increase in turbulence, due to strong winds or flow pulses. Except in cases where sudden flow pulses are caused by controlled artificial water discharges, most such events are subject to the vagaries of weather. Hence timing can be difficult to precisely predict and monitoring must be largely responsive rather than prescheduled. Nevertheless, high risk sets of conditions can be identified in advance so that investigators know when assessments will most likely be needed. These factors are discussed in subsequent sections, but include sudden cold snaps, periods of unusually strong wind, unseasonable rain storms and pre-flush rain events.

A.11.2 Time of Day

As can be seen in the examples in A.1-A.10, the DO concentrations in the near-surface layers of most very slowly flowing and/or lentic waters fluctuate substantially over the course of any day. This is correctly termed diel periodicity or diel cycling although it is sometimes incorrectly referred to as diurnal cycling, giving the false impression that the variations only occur during daylight hours. During fine calm weather and under stable flow conditions, the timing of the daily DO minimum and maxima can generally be predicted with reasonable confidence, although this can become a little more complicated at sites that exhibit diurnal stratification (examples of which are shown in A.1-A.10).

As a general rule, in clear exposed waters, photosynthetic DO production begins to exceed respiratory oxygen consumption almost as soon as the sun rises, so the daily minimum generally occurs at dawn. The amount of light required to achieve net DO production can be somewhat higher in waters that are turbid or coloured, and in situations where background respiration rates are very high. In such situations the daily DO minimum may not occur for some hours after sunrise, and further delays can be introduced by shading from high banks, dense riparian vegetation and/or emergent aquatic plants. Nevertheless, the DO levels in most healthy lentic waters have usually reached a minimum by 8:30 to 9:00 a.m., provided that conditions are fine.

There are however, some sites that are so turbid and/or which have such high oxygen demand that DO levels do not begin to rise until as late as 10:30 a.m. Such sites often become anoxic during the night and remain that way until late in the morning, meaning that the DO minimum can actually be detected anytime during the early morning. Note however, that once production begins at these sites, the rates of DO increase can be enormous, and in extreme cases concentrations can increase from 0 to 100% in less than 30 minutes (Burrows 2000).

A.1-A.10 includes some examples of sites that are diurnally stratified (i.e. sites where a separate layer of warm oxygenated water, which forms at the surface during daylight hours, cools sufficiently to mix during the night). In cases where the bottom waters are hypoxic, which normally means that the benthos contains a lot of organic matter, the timing of the DO minimum at the surface can depend on when the overnight mixing occurs. (Such sites normally have muddy bottoms).

In flowing waters, the timing of the DO cycling is partially dependent on instream processes that occur upstream of the monitoring site, and this can lead to unexpected results in cases where conditions vary along the course of the stream. For example the high levels of DO produced during the middle of the day at a productive upstream site may cause a DO maximum to occur during the night or even early the next morning at a less productive downstream site. This effect is commonly observed in lagoons and waterholes that have been infested by dense floating weed mats, but which are located downstream of productive open waters (and in supplemented systems this includes the weirs and impoundments from which supplies are drawn).

Diel cycling is a natural process to which native aquatic fauna are well adapted, however, the DO concentrations at many sites regularly fall to life-threatening levels for a short time each morning and there is a strict limit to how long sensitive species can tolerate such hypoxic conditions. Accordingly any factors that delay the daily resumption of instream DO production or prevent it entirely, present a significant hypoxia threat. As mentioned above, turbid and coloured waters suffer delays on a day to day basis, and as a result daily hypoxia exposure may be prolonged at such sites. Many such sites manage to maintain high photosynthesis levels within the water column during most of the dry season thanks to a regular supply of strong unabated sunlight. Waters of this kind can suffer severe bouts of hypoxia if sunlight intensity falls due to the development of overcast conditions. The daily DO minimum still usually occurs early in the morning under such circumstances, but average concentrations, and therefore daily minimum values, often decline from day to day while conditions remain cloudy. (Effects of this kind are discussed in the light climate section A.11.8). Fortunately photosynthetic DO production can be so rapid that in many cases the sun only needs to break through the clouds for a short time each day to prevent hypoxia from becoming critical.

A.11.3 Baseflow and Hydrodynamics

Re-aeration requires oxygen from the overlying air to diffuse into the thin layer of water that is in contact with the atmosphere. Diffusion rates are proportional to the concentration gradient at the air-water interface. Put simply, oxygen from the air enters a hypoxic waterbody substantially more rapidly if the DO concentration at the water surface is very low than it does if the surface concentration is only moderately low. Of course once sufficient oxygen has diffused into the surface layer to achieve saturation there is no net movement of oxygen into the water. In order for re-aeration to proceed the very thin oxygenated surface layer must be replaced with hypoxic waters from the underlying water column; a process referred to as surface renewal.

Although there are some minor exceptions (see for example a.9)) DO concentrations usually decline through the water column with increasing distance from the surface, and since oxygen uptake rates at the surface are inversely proportional to DO concentration, surface renewal is generally most effective when deep waters are carried to the surface. Mixing of this kind requires energy and turbulence, and in lotic systems these are usually provided mainly by stream flow.

Other factors being equal, waterbodies that consistently support swift turbulent flows are less prone to hypoxia than lentic waters because they are well-mixed and efficiently re-aerated, and also tend to rapidly flush away organic matter and plankton biomass. The effects in question are not just a function of hydrology and cannot be evaluated by simply referring to hydrographic data. The factors of concern are hydrodynamic characteristics such as water residence time, surface renewal rate, turbulence and current velocity, all of which vary substantially over space and time within drainage systems. Basically, flow characteristics can only be meaningfully assessed at the scale of the individual habitat or hydrogeomorphic unit and in the context of seasonal and long term variability patterns. For example many perennial river reaches comprise one single relatively homogenous lotic waterbody during the wet season, but during the dry season they retract into a heterogeneous series of individual hydrogeomorphic units such as riffles, runs and pools, each of which has its own unique flow characteristics. (Riffles for instance always flow swiftly and are well-aerated even if discharge rates are low, while large deep pools may always flow sluggishly even if discharge rates are high).

The majority of northern rivers and streams are intermittent, so by the end of the dry season they have actually become fragmented into a number of individual lentic waterholes. Spatio-temporal heterogeneity of this kind makes it difficult to determine the most appropriate operational boundaries of a site or waterbody. However, since hypoxia risks are greatest when flows reach their seasonal minimum, it is most appropriate to focus primarily on late dry season conditions when determining the locations, boundaries and characteristics of sites. If the selected sites include more than one type of hydrogeomorphic unit and/or hydraulic habitat, it is important to record the number, type and relative size of each, and determine which are most likely to posses the poorest re-aeration and mixing characteristics.

This can be done by applying the following simple qualitative classifications to assess the status of each unit under late dry season baseflow conditions:

Hydrogeomorphic Unit	**Approximate surface current velocity (dry season baseflow)**	**Relative Risk**
Falls, cascades, rapids and riffles	all	Very Low
Waters less than 2 m deep	>0.5 m/sec	Low
Lotic waters less than 1m deep Lotic waters 1 to 2 m deep Lotic waters greater than 2 m deep	visible flow <0.05 m/sec 0.05 to 0.5 m/sec >0.5 m/sec	Low to moderate
Lentic waters less than 1m deep Lotic waters 1 to 2 m deep Lotic waters greater than 2 m deep	nil visible flow <0.0.5 m/sec 0.05 to 0.5 m/sec	Moderate
Lentic waters 1 to 2 m deep Lotic waters greater than 2 m deep	nil <0.0.5 m/sec	High
Lentic waters greater than 2 m deep	nil	Very high

Human interventions such as river regulation, construction of dams and weirs, water extraction, and supplementation, which can significantly alter the hydraulics of natural waterbodies, can also substantially alter hypoxia risks. Although there are those who would argue that any deviation from natural is undesirable, not all of these alterations are unequivocally negative. Flow supplementation can for example enhance mixing and re-aeration and flush away poor quality water, thereby alleviating many of the hypoxia problems that would otherwise develop in stagnant waterholes. However, this may be at the cost of increased risks to other parts of the system that don't receive flow supplementation. For example, deep impoundments, and especially weirs, are often much more hypoxia-prone than the natural stream channels they inundate, and the river reaches downstream of them often experience significant reductions in the intensity, duration and/or frequency of flows, leading to higher risk conditions.

The relative risk categories suggested above are premised on the assumption that other factors are equal and this includes the proviso that the quality of the water is not significantly altered by inflows. This condition is normally only met under natural steady baseflow conditions (or in altered systems where levels of supplementation are high enough to completely mask the natural baseflow water quality signature). This therefore excludes storm flows (which inevitably contain elevated concentrations of sediment, organic matter and nutrients) and most flows that are derived from anthropogenic point sources. Even in systems with unaltered hydrology there can be situations where the benefits of increased flow are undermined because the flow carries poor quality water into a site. This is not a serious issue in most catchments and in the few cases where it is, the source of the problem is usually fairly obvious. For example there are a number of low-lying watercourses on the coastal floodplains of north Queensland that have become severely hypoxic, and in some cases virtually anoxic, due to exotic weed infestations. Flows emanating from these sites can substantially degrade the DO status of downstream receiving waters. This is a significant management issue in these catchments because it means that in order to protect a priority site located in the lower reaches of a drainage system, it may be necessary to remove most of the weed infestations from the upstream reaches.

All of the preceding comments relate to relatively stable flow conditions. The oxygenation of natural waters depends on the equilibrium between re-aeration, mixing, inflows, outflows, respiratory consumption and photosynthetic production. This can be such a delicate balance that is easily upset by any sudden changes in conditions. Accordingly, regardless of the quality of the water, sudden flow pulses have the potential alter the system so rapidly that it does not have time to adjust, and in some types of waterbody this can result in severe DO sags. These kinds of effects are discussed in subsequent sections.

A.11.4 Wind

In lentic waters, internal processes such as convection may at times help promote mixing and surface renewal, but in the absence of stream flow, wind is the major external source of mixing energy. As a general rule turbulent wind-mixed waters are better aerated and therefore less prone to developing severe hypoxia problems than very calm ones. Even a light breeze can significantly enhance DO concentrations within the critical near-surface layer of the water column of some waterbodies.

Wind exposure must be assessed in the context of the waterbody’s shape and fetch. For example large open waters with significant wind fetch can be mixed by the wind regardless of its direction, but long narrow lagoons may only be significantly affected if the wind comes from a particular direction. If waterbodies of this kind stratify they may be prone to turnovers (i.e. severely hypoxic bottom waters may be suddenly brought to the surface) whenever the wind blows strongly from a certain direction.

In order to interpret DO monitoring data properly it is necessary to know what the wind conditions were like at the time when measurements were taken. However, due to the substantial fluctuations in wind speed and direction that can occur over the course of a day, it can be surprisingly difficult to collect information that adequately summarise the conditions that are encountered during routine monitoring trips. In our own monitoring work we have found that the most reliable results are obtained by asking field personnel to qualitatively assess and/or photograph the condition of the water surface, rather than attempting to measure wind speeds and directions. This can be accomplished by applying simple qualitative descriptors such as glassy, smooth or rippled, to describe both instantaneous and daily average conditions.

Sites that are frequently subject to wind mixing generally benefit from the increased aeration; however, there are some exceptions to this rule. Shallow waterbodies with muddy organic rich bottom sediments for example may become turbid and heterotrophic (meaning that oxygen consumption exceeds oxygen production) due to wind mixing, and there is potential for respiratory consumption rates to increase to the point where oxygen sags can occur whenever conditions become calm again. Moreover, as is the case with flow, wind anomalies such as those associated with storms can turnover strongly stratified waterbodies (discussed later) potentially causing severe, though usually transient, oxygen sags.

Human interventions that alter the depth and morphology of waterbodies can also substantially change the ways in which they respond to wind mixing. The re-aeration characteristics of hypoxia-prone stagnant stream sites, especially narrow ones, can also be substantially influenced by riparian vegetation, which can act as a wind break. If revegetating high risk sites of this kind, it is important that appropriate species and planting densities are employed to ensure that the waterbody is not denied access to the wind – we have encountered cases where pre-existing hypoxia problems in lagoons have actually been exacerbated by overly enthusiastic riparian revegetation works (Butler and Crossland 2003).

A.11.5 Waterbody Size and Shape

Since re-aeration is contingent on contact between the water and the air, the surface area to volume ratio of a waterbody is an important determinant of the amount of surface renewal and mixing that is required in order to maintain normoxic conditions within the water column. As a rule of thumb, other factors being equal, hypoxia risks are inversely proportional to the surface area to volume ratio; large shallow lakes and waterholes generally being substantially less prone to severe hypoxia than narrow deeply incised lagoons, unless the high surface area waterbodies are very shallow and in intimate contact with highly bioactive bottom muds. Low surface area waterbodies generally have very strong thermal stratification tendencies, and as shown in A.8, surface mixing in local examples of such sites can be extremely constrained, sometimes extending to depths considerably less than a metre below the water surface.

High surface area waterbodies also have greater wind fetch and can exploit the energy of the wind to promote mixing and re-aeration. The wind fetch of low surface area, channel-form waterbodies depends on their shape and their orientation to prevailing winds. Sinusoidal channels may contain some reaches that frequently capture prevailing winds and others that do not, so mixing and re-aeration characteristics may vary substantially along the length of the waterbody. For detailed local-scale investigations, especially at priority management sites, it is important to determine if waterbodies of this kind contain poorly mixed sections that are more vulnerable to hypoxia problems than others.

Size and bathymetry are also key determinants of the risks associated with localised instream disturbances and/or contaminant inputs. By the end of the dry season many northern river systems have retracted into a few small permanent waterholes and lagoons. These low volume waters have very limited capacity to dilute any contaminants that are introduced into the water column and this makes them particularly susceptible to the development of hypoxia and other water quality problems, especially during the hot months towards the end of the dry season. The vast majority of these high risk sites occur in free-range grazing areas, so at regional scales, domestic and feral livestock are the most common source of such problems.

In irrigated agricultural areas the introduction of tailwater to sluggishly flowing or stagnant waterbodies can potentially cause similar problems. However, these inputs can also concomitantly affect flow rates and therefore have the potential to enhance re-aeration. This can create a somewhat paradoxical situation where low inputs of tailwater can cause more severe problems than large inputs. Complete prevention of tailwater inputs might improve the situation in some cases, however, streams that have been supplemented by irrigation runoff for a number of years can accumulate such considerable organic loads and/or aquatic weed infestations that they rely heavily on the mixing and re-aeration provided by artificial flows to maintain adequate oxygenation.

Many such systems would be likely to suffer severe hypoxia problems if they were allowed to stagnate, and since weeds have usually already gained a foothold and agricultural runoff will still reach them during storm events, restoring their natural hydrology is very unlikely to return them to any semblance of their natural state. In such cases the best possible management outcomes would be achieved by providing an environmental flow allocation so that good quality irrigation water can be used to replace the poorer quality tail flows (see Butler 2005, Burrows and Butler 2007).

A.11.6 Substratum Type

Healthy natural waterbodies constantly consume oxygen, hence the need for constant re-aeration and/or re-oxygenation. Most of the biota within a waterbody utilise DO to respire, but in local waters the majority of the oxygen consumption can be attributed to either photosynthetic organisms or heterotrophic microbial biomass (i.e. microbes that grow by consuming organic matter rather than producing their own). Except for brief periods when sunlight availability may become limited (discussed later), autotrophs (i.e. photosynthetic organisms) generally produce sufficient surplus oxygen during the day to more than meet their own respiratory needs during the night. Accordingly, although it is autotrophs that drive diel periodicity and determine the amplitude of daily DO fluctuations, it is the constant respiratory consumption of microbes and other heterotrophs that determines the average and daily minimum DO concentrations – i.e. it is microbes that normally determine how hypoxic the water becomes.

Most microbes attach themselves to solid surfaces such as sediment particles, and because very small particles have a very high surface area to volume ratio, sediments containing very fine-grained sediments (i.e. muds) support much denser microbial populations than sediments dominated by coarse grained particles such as sand and gravel. Microbes also require an organic substrate to support their nutrition, so sediments that contain a lot of organic matter host larger microbial populations than inorganic ones.

Persistently turbid waters contain significant concentrations of suspended particles that are too small to settle. These particles originate from catchment soils or resuspended bottom muds. When they first enter the water column, these particles are quite microbially active and can therefore exert a significant oxygen demand. (The experimental results shown in A.11.9 provide some indications of the kinds of effects that fresh microbially active sediments can have on DO concentrations). However, the readily bioavailable organic component of this allochthonous suspended material is rapidly consumed, so the oxygen demand associated with it is seldom sustained for long.

Even in very turbid waters the overwhelming majority of the fine sediment and particulate organic matter contained within a waterbody is stored in benthic sediment deposits, and these are often the principle source of oxygen demand on the overlying water column. The ways that this demand influences the distribution of DO within the water column vary depending on the waterbody's mixing characteristics and are discussed in the next section, but regardless of the mechanisms involved, the composition and thickness of the benthic sediment layer can be a key determinant of overall oxygen demand and therefore hypoxia risk.

Fine sediment deposits that occur in shallow waters are easily resuspended, and at exposed sites such as lakes and ponds, windy conditions can cause chronic turbidity to develop in waters that were previously clear. The probability of this happening increases towards the end of the dry season when water levels approach their annual minimum. The resulting decreases in water clarity can substantially alter the metabolism (i.e. the balance between photosynthesis and respiration) and therefore the DO status of the water body. Wind resuspension is less of a problem in more sheltered locations such as riverine waterholes; nevertheless some such sites can still suffer acute episodic increases in turbidity if unusually strong winds develop.

For the purposes of assessing relative risks as a precursor to monitoring site selection, it is only necessary to discriminate between waterbodies, and/or sections of waterbodies, that exhibit fairly striking differences in the amount and type of sedimentary materials – sites that contain relatively inert bottom materials such as coarse sand and gravel being the least at risk, and those that contain large amounts of fine organic muds with the potential to be highly bioactive, being at highest risk.

For detailed work, especially when dealing with shallow heterogeneous waters, it may be necessary to divide the waterbody into different zones with distinctive sediment characteristics. (The potential importance of this is obviated in some of the examples in A.1-A.10). When carrying out such assessments it is imperative to recognise that the substrata of many waterbodies in this region are subject to seasonal and/or episodic layering effects, and that as a result, many of the techniques that are conventionally used to assess the composition of bottom sediments may not be completely reliable.

Specifically, the composition of the primary substratum of most streams is governed by large-scale flood events which remove the fine sediments from most parts of the drainage system leaving behind coarse grained sediments such as sand and gravel, or in some cases exposed bedrock and stone. However, during the prolonged periods between these high flow events this primary substratum often becomes covered by a secondary layer of much finer sedimentary materials comprising mainly flocculated colloids and organic detritus. These materials can be quite bioactive and significantly influence the DO status of both the overlying water column and benthic habitats. It is therefore important to record the presence of such layers. However, because the material is very light and seldom consolidates, it is very difficult to collect using conventional sediment grab samplers.

A.11.7 Waterbody Stratification

The water column of calm, poorly mixed waters often becomes divided into separate layers (i.e. strata) that do not intermix. This stratification results from differences in water density – the lowest density waters tending to float at top, while high density waters sink to the bottom. In inland waters these density differences are generally caused by inequalities in water temperature, termed thermal stratification, but in near-coastal waters they may be also be caused by differences in the concentrations of dissolved salts, termed salinity stratification. Regardless of water temperature, the density of seawater (which has a salinity of about 35 psu) is considerably greater than that of freshwater (which in this region usually has a salinity of significantly less than one psu), so salinity is normally the prime driver of stratification in systems that contain mixtures of both types of water. However, in inland waters that are not influenced by seawater, stratification is almost always thermal.

Thermal stratification occurs because the sun transfers more heat to the near-surface waters than it does to deeper waters. Once the surface layer heats up, its density falls to the point where it cannot sink into the underlying water until it either cools or gets forced downwards by an external force such as that provided by wind or water flow. In the absence of such forces, the warm water remains near the surface and the cooler waters beneath are trapped near the bottom, unable to obtain enough heat from the sun to be able to rise to the surface. Sometimes the surface layer only stays warm during the hot daylight hours and the water column can become mixed each night (a process called diurnal stratification). However, deep waters (or more specifically those that are deep compared to their surface area) can maintain stable thermal stratification most of the time, especially during the warmer months towards the end of the dry season.

There is always some mixing within the surface water layer (termed the epilimnion – limnion being the correct scientific term for the water column); although in small waterbodies this may be limited to depths of considerably less than a half a metre below the water surface. The epilimnion can obtain oxygen from the overlying air, receives more light than deeper waters allowing it to better sustain photosynthetic DO production, and is not in direct contact with the oxygen consuming sediments contained on the bottom. Accordingly, except in relatively shallow waterbodies where the bottom water layer (often referred to as the hypolimnion) receives sufficient light to support dense stands of submerged macrophytes on the bottom, the surface layer is almost always better oxygenated than the underlying waters. Though always warmer than the hypolimnion, this mixed surface layer is constantly heating and cooling, and may also be exposed to external mixing forces. Consequently, the depth of surface mixing (i.e. the thickness of the surface layer) can vary substantially over the course of a normal day, and over longer time frames. The surface mixing depths of many shallow waterbodies increase so substantially overnight that they almost become fully mixed – basically, in shallow waters the distinction between diurnal and stable stratification is often somewhat indistinct.

The upper and lower water layers of a deep waterbody are typically separated by a thermocline; a layer of water within which temperatures decline rapidly with increasing depth. Many freshwaters in this region are so shallow and poorly mixed that a distinct thermocline is maintained through the entire water column during the heat of the day but not during the cool of the night. Such waters do not form a distinct bottom layer as such, but except in cases where submerged macrophyte beds are present, they do normally exhibit pronounced oxygen gradients with concentrations generally decreasing substantially with depth. Under such circumstances bottom waters are hypoxic most of the time because the thermocline only breaks down during the night when DO levels throughout the entire waterbody are approaching their daily minimum.

Except when they contain dense macrophyte assemblages, hypolimnetic waters (i.e. those that lie below the thermocline) are generally severely hypoxic, and sometimes anoxic. This is because they are constantly in intimate contact with oxygen-demanding bottom sediments, are unable to access the oxygen contained in the air and don't usually receive enough light to support much photosynthetic oxygen production.

Even in the tropics, and especially in the dry interior, overnight temperatures can become cool enough during winter for the surface water layer of stably stratified waterbodies to eventually sink, suddenly forcing the hypolimnetic water to the surface. Such an event is called a turnover. Hypolimnetic waters, which have usually been severely hypoxic for a long time, often contain significant concentrations of oxygen-demanding substances such as hydrogen sulphide, reduced forms of iron and manganese, methane, ammonia and partially decomposed organic matter. These can rapidly consume the DO contained within the newly mixed water column, potentially leading to quite severe episodes of hypoxia. The oxygen demanding materials are usually consumed quite rapidly, so normoxia is generally restored if the water column remains mixed for a significant period after the turnover, however, many components of the ecosystem can suffer extensive damage in the interim.

Any stratified waterbody that normally experiences calm conditions can turnover if the water column suddenly becomes turbulent. This can be caused by unusually strong winds (which also promote mixing by cooling the water surface) and/or flow pulses. Flow pulses associated with unseasonable winter rain events are particularly likely to induce turnovers because rainwater runoff is significantly cooler, and therefore denser, than receiving waters, and can preferentially flow into the bottom of a waterbody (a process called underflow).

The relative risks of adverse effects from a turnover event can be estimated in qualitative terms by conducting depth profiles and calculating the depth averaged DO concentration of the entire waterbody. This is the DO concentration that would theoretically result if the water column was to suddenly mix without receiving any additional re-aeration or experiencing any additional oxygen demand. In practice neither of these provisos are actually met during a turnover event, nevertheless the calculated concentration still provides a relative measure of risk. DO profiles should ideally be conducted during the early morning when concentrations are at their daily minima. It is noteworthy that when this is done, some natural lagoons such as that shown in A.8, report extraordinarily low depth-averaged concentrations indicative of very high hypoxia risks.

Turnovers are a natural occurrence but their frequency and severity have been increased by a variety of anthropogenic factors. The construction of impoundments and artificial deepening of natural waterholes has greatly increased the number of stratified waterbodies in many catchments, and baseflow reductions in regulated river reaches have also increased the stability of stratification in natural lagoons and waterholes. At broader scales anthropogenic contaminants have led to a general decline in the quality of the water and sediments contained within the hypolimnion of stratified waters, thereby increasing the potential damage that can be caused in the event of a turnover.

The risks of turnovers resulting from flow pulses need to be factored into the operation of water supplementation schemes. Artificial transfers of water from one impoundment to another, and/or sudden releases of dam waters into natural stream reaches that contain deep waterholes can cause turnovers if the water is delivered too rapidly. Conversely, more gradual releases can actually decrease the potential risks associated with a turnover event by increasing the depth of surface mixing, thus reducing the volumes of hypoxic water contained within the system.

A.11.8 Water Transparency and Light Climate

Waters that are sufficiently transparent to allow sunlight to reach the bottom are termed "optically shallow", while those that are not, are said to be "optically deep". An optically shallow waterbody can actually be quite deep if it contains very clear colourless water, and conversely, an optically deep waterbody can actually be quite shallow if the water is highly turbid and/or coloured.

The optical depth of a waterbody can be estimated by measuring the ratio of the total water depth (Z) to the euphotic depth (Z_{eu}) – the latter being the depth at which light intensity is reduced by 99%. Optically shallow waters have a low Z : Z_{eu} ratio indicating that the entire waterbody, including the benthos, is photic and therefore able to support photosynthetic DO production.

Waterbodies that are optically very shallow (i.e. that have Z : Z_{eu} ratios significantly below 0.5) do not need to receive strong direct sunlight to be able to sustain high photosynthesis rates. Accordingly their oxygen production is not strongly affected by overcast conditions or moderately dense shade – small rainforest streams with a closed shade canopy being the only optically shallow waters in this region that regularly experience light limiting conditions (due to the combined effects of very dense shade and frequent heavy cloud cover).

The oxygen production levels in very turbid, optically deep waters with euphotic depths of only a few centimeters may also be unaffected by changes in incident light intensity, simply because the majority of the water column never receives enough light to support photosynthesis anyway. However, the majority of regional waters fall between these two extremes and can exhibit significant DO fluctuations in response to the variations in incident light intensity that occur if, for example, the sun is obscured by passing clouds. In the authors' experience productive exposed waterbodies with Z : Z_{eu} ratios in the order of 0.8 to 3 are particularly sensitive to this factor and can experience significant declines in oxygen production whenever conditions become overcast. Many wetlands in this region experience fine weather conditions for months at a time during the dry season and consequently they develop a very high biomass of light-dependent plants. In these systems, the arrival of dense cloud cover can potentially bring about DO sags severe enough to cause fish kills.

A.11.9 Experimental Demonstration of the Effects of Benthic Sediment and Light Climate on the Concentrations of DO in a Water Column Dominated by Submergent Macrophytes

Due to the high spatio-temporal heterogeneity of natural waterbodies and the large number of biophysical variables that influence DO, it can be quite difficult in field studies to confidently discriminate the effects of particular driving variables such as light climate and benthic sediment composition. However, the controlled outdoor experiment discussed below (Butler, Pearson, Crossland and Manwaring, unpublished) demonstrates the kinds of effects that can be expected.

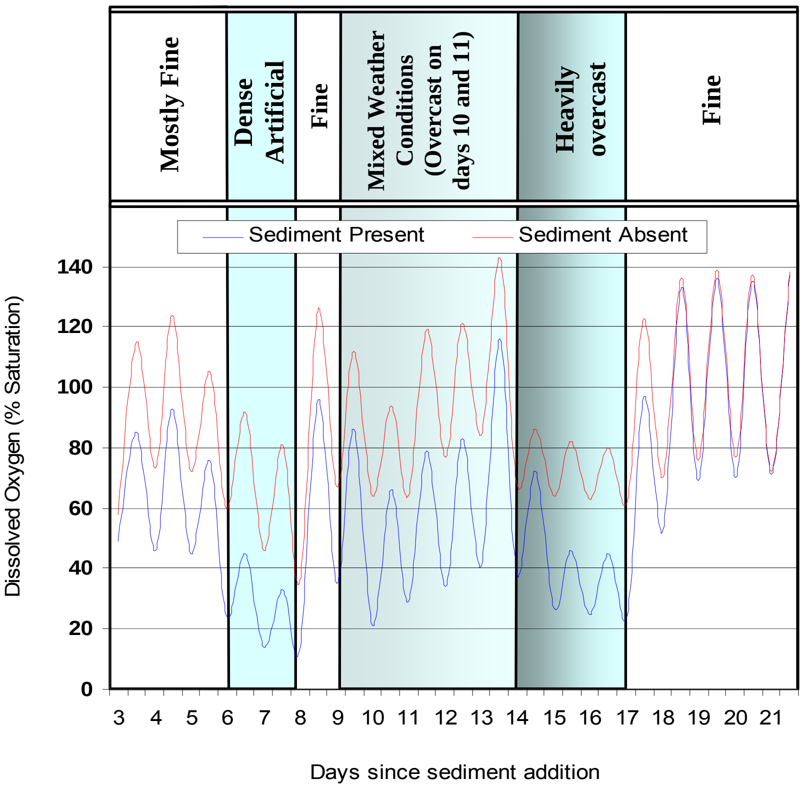
The experiment utilised twelve glass tanks each containing clear water (turbidity <<1 NTU and colour <1 TCU) and 10g/L of *Ceratophyllum demersum*, a very common local submergent macrophyte species (This biomass concentration is approximately equivalent to that maintained within a moderately dense natural macrophyte bed). Plants were exposed to 1 of 3 sediment treatments: (1) plants buried under a 3 to 4 mm thick layer of flocculated sediment particles, (2) plants placed on top of a layer of sediment, and (3) plants not exposed to sediment. The experiment was a randomised block design with each treatment being replicated 3 times.

Sediments were collected from a local dam with a pristine catchment and were pre-treated with a flocculant to ensure that clays and colloids settled and did not influence water transparency during the experiment. Fertiliser was applied as necessary to ensure that the concentrations of nitrogen and phosphorus in the water column of each tank were similar at the start of the experiment and were not growth-limiting during the early stages of the experiment. Re-aeration was inhibited by placing a layer of clear plastic on the water surface of each tank. Tanks were placed in direct sunlight, and underwater light climates were adjusted to marginal growth limiting levels by completely covering them with 3 layers of black plastic sheeting. This created light levels and DO fluctuations very similar to those observed in the near-surface to mid-water layers of many moderately turbid natural wetlands.

The plants proved to be so impervious to the effects of smothering that there was no statistically significant difference between treatment 1 (plant buried under a thin layer of sediment) and 2 (plant placed on top of sediment), so the data were pooled. The DO results plotted in Figure A.11.9 are mean values obtained from the 6 replicates in which sediment was present and the 3 replicates in which sediment was absent.

Since the sediment used in this experiment came from a productive waterbody, it contained significant amounts of natural organic matter. This increased the respiratory demands in all tanks that contained sediment, decreasing mean DO concentrations by about 30%.

Figure A.11.9 **The effects of cloud cover and benthic sediment on the DO levels of waters containing moderate concentrations of submergent plant biomass.**



Under fine weather conditions, the DO levels in the "sediment absent" treatments fluctuated from 110 - 130 % in the mid afternoon to a minimum of about 70 % in the early morning. This is very similar to the pattern that is often observed in healthy natural waterbodies that contain coarse grained inorganic benthic sediments and moderate to dense assemblages of vibrantly growing submergent macrophytes and/or filamentous green algae. Under the same lighting conditions the DO concentrations in the "sediment present" treatments were initially always under-saturated, ranging from a daily minimum of about 45% to maxima of 85 to 95%. This is strongly reminiscent of many of the moderately productive waterholes that occur in sandy-bottomed river channels in this region, although many such waterholes are more heterotrophic than this and experience significantly lower daily minimum DO levels. In this experiment the differences between treatments became negligible after about 19-20 days indicating that the organic matter contained in the added sediment had largely been consumed by that time.

Reductions in the intensity of incident light due to artificial shading and natural cloud cover led to significant declines in the amplitude of diel cycling and mean DO concentrations, with levels gradually falling from day to day in cases where light levels remained consistently low. Notably DO concentrations in the sediment-present treatment fell to a minimum of only 11% after 3 consecutive days of unabated shading, and the maximum on that day was only about 30%. The sediment-absent treatment also exhibited substantial DO declines under low light conditions but concentrations never fell below 35% at any stage of the experiment.

A.12 DISSOLVED OXYGEN MONITORING METHODS

A.12.1 Using the New Trigger Values

The effect-based trigger values (TV's) provided in the DO guidelines are designed to be used in two ways:

1. Identifying situations where it would be necessary to conduct more detailed assessments before being able to determine if DO concentrations are adequate to meet the needs of sensitive species.

    Compliance with chronic and acute TV's is tested using minimum daily dissolved oxygen (MDDO) values. Estimates that are acceptable for initial screening purposes can be obtained by conducting spot readings, but except in some cases where flows are present or weather conditions are unusual, this can only be accomplished by monitoring very early in the morning, usually within a few hours of sunrise. The precise timing needed at each site is best determined by conducting more detailed monitoring on a few occasions (for example by deploying a data logger) before commencing routine monitoring. Results obtained from other similar sites in the region can also provide an adequate basis for prediction, and the examples provided in A.1-A.10 will be useful in many cases. However, it is important to ensure that study sites really are similar to reference sites, and that flow rates are identical. The factors which need to be considered when assessing between-site similarities are detailed in earlier sections of this appendix.

    TV breaches trigger the adoption of more intensive monitoring to determine variability over time. Note that breaches of the chronic TV (CTV) are likely to be a common occurrence, especially when dealing with productive lentic waters. This does not indicate that the TV is too conservative, but rather that DO concentrations in many waters really are low enough to affect the animals that inhabit them. Since the CTV is designed to protect against subtle sub-lethal effects that develop gradually over time, compliance is best evaluated using long-term median MDDO values rather than individual spot measurements – some breaches will almost certainly occur from time to time at most sites, for example when conditions become overcast, but these are of little significance provided that such episodes are brief and infrequent, and the Acute TV (ATV) is not breached.

    In cases where CTV breaches are recorded at many sites, secondary screening can be done by taking the daily DO maximum into consideration; the highest risk sites being those that exhibit the greatest deviations from saturation (either undersaturation or supersaturation are significant, the latter being indicative of poor mixing and/or very high productivity, both of which are high risk factors). If diel variability data have not been collected during the initial screening surveys, maxima will need to be determined by carrying out follow-up monitoring. The required timing can be determined by referring to the same reference data that was used for the MDDO.

    Estimates of the duration and severity of TV breaches obtained by this means provide an adequate basis for comparing the relative risks at different sites, and will help investigators determine which sites, if any, deserve closer investigation. Risk is not the only basis for arriving at this decision; many other factors such as the objectives of the monitoring program and the ecological importance, socio-economic value, management significance, representativeness and intactness of the sites also have an important bearing. Accordingly, the assignment of final priorities is left to the discretion of individual investigators.

    Compliance with the ATV needs to be assessed differently, because in this case each individual breach has the potential to be life-threatening for some species. Sites that report severe and/or regular ATV breaches should be investigated thoroughly by monitoring continuously over the course of at least one or two days to obtain more accurate indications of the extent of the breach. It is critical to ensure that monitoring is carried out during the critical overnight period, so dataloggers will usually be needed.

2. Calculating values for the three new risk-based monitoring parameters that have been introduced in these guidelines.

    The new parameters, namely the Effective Daily DO (EDDO), the Acute DO Deficit (ADDO) and the Diurnal Acute DO Deficit (DADOD), have been proposed as a means of integrating the effects of variations in the severity and duration of the TV breaches that occur at sites, especially when there is significant diel cycling. Values for each parameter are calculated by analysing diel monitoring data in the context of the relevant TV to obtain a single value indicative of the risks that accrue over any given 24 hour period. EDDO values relate to chronic effects, ADDO values relate to acute effects resulting from normal diel cycling (i.e. to situations where low DO values occur during the cool of the early morning when fish can rise to the surface in comparative safety) and DADOD values relate to situations where acute hypoxia occurs during the heat of the day.

    These parameters are intended to be used in the same way as any other water quality parameter that yields a single value indicative of the conditions at a site on the day that samples or measurements were taken. They can be included in routine monitoring programs and used to evaluate longer term trends and/or to compare between sites. Once reference data are available (and collection is currently in progress) it will be feasible to derive referential trigger values by employing the methods recommended (for natural stressors) in the National Water Quality Guidelines (ANZECC 2000).

    EDDO values can only be calculated using a full 24 hour dataset and will therefore usually demand the use of datalogging equipment. ADDOD monitoring only requires measurements to be collected during periods when the ATV is being breached, and the DADOD only makes use of data collected at times when ATV breaches occur during daylight hours. Since the DO levels at many sites will only breach the ATV for a short time it may be feasible to employ manual monitoring methods in some instances.

A.12.2 When to Monitor

As mentioned above, the MDDO readings that are used for initial screening purposes must be collected at the time of the day when DO reaches its diel minimum. There are a few large wind-exposed waterbodies and some turbulent perennial streams that are sufficiently well-aerated to ensure that water is almost always very close to equilibrium with the air, and as a consequence these sites seldom exhibit strong diel cycling. These are the only healthy waters where it is possible to assess MDDO values by taking a single randomly-timed reading, but because they are so well aerated, they are also the least likely to breach TV's and/or to warrant DO monitoring. In some catchments there are also many wetland habitats that have been so successfully invaded by exotic weeds that the water surface is now entirely covered by mats of vegetation. These sites are so severely and persistently hypoxic that it wouldn't usually matter when readings were taken. However, because they are in such obviously poor condition, there would be little to gain from monitoring DO at these sites until the weed infestations are brought under control.

All other waters with the potential to suffer from hypoxia also have the potential to experience significant diel DO fluctuations, and this precludes the use of randomly timed spot measurements. Random spot DO readings are often included in routine monitoring programs on the premise that some information is better than none. This is not a defensible practice, especially when dealing with the kinds of waterbodies where DO monitoring is most needed, because the results obtained are so misleading that they are most likely to misdirect management attention. Ostensibly this is a situation where no information is better than misinformation.

Water quality practitioners have historically attempted to ameliorate the problems associated with spot measurements by ensuring that the readings taken at any particular site are always collected at the same time of day. This strategy simply does not work (in the kinds of waters that are in question here at least) unless spot readings are carefully timed to target a known or predicted DO state (such as the daily minimum or maximum) as recommended earlier.

MDDO values can vary substantially during the year and it is important to ensure that monitoring achieves adequate coverage of high risk conditions. Notably it is impossible to assess compliance with the ATV unless this is done. The sets of biophysical conditions that need to be targeted in order to accomplish this objective are detailed in earlier sections of this report. They vary somewhat depending on circumstances and waterbody type, but as a general rule, virtually all sites are in a vulnerable state during the stressful warm months of the pre-wet season; especially when the first rains arrive, leading to overcast weather and pre-flush stormwater inflows.

The EDDO is designed to aid in assessing the same sorts of long term chronic effects that are of interest in any routine ambient water quality monitoring program. Accordingly, the regular-interval and/or random monitoring schedules that are usually employed in other ambient monitoring applications are equally appropriate for EDDO monitoring, and data should be interpreted using the same kinds of statistics (e.g. seasonal means or medians). The only real deviation from conventional ambient monitoring practices is that, instead of collecting a sample or spot measurement each time a site is visited, personnel will need to deploy a datalogger and then return at least a day or so later to retrieve it.

ADOD and DADOD values on the other hand are used to assess the risks associated with brief transient events, any one of which could have the potential to cause catastrophic problems such as fish kills. The precise timing of such events is usually subject to the vagaries of the weather, so if they are to be detected, dataloggers may need to be deployed for prolonged periods during high risk stages of the year. Since there is normally a limited supply of dataloggers it is important to ensure that they are deployed at precisely the right location.

A.12.3 Where to Monitor

Previous sections detail the biophysical factors that drive temporal variations in the ways that DO is distributed throughout individual waterbodies. This information provides some basis for predicting which of the different habitats contained within a waterbody are most likely to be at risk of hypoxia. Nevertheless, whenever dealing with high priority sites it would be advisable to carry out surveys to determine which zones within the waterbody are most prone to hypoxia, especially during events when conditions become less predictable.

If assessing the risks of acute lethal effects (i.e. fish kills) associated with brief transient episodes of hypoxia, we suggest taking readings in the most highly oxygenated sections of the waterbody, because that is where the fish will go, if and when they need to. However, if very swift flows develop, fish may be forced into backwaters and edge habitats, and that is where monitoring is best carried out. Since DO levels often decline rapidly with depth, meaningful results will only be obtained if DO sensors remain within the near-surface layer whenever water levels rise and fall. This usually necessitates the use of DO sensors equipped with floats, and protective enclosures or cages to prevent them from being washed away.

When carrying out ambient monitoring and/or assessing the potential for chronic sub-lethal effects it is important to ensure that DO readings are taken within some of the more hypoxia-prone microhabitats that fish must regularly exploit in order to prosper. The surface layers of open stretches of water are often the most oxygenated, but fish spend much of their time hiding or foraging in and around more sheltered edge habitats and backwaters where DO levels are usually significantly lower, and/or within macrophyte beds where DO levels often fluctuate wildly. Fish are well attuned to diel variations in the distribution of DO and can adjust their movements accordingly. It is therefore usually valid to focus on monitoring the mixed surface layer of the water column unless that layer is too thin for fish to utilise without exposing themselves to significant risk (of predation or overheating, for example). Unless there are compelling reasons to adopt alternative tactics (such as a detailed study of fish habitat quality and availability), we suggest taking readings from a depth of 25 to 30 cm below the water surface, as this maintains consistency with the conventions that are followed in most water quality monitoring programs.

This first version of the DO guidelines is designed to be used mainly as an aid for assessing fish habitat values and/or the condition of benthic edge habitats. Unlike many benthic species, fish can easily rise in the water column to obtain the DO they require. The DO concentrations available to fish can therefore be determined by simply carrying out readings in the mixed surface layer of the water column, without the need to carry out depth profiles. DO data collected from the mixed surface water layer can also be used to assess the hypoxia status of benthic edge habitats (i.e. the parts of a water body where the bottom meets the oxygenated surface water layer), provided that these areas are not choked by emergent or floating vegetation. However, the fact that these are likely to be the least hypoxia-stressed benthic habitats in the waterbody must be taken into consideration when interpreting findings.

It should be noted that edge habitats of this kind can be important as they provide a place where bottom-dwelling fish can access the DO available in the mixed surface layer without needing to leave the bottom.

A.12.4 Assessing Benthic Habitats

The DO levels in the benthic habitats of most hypoxia-prone waterbodies are naturally very low; in fact many species occupy microhabitats that become severely hypoxic even in very well-oxygenated waters. For example we have often detected DO concentrations of less than 30% within litter packs on the bottom of pristine rainforest streams (a benthic habitat that supports exceptionally diverse and abundant macroinvertebrate communities), even though conditions in the overlying water column are always normoxic. It is intuitively obvious that such organisms must be very tolerant of hypoxic conditions, and the weight of available evidence supports this contention, though hard data are surprisingly scant. Preliminary tests conducted prior to the commencement of the experimental program that ultimately led to the development of these guidelines (Pearson *et al.* 2003) indicate that the acute asphyxiation limits of the most sensitive benthic species are approximately equivalent to that of fairly tolerant fish species, and that the majority of benthic species are significantly more tolerant than most DO-dependent fish. Accordingly, the TV's provided in these guidelines will almost certainly be overly conservative if applied to the benthic fauna that inhabit deep, naturally hypoxic waters.

However, this is not the main impediment to benthic habitat assessment. The main problem is that the DO variations below the mixed surface layer, even in the deeper parts of quite shallow waterbodies, are so complex that it is a major undertaking to evaluate the DO status of the entire water column, let alone the benthic microhabitats that macroinvertebrates and microfauna generally inhabit. To date there are no established methods of overcoming these complications, so the task of properly assessing the hypoxia status of benthic habitats is a particularly challenging endeavour that should only be attempted by experienced limnologists with considerable resources at their disposal.

A.12.5 Depth Profiling

As mentioned earlier, for routine ambient compliance testing and/or event monitoring, it will often be adequate to rely mainly on DO measurements taken in the near-surface layer of the water column. However, for detailed ambient investigations, fish habitat evaluations and/or predictive risk assessments, it will usually be necessary to survey deeper waters as well.

This is particularly important if attempting to predict the potential consequences of a turnover event in a stratified waterbody, as described in section A.11.7. In this case it is necessary to obtain a reasonably accurate estimate of the depth-averaged MDDO concentration. Since stratification patterns can vary throughout a waterbody, accurate results can usually only be obtained by conducting depth profiles at a number of different monitoring stations representative of each of the different zones within the waterbody. This must be done during a relatively brief period early in the morning, and it can be logistically difficult to carry out enough depth profiles to describe spatial variations within the limited time available.

These difficulties can be minimised by adopting the following tactics: 1) start profiling an hour or two before sunrise, because conditions change much more gradually at that time of the day than they do once the sun has risen; 2) before moving on to deeper limnetic zones, conduct profiles in shallower zones first, and especially those with macrophyte beds because conditions can change more rapidly in these locations once the sun has risen; 3) use instruments with built in memories so that data do not have to be manually recorded (noting that this is only really feasible if the sensor has depth measuring capabilities); 4) if using membrane electrodes ensure that they are equipped with a thin high-diffusion membrane in order to reduce the time taken for readings to stabilise, and; 5) further reduce reading equilibration times by immersing the sensor in an insolated container of water when moving between stations, and then lower the entire container into the water column so that the sensor is never exposed to the air at any time during surveys.

A.12.6 Background Data Collection

It is only necessary to collect DO measurements to be able to test compliance with the TV's. However, in order to have any chance of understanding why DO concentrations are the way they are, and perhaps more importantly, to be able to determine if results can validly be compared with reference data from other sites, it is necessary to collect qualitative data indicative of the status of each of the risk factors discussed in Section A.11. As explained in these sections, DO is very strongly influenced by a wide variety of localised factors such as hydrodynamics, depth, shape, geomorphology, and the amount and type of instream biomass. This means that two sites need to be an exceptionally close physical match before one can be validly used as a reference for the other. Some of the key variables involved can change quite dramatically over time, so even if the two sites are a perfect physical match, it is imperative to ensure that the data available for each site are representative of precisely the same combinations of prevailing conditions. It would, for example, make no sense to compare two physically similar sites if the weather had always been fine and windless when data were collected at one site, but cloudy and/or windy when they were collected at the other. Results would be particularly misleading if one site had been flowing at the time when data were collected and the other had not.

Problems of this kind can be avoided by applying data classifications before carrying out analyses – i.e. by separating data collected under one set of environmental conditions from those collected under another. This is not difficult to accomplish but it is only feasible if prevailing conditions are recorded properly every time a reading is taken. This only needs to entail the collection of qualitative data and can be accomplished by using simple nominal categories to describe any major differences in key variables. Examples of these are given in section A.11. Routine collection and collation of biophysical background data of this kind is strongly recommended for any DO monitoring program (and in fact any ambient water quality monitoring program).

Multi-parameter probes capable of simultaneously recording DO, temperature, pH and conductivity can provide much useful information about the factors that drive DO status. These data are particularly useful for identifying changes in flow conditions during events, as illustrated in the example shown in A.10.