Water Quality Tolerances of Aquatic Biota of the Murray-Darling Basin

November 2009

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A report prepared for the Murray-Darling Basin Authority by the Murray-Darling Freshwater Research Centre

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Cover Photo: Carp killed by a deoxygenation event in Colligen Creek, NSW; February 2009
Photo Credit: Kerry Whitworth, MDFRC

Citation: Watson G. O, Bullock E. K , Sharpe C & Baldwin D. S (2009). Water Quality Tolerences of Aquatic Biota of the Murray-Darling Basin. A report prepared for the Murray-Darling Basin Authority by the Murray-Darling Freshwater Research Centre

The Murray-Darling Freshwater Research Centre is a joint venture between the Murray Darling Basin Authority CSIRO Land and Water and La Trobe University that aims to answer locally relevant research questions to help guide water managers, and ultimately improve the health of our rivers, lakes and wetlands.

Executive Summary

The purpose of this report is to inform target setting for key water quality parameters for the Basin Plan, being developed by *the Murray-Darling Basin Authority*. This report examines the thresholds of biota found in the Murray-Darling Basin to water quality parameters. It is based on an exhaustive review of both the peer-reviewed and non-peer reviewed literature.

In developing this report we have focused only on studies which specifically report on species found in the Basin. Frequently water quality thresholds reported in the Australian literature are based on North American or European species. It is inferred that Australian species should respond in an analogous manner to their northern-hemisphere relatives.

Compared to North America and Europe, there are relatively few studies on chemical thresholds of MDB endemic species. (The notable exception is salinity tolerances; however, as salinity thresholds are covered under a separate study, we have limited our discussion on salinity to a brief overview of thresholds). Unfortunately the paucity of data leads to uncertainty in the risk assessment process.

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Definitions

Most parameters are measured in terms of concentration with the following relationship: mg/L = ppm = 1000ug/L = 1000 ppb

Lethal effects = mortality caused by a specific parameter threshold.

Sub-lethal effects = individuals do not die but the effects of the parameter cause changes to other areas of metabolic function such as reproduction and growth performance.

Short term or acute = measure both lethal and sub-lethal effects that occur after a period of exposure that is short term relative to the organisms life span.

Chronic effects = measure both lethal and sub-lethal effects that occur after a period of exposure that is longer term relative to the organisms life span.

Direct effects = where lethal and sub-lethal effects are directly attributable to the parameter present.

Indirect effects = where an organism is not directly affected by the parameter concentration itself but where something else in the environment (such as prey) is affected and the prey response affects the organism at either a lethal or sub-lethal level.

LC50 value = threshold where 50% of individuals over a specified period are killed.

EC50 value = concentration of a drug, antibody or toxicant which induces a response halfway between the baseline and maximum after some specified exposure time

Salinity is measured in a variety of ways and describes the total concentration of inorganic ions in the water. Typically it is measured in EC (electrical conductivity) units and expressed as uS/cm. A more accurate measurement expresses salt as Total dissolved salts (TDS) and is measured in mg L^{-1}. In general salinity levels above 3 000 mg L^{-1} (4500 EC) distinguishes 'fresh' from 'saline'.

LOEC = lowest observed effect concentration.

NOEC = no observed effect concentration.

Introduction

The purpose of this report is to inform target setting for key water quality parameters for the Basin Plan, being developed by *the Murray-Darling Basin Authority*. This report examines the thresholds of biota found in the Murray-Darling Basin to water quality parameters. It is based on an exhaustive review of both the peer-reviewed and non-peer reviewed literature.

In developing this report we have focused only on studies which specifically report on species found in the Basin. Frequently water quality thresholds reported in the Australian literature are based on North American or European species. It is inferred that Australian species should response in an analogous manner to their northern-hemisphere relatives. Given the highly variable nature of river flows in this continent (Beare and Heaney 2002) and by inference the large range in concentrations in chemicals that endemic species could be exposed, such generalisations may not necessarily be valid (e.g. see Julli *et al.* 1990). However, compared to North America and Europe, there are relatively few studies on chemical thresholds of endemic species. (The notable exception is salinity tolerances; however, as salinity thresholds are covered under a separate study, we have limited our discussion on salinity to a brief overview of thresholds). Unfortunately the paucity of data and the variability in response of different species can lead to uncertainty in the risk assessment process - as Calow and Forbes (2003) note "… we rarely have enough information on the species sensitivities in particular communities to place much confidence in the precise risk probabilities generated." In particular, there are almost no data on the impact of specific constituents on key biogeochemical processes in the MDB – nitrification, denitrification, decomposition etc.

Background Principles

Biotic Responses

An organisms response to toxicants can follow one of two trajectories; dose dependency or hormesis. Dose dependency (figure 1) is a simple concentration response where no adverse effect occurs in an organism exposed to a toxicant until a threshold (lowest observed effect) is reached. Further increases in toxicant concentration produces dose dependent adverse responses in the organism (inhibition to reproduction, growth etc) until the organism dies. Hormesis (figure 2) describes the condition where low concentrations of a toxicant are regarded as beneficial to an organism and actually give positive stimulation to growth characteristics. A concentration is eventually reached however where the toxicant no longer stimulates but brings adverse outcomes for the organism which may continue in a dose dependent manner until the organism dies.

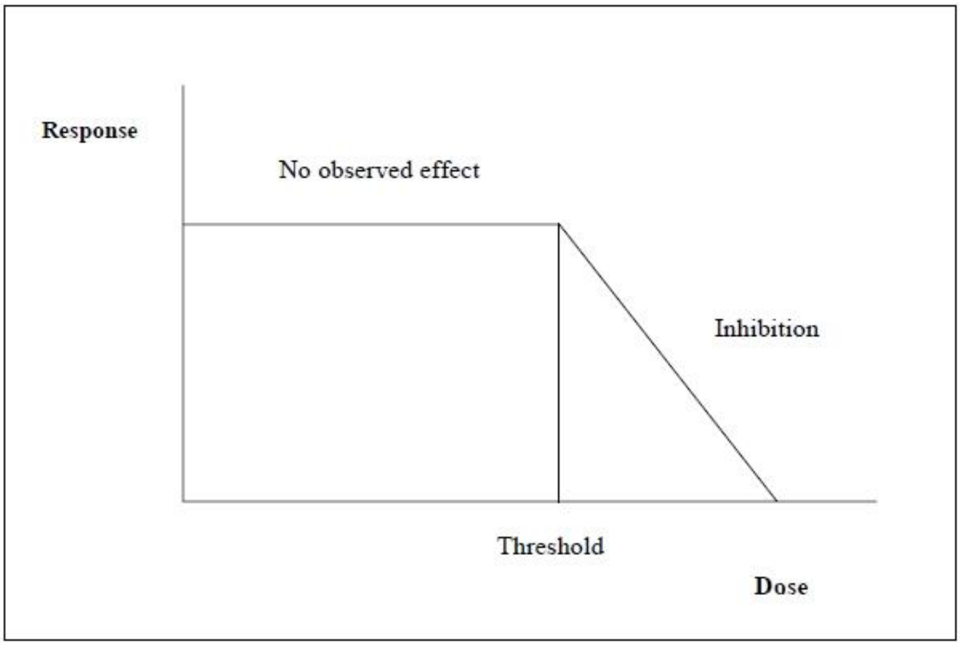

Figure 1: Dose-response relationship of a toxic agent with an aquatic organism (Zalizniak 2006).

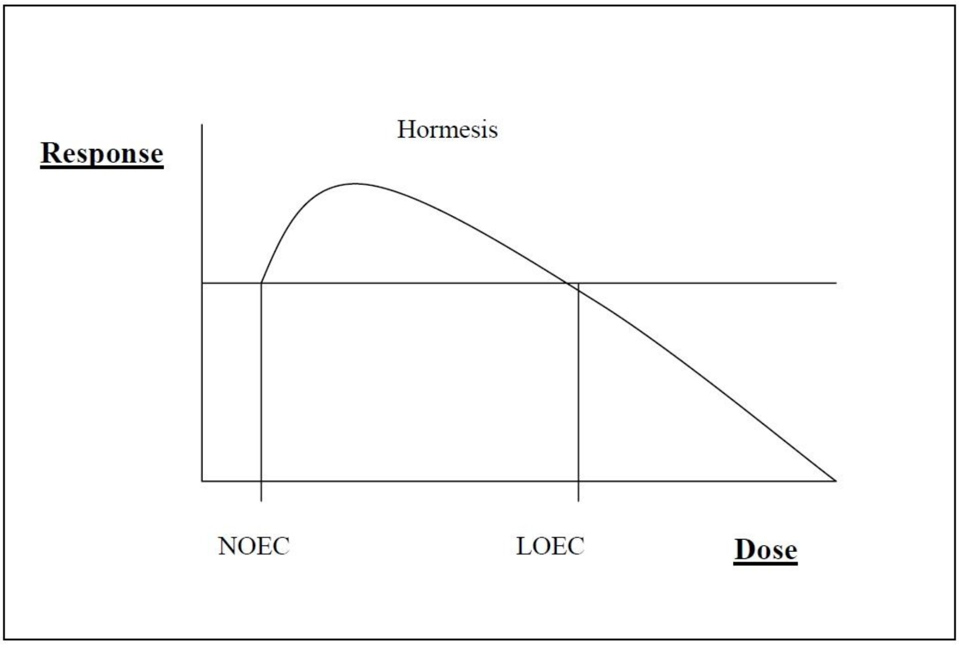

Figure 2 : Dose response curve showing hormesis occurring below LOEC NOEC = no observed effect concentration, LOEC = lowest observed effect concentration. (Zalizniak 2006).

Interactive Effects

Ecological threshold testing is traditionally performed using a single parameter or contaminant approach. While this gives direct cause and effect correlations, these chemicals or parameters rarely act in unison or exist on their own. Since rivers and wetlands are often 'sinks' for environmental contaminants, chemicals exist as mixtures. It is the interactive effects of these mixtures, and further influences from prevailing physical and chemical parameters that determine the ultimate water quality of an aquatic environment. For example, there is evidence that some contaminants, such as chlorpyrifos, can interact synergistically, additively or antagonistically, with their metabolites or break down products (Ca´ ceres *et al.* 2007). The resulting tolerance thresholds can have a variance that cannot be anticipated from single chemical or parameter studies.

Measuring Tolerance

Tolerance reporting generally describes the level that causes mortality for specific taxa. It is a trigger value at a specified level rather than the varied levels and pulses that naturally occur in rivers and wetlands. They are often reported as LC_{50} values which are relative and defined against a defined exposure period. Difficulties can arise when extrapolating LC_{50} results into natural systems as exposure periods vary in the real world.

High LC_{50} concentrations over short durations give no indication of an individual organisms tolerance at lower concentrations over longer periods.

LC_{50} values however do assist in developing cause-and-effect relationships. They have relevance where system contamination exposure follows a similar pattern and as such may assist in managing and understanding the potential impacts of pulses or short term exposures and provide guidance if establishing upper pulse thresholds.

Resilience and Recruitment

LC_{50} values however, do not provide any information on sub-lethal effects such as reduced reproductive capacity. Early life stages can be particularly sensitive to moderately small elevations in contaminants such as salinity (Watson *et al*. 2008). Managing environmental stress using adult threshold data can overestimate the viability of aquatic communities either by under-estimating the importance of sub-lethal effects or indirectly through for example, loss of habitat and prey items. Protection of early life stages is vital for recruitment success consequently, concentrations of contaminants lower than reported LC_{50} values may still have long term detrimental effects of species survival. However, not all organisms need to reproduce every year to maintain viable populations. In this case issues around resilience and habitat refugia become more important as does adult life form threshold data.

Water Quality, the *Basin Plan* and Climate Change

The impact of a given chemical compound on a particular aquatic organism depends on exposure to the chemical, which is a function of concentration and, to a lesser extent time. Water Quality targets for catchments (particularly salt) have in the past been based on loads. This can potentially lead to a paradox when framing the Basin Plan. If the target is to reduce the load of a specific chemical constituent leaving a particular catchment, it can lead to the increase in concentration of that constituent within the catchment. Consideration should be given to exporting specific constituents from catchments when conditions favour their export; for example during a large dilution flow, or at a time when the constituent posses less a risk for the ecosystems (e.g. nutrients during colder months).

Also, when setting targets, future climate change predictions should also be considered. Much of the Murray-Darling Basin is expected to be drier and hotter into the future. A drier climate means that the concentration of a particular contaminant can increase because of evapo-concentrations. Also as temperatures approach the thermal tolerances of a particular organism, they may become more susceptible to the impacts of a particular contaminant (synergistic effects). Generally speaking we have little available data on the synergistic effects of temperature with other contaminants on aquatic organisms in the MDB. However, a series of studies are currently underway linking the synergistic (sub-lethal) effects of temperature with either dissolved oxygen, salinity etc on the a small native fish – the Purple spotted gudgeon (Rick Stoffels, Murray-Darling Freshwater Research Centre, Pers. Comm).

The water quality tolerances of aquatic organisms in the Murray-Darling Basin are listed in the accompanying table. The table lists the reported thresholds of a particular physico-chemical parameter (specifically pH, dissolved oxygen, temperature, turbidity or suspended solids and dissolved organic), nutrients, agricultural chemical (herbicide, pesticide, fungicide or surfactant) or heavy metal on particular species. Species are grouped into specific taxonomic groups (Fish, birds, frogs, aquatic plants, aquatic mammals, macro-invertebrates, micro-invertebrates etc) and, if the study was done a particular life stage this has been noted. We have also noted the status of a particular species under the *Environment and Biodiversity Conservation Act (Cmth)*. Two types of data are shown – either measured lethal and sub-lethal effects or ranges where the organism has been found in the environment. Where gaps exist in the data (and there are many) we have not been able to find data relevant to the Murray-Darling Basin. The following gives a brief overview of the results.

Physico-chemical Parameters

Water quality is inherently variable, and it is natural for parameters to fluctuate and constantly change. Even so, this fluctuation occurs naturally within certain limits, and one could loosely say it is balanced. The introduction of external pressures from both natural and human influenced events and their effects can alter this balance. While this is true for all river systems, it is especially important within the Murray-Darling Basin. The basin is Australia's most important agricultural region, supporting 40% of Australian farms (approximately 470,000ha) for which 3,780 GL are diverted annually for irrigation (Anonymous, Discover Murray Webpage, http://www.murrayriver.com.au/about-the-murray/murray-darling-basin, accessed November 16th 2009). The quality of water impacts the productivity of these farms and vice-versa, for any impact upon the land within the basin ultimately impacts upon the river systems themselves, and in turn biota within them.

Physicochemical parameters such as temperature, pH, conductivity, turbidity, suspended solids, dissolved oxygen (DO), and dissolved organic carbon (DOC) can have a significant impact upon the biota of a waterway if present in concentrations or levels outside a tolerable range. The known ranges, lethal and sub-lethal effects are discussed hereafter with reference to specific examples. Data given are exclusively from studies carried out where samples were collected within the Murray Darling Basin, or within close proximity to the basin if data is deemed necessary for inclusion. No data from overseas studies were considered.

Salinity

Salinity is not a major focus of this study but the report would not be complete without at least some general comments on salinity tolerances.

Salt is a natural component of the Australian landscape, to which a number of biota inhabiting rivers and wetlands are adapted. However, freshwater ecosystems in Australia are now becoming increasingly threatened by rising salinity as a consequence of rising saline groundwater and modification of the natural hydrological regime reducing the frequency of high flow (flushing) events.

Salinity and its response in aquatic organisms is undoubtedly the most studied parameter in the Australian literature. Morris *et al.* (2002) compiled a salt sensitivity database for more than 1200 species of Australian taxa while another more recent salinity review addresses data collected since the compilation of the salinity sensitivity database (Watson *et al.* 2008). Most noteworthy is data suggesting that freshwater ecosystems are being negatively impacted by salinity levels less than 1000 mg/L. Our understanding of salinity tolerance has been historically dominated by adult life form data but recent research (last 5 – 10 years) finds early life stages of biota generally more sensitive than their adult counterparts. The differences can be quite significant, for example Murray Cod have a reported 12 day critical egg development period with mortality occurring at levels over 340 mg/L while their juvenile fingerling counterparts have an LC50 of 13,700 mg/L (Chotipuntu 2003).

Table 1: General Biotic Salinity Thresholds (with some selected examples)

Biotic Grouping	**Threshold mg L^{-1} NaCl** (all figures approximate roundings)	**Source**
Bacteria	mostly unknown. 7500 EC (5000 mg L^{-1}) can cause shifts in specialist microbial processes	Hart *et al.* (1991); Metzeling *et al.* (1993), Baldwin *et al.* (2006)
Macro algae	4500 – 7500 EC (3000 – 5000 mg L^{-1}): important upper threshold but mortality as early as 1500 EC (1000 mg $^{-1}$)	Garcia (1999)
Microinvertebrates	7500 EC (5000 mg L^{-1}): an important upper threshold but affects apparent at 1500 EC (1000 mg L^{-1})	Nielsen *et al.* (2003) James *et al.* (2003); Brock *et al.* (2005)
Macroinvertebrates	7500 EC (5000 mg L^{-1}): an important upper threshold but affects apparent < 1500 EC (1000 mg L^{-1}). Several families capable of saline concentrations well above previously reported figures.	Horrigan (2005); Kefford and Nugegoda (2005), Dunlop (2005), Hart *et al.* (1991), Cameron (1991), Horigan *et al.* (2007) Kefford *et al.* (2007)
Frogs (tadpoles)	3000 – 7500 EC (2000-5000 mg L^{-1}): range for disappearance but affects apparent < 1500 EC (1000 mg L^{-1})	Francis (2003), Flowers (2004), Quincey (1991); Christy and Dickman (2002); Chinathamby *et al.* (2006),Baumgarten (1991)
Macrophytes	6000 EC (4000 mg L^{-1}): an important upper threshold but community with sub-lethal affects ≤ 1500 EC (1000 mg L^{-1})	Bailey and James (2000), Hart *et al.* (1991);James *et al.* (2003), Nielsen *et al.* (2003), Brock and Lane (1983), Brock and Shiel (1983); Garcia (1999)
Riparian vegetation	> 30 000 – 52 000 EC (20 000 – 35 000 mg L^{-1}): is an upper threshold but water logging must be considered	Hart *et al.* (1991), Rawat and Banaerjee (1998), Overton and Jolley (2004), Overton and Jolley (2004)
Fish	13 500 – 60 000 EC (9000 – 40 000 mg L^{-1}) (and greater): upper limit for adults but early life stage mortality possible < 1500 EC (1000 mg L^{-1}).	Chotipuntu (2003), MDBC database (2008)*, LWA database (2008)#, James *et al.* (2003), ,
Waterbirds	large gaps in knowledge but will invariably involve indirect effect dynamics. waterbirds	
Nutrients	Nutrient release can be affected above 1200 mg L^{-1}	Baldwin *et al.* (2006)

Temperature

When studying freshwater biota, temperature tolerances usually regard the direct effects of water temperature upon an individual or group, or occasionally the processes and behaviours that can be indirectly affected by temperature. It can be measured using a range of devices, and the results in Australian studies are almost always given in degrees Celsius (°C). An organism's temperature tolerance has both an upper and a lower limit beyond which normal bodily function is impaired or ceases completely. This varies between species, and can also vary within species if they are found in different locations (e.g. temperate vs. tropical). Tolerance can also vary if the organism has been acclimatised to a particular temperature before an experiment takes place, which makes true temperature tolerance difficult to determine.

There have been no studies conducted within the Murray-Darling Basin that we have found that report the water temperature tolerance of birds, algae, macrophytes or trees.

Fish

In regards to Murray-Darling Basin species; LC50, acclimatisation and capability of movement, presence/absence, mortality, upper and lower critical temperature and critical spawning temperature were determined for several species. (Pusey *et al.* 2004; Patra *et al.* 2007; McNeil *et al.* 2009) temperature tolerance is too subjective to give any overall tolerance values, but from the information available it seems that fish are more tolerant of lower temperatures after a period of acclimatisation (e.g. at 10-15°C). It can also be seen that few species found in the Murray Darling Basin could tolerate temperatures over about 35 °C. For example, the recognised upper tolerance limit for Murray cod is 33 °C (Koehn and O'Connor, 1990).

Critical spawning temperature ranged from 11 – 26.5 °C, but the source of this information (observed or experimental data) was unclear and highly variable between species (Koehn *et al.* 1990; Pusey *et al.* 2004).

Macroinvertebrates

It is assumed that the temperature tolerances of Australian macroinvertebrate species are similar to that of their overseas counterparts. Early studies of temperature tolerance regarding aquatic invertebrates have mainly been conducted in the USA, although some work has been done in New Zealand. In studies relevant to the MDB, lethal temperature ranged between 20.5°C and 33.8 °C overall, but lethal temperatures seem to be lowest in mayflies, suggesting an inability to handle high temperatures. Hatching success was greatest in 'mid range' temperatures, and almost always unsuccessful outside of a 5°C -30 °C range. (Wallb 1977; Brittain 1991; Brittain and Campbell 1991; Parnrong and Campbell 2003; Davies *et al.* 2004; Rees *et al.* 2004; Schreiber *et al.* 2003; Zukowski and Walker 2009)

Zooplankton/Microcrustacea

Generally, age at production of first brood of *Moina australiensis* (Moinidae: Diplostraca) was found to decrease with increasing temperature (Anderson-Carnahan 1994).

Cyanobacteria

Water temperature can influence whether or not a bloom of cyanobacteria occurs. Blooms of *Anabaena sp.* do not occur at temperatures less than about 10 ^{0}C and *Microcystis sp* below about 15 ^{0}C. (Robards and Zohary 1987; and references therein).

Baker and Bellifemine (2000) conducted an experiment in order to determine the effect of temperature on the akinete germination of *Anabaena circinalis*. It was discovered that over 24 hours there was no akinete germination at 10°C. Over the same time frame maximum germination (15.4%) occurred at 25°C, and germination decreased from 8.5% at 35°C to less than 1% at 38°C. Over 48 hours there was 19.4% germination at 20°C and there was 11.9% germination at 5°C.

pH

pH is a measure of the acidity or alkalinity of a given substance on a scale of 0 to 14, where 0 is the most acidic, 14 is the most alkaline (or basic), and 7 is 'neutral'. The pH scale is logarithmic, and the pH of a substance is measured by the concentration of hydrogen ions within it. The amount of these ions is determined by the concentration and nature of dissolved salts and organic matter within the substance (Lanigan 1998). Figure 3 shows the pH of various common substances, and we can see that the pH of rainwater is slightly acidic. The same is not true of river water, which can vary between being moderately basic and moderately acidic, depending upon internal and external influences. Acidification is an emerging area of concern for waterways in the Murray-Darling Basin, with wetlands being reported with pH levels as low as 1.6.

There have been no studies conducted within the Murray-Darling Basin that we have found that report on the pH concentration tolerance of frogs, birds, macroinvertebrates, zooplankton and microcrustacea, algae, cyanobacteria, or trees.

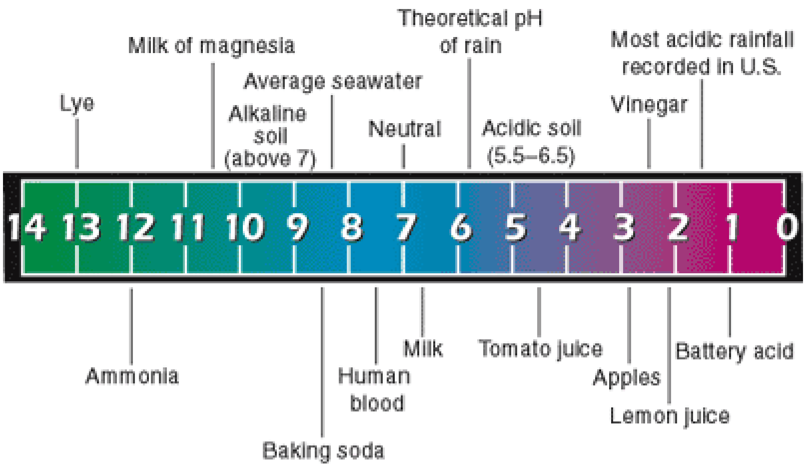

Figure 3: The pH scale displaying the various pH levels of common substances (from Anonymous. PEARL Webpage http://www.pearl.maine.edu, accessed November 17th 2009)

Fish

Only observational data from Pusey *et al.* (2004) was available for the pH tolerances of fish species. While most fish appeared to have a relatively narrow tolerance range (see accompanying table), some species were quite resilient. For example, *Retropinna semoni* (Australian smelt) has been observed in water with pH ranging from pH 3.7 - 9.8.

Macrophytes

Only one statement could be found linking macrophytes with pH tolerance: 'Species richness was negatively correlated with pH (P = 0.003)' (Porter *et al.* 2007). The authors' main concern however was the effect of salinity on macrophytes.

Dissolved Oxygen (DO)

Dissolved oxygen is simply a measure of the amount of oxygen dissolved in water. This is obviously important for the survival of taxa dependant upon oxygen transfer through the skin or gills with no supplementary atmospheric transfer, as dissolved oxygen levels below levels needed to sustain the body would quickly be fatal. Dissolved oxygen concentration is commonly expressed in mg/L or as a percentage concentration within a sample. The concentration of dissolved oxygen within a waterway can have an interactive effect with temperature as the dissolution of oxygen in water is negatively related to temperature; the higher the temperature of a waterbody, the less dissolved oxygen it can hold (Lanigan 1998).

There have been no studies conducted within the Murray-Darling Basin that we have found that report on the dissolved oxygen concentration tolerance of frogs, birds, zooplankton and microcrustacea, algae, cyanobacteria, macrophytes or trees.

Fish

Native fish and other large aquatic organisms require at least 2 mg O_2 L^{-1} in the water to survive, but may begin to suffer at levels below 4-5 mg O_2 L^{-1} (Gerhke *et al.* 1988). However, this can at best be seen as a guide. The low range LD_{50} of *Tandanus tandanus* (eel-tailed catfish, freshwater catfish) was found to be 0-2% saturation (Pusey *et al.* 2004). Many small native fish can withstand short term exposure to water with less than 1 mg/L.

Macroinvertebrates

Anecdotally, macro-invertebrates appear to be able to withstand water with low dissolved oxygen concentrations (Rees *et al.* 2004) but there are scant experimental or readily accessible observational data to back up these claims.

Turbidity and suspended solids

Suspended solids refer to suspended material present in the water column (Lanigan 1998) and is generally measured gravimetrically. Turbidity, obviously related to suspended solids is a measure of water clarity, and indicates the presence/absence of suspended materials, particularly sediment and fine clays within the water column. It is measured in Nephelometric turbidity units (NTU) or alternatively may be expressed as depth that a sechi disk can still be observed (secchi depths). Abundance of microorganisms such as zooplankton or phytoplankton can also contribute to increased turbidity levels (Crabb 1997). The rivers of the Murray Darling Basin, particularly the Darling River, are naturally turbid, although turbidity has increased dramatically with European settlement. The effect of carp especially exacerbates turbidity problems within the waterways of the Murray Darling Basin through their feeding habits, which stirs up suspended sediments that have previously settled to the riverbed.

Suspended solids can aid the transport of other materials into a waterway via attachment (Lanigan 1998). These materials may include chemicals such as pesticides and herbicides, or nutrients particularly phosphorus and nitrogen.

We could not find any studies conducted within the Murray-Darling Basin that report on the turbidity tolerance of frogs, zooplankton and microcrustacea, algae, cyanobacteria, birds or trees.

Fish

Almost all data available for the turbidity tolerances of fish are observational only. Native fish have been reported in water with turbidity values as high as 680 NTU.

Macrophytes

While no studies have identified turbidity tolerance of native aquatic plants, but there is no doubt that turbidity effects aquatic macrophytes – particularly submerged species. As Reid *et al.* (2007) noted: '(the) sequence of events in the derived record suggests that a period of high abiotic turbidity, leading to a critical reduction in water transparency and caused by widespread erosion during the late 19th century, is the most likely factor contributing to loss of submerged vegetation from this billabong'.

Macroinvertebrates

Again little data exists for tolerance of macroinvertebrates to turbidity. In one of the few exceptions the snail *Potamopyrgus antipodarum* (Hydrobiidae) was observed in waters with suspended solids in the range 1.0-72mg/L.

Dissolved organic carbon (DOC)

Dissolved organic carbon refers to fine organic material from various sources suspended within the water column of an aquatic system. Dissolved organic carbon is linked to dissolved oxygen. Microbial utilization of dissolved carbon can lead to low oxygen tension. Often the two are associated in blackwater events. The severity of a blackwater event will depend on the amount of dissolved organic carbon in the water column and the water temperature; the higher the water temperature the greater likelihood of negative environmental impacts (Howitt *et al.* 2006).

The only data availability on DOC toxicity on aquatic organisms has been some recent work on small native fish.

Fish

Information on tolerance of DOC (as red gum leachate) is only available for 3 species of fish: *Galaxias olidus* (Mountain galaxias), *Hypseleotris klunzingeri* (western carp gudgeon), and *Nannoperca australis* (Southern pygmy perch), where 80-99mg/L DOC caused mortality (McMaster and Bond 2008).

Nutrients

Waterways contain a wide range of nutrients, all of which are essential for the function and health of that waterway. Two of the most important nutrients with regard to water quality are phosphorous and nitrogen.

Phosphorus

Phosphorous is naturally occurring, derived from the weathering of rock and soils. It is important for plant growth, and is also a component in many common household products (toothpaste, detergent etc.) (Lanigan 1998). The water soluble nature of these products means that they often find their way into our rivers, lakes, and wetlands where in excess phosphorous can present a problem for biota. This can occur via direct effects, or through the development of algal blooms as a result of eutrophication (excessive nutrient load). Total Phosphorous (TP) is a measure of the total dissolved and particulate phosphorous present in a sample or waterway (Lanigan 1998).

No toxicity data is available for phosphorous in the MDB. Rather the largest issue is associated with the development of noxious algal blooms. TP values of greater than about 10 µg/L are capable of sustaining a blue-green algal bloom (Oliver and Ganf 2000).

Nitrogen

Nitrates can become troublesome when present in excess in aquatic systems. They are a limiting factor for plant growth and their use as fertilisers in agricultural systems often results in transport to rivers and streams as runoff. Readily available nitrate can drive algal blooms. We could find no Australian studies for the effects of nitrates on amphibia however; non-Australia studies indicate an enormously wide range of effect-concentration data. For these taxa, mortality has been reported to occur from ≤50 mg NO_3/L to over 1000 mg/L with 100 mg/L regarded as a general no effect threshold (Mann *et al.* 2009). Moderate nitrate enrichment can increase amphibian habitat and food resources as aquatic macrophytes and algal communities become stimulated (Mann *et al.* 2009).

Given the natural variability of nitrogen (and phosphorus) across regions of the country, site specific guidelines need to be developed. Region specific guidelines have been developed for Victoria (table 2).

Table 2: Nuisance plant growth. From Rutherford *et al.* 2000.

	Total phosphorus mg/L	Total nitrogen mg/L
Indicative for potential nuisance growth (Australia wide)	0.001 - 0.01	0.1 - 0.75
Vic highland rivers	0.02	0.15
Vic Murray foothills	0.03	0.2
Vic Murray plains river region	0.05	0.6
Vic north-west river plains	0.05	0.9

Agricultural Chemicals

Urban, agricultural and industrial developments have altered the pre-European chemical profiles of Australian soils and waterways, however, there has been very few toxicological studies targeting their effects on Australian fauna. Much of the studies on organisms found in the Murray Darling Basin have focussed on three chemicals – Endosulfan, Chloropyrifos and Glyphosate

Endosulphan

Endosulphan run-off from cotton fields has been recorded exceeding 7 mg/L although overspray contamination has been recorded 10-fold higher (Edge *et al.* 1999). This exceeds biotic tolerances of many organisms. The Australian and New Zealand Environment and Conservation Council (ANZECC 1992) water quality guideline for endosulphan is an order of magnitude lower at 0.01 mg/L. This value however, this is based on non-Australian studies since direct Australian studies are limited (Harford *et al.* 2005).

Difficulties arise when comparing toxicity employing different methodological approaches. 'Water only' exposure is more toxic than treatments added with sediment slurries since sediment is assumed to bind and reduce the bioavailability of the pesticide (Hose *et al.* 2002). Consequently, caution is needed when extrapolating data to the field and extrapolation will need to be tailored to the transport mechanism of the pesticide. For example, air-borne boom spray contamination of water bodies will not be transporting sediment whereas surface run-off will. The level of suspended sediment in the water column will also presumably influence the outcome.

Endosulphan has been suspected with fish kills and is thought to increase the incidence of disease in feral fish populations (Harford *et al.* 2005). It is however less persistent in the environment than other OC's. European carp have lower lethal sensitivities (96-h LC50 of 0.1 µg/L) than the native fish so far tested. For example silver perch and eastern rainbowfish have 96-h LC50 values of 2.4ug/L (Suderam *et al.* 1992).

Sub-lethal endosulfan concentrations are known to reduce the temperature tolerance of silver perch, eastern rainbowfish, western carp gudgeon, and rainbow trout (Patra *et al.* 2007) which has implications as global warming becomes apparent. Immunotoxicological tissue experiments using head kidney cells incubated with endosulphan have found decreased immune function in some native fish due to exposure. The order of sensitivity follows; Murray cod = rainbowfish > golden perch > silver perch (Harford *et al.* 2005).

Endosulfan is generally less toxic to invertebrates than to fish (Chapman *et al.* 1993). Several Australian macroinvertebrate studies have been conducted including a simulated field experiment where community composition was altered after both 12 and 48 hours of endosulphan exposure to 48.87 mg/L and 6.87 mg/L respectively (Hose *et al.* 2003). Consequently short term exposure is likely to cause ecological shifts to aquatic communities. Macroinvertebrate drift can represent an avoidance strategy to escape unfavourable conditions and is only detectable in field based experiments. Interestingly some taxa have been noted to drift at concentrations (6.14 µg/L) that have not disturbed benthic communities (Hose *et al.* 2002).

The extent to which endosulphan poses threat to frog populations is not clear, however many Australian frog species breed during cotton spraying periods (Broomhall and Shine 2003). We found only one Australian study investigating pesticide tolerance (Broomhall and Shine 2003). An endosulphan exposure of 1.3 ug/L over a 96 hr test period caused 17% mortality and decreased growth and feeding of tadpoles while survivors were more vulnerable to predation. These sub-lethal effects highlight that even short term exposure may have long term effects.

Chlorpyrifos

Chlorpyrifos is a broad-spectrum organophosphorus insecticide that has been used in Australia for over 35 years. Used widely for crop, orchard, turf and termite protection Australia's year 2000 level of consumption was 1000 tonnes per annum. It inhibits an enzyme (acetylcholinesterase) involved with nerve impulses at neural junctions and has been long recognised as highly toxic with links to fish kills at several parts per billion (NRA 2000). Its effects on other aquatic organisms are relatively well known with invertebrates, especially crustaceans and larval insects are the most sensitive. The ANZECC and ARMCANZ (2000) water quality guidelines can be been significantly breached with field concentrations reported up to 0.525 mg/L (Humphrey and Klumpp 2000).

Acute toxicity ranges from 0.0013 mg/L for the common carp (*Cyprinus carpio*) to 1.018 mg/L for mosquito fish (*Gambusia affinis*) (Barron and Woodburn 1995). Australian and introduced fish are sensitive to chlorpyrifos at low concentrations with European carp cited as most sensitive (96-h LC50 of 0.1 µg/L). The native eastern rainbow fish and silver perch are moderately more tolerant (96-h LC50 of 2.4 µg/L) (Sunderam *et al.* 1992). Australian guidelines have relied heavily on American and overseas studies. The general expectation is that 1 µg/L represents the lower threshold for native fish which is well above acute LC50s for many invertebrates (NRA 2000).

Little Australian specific data is available but a 21 day exposure at 1 µg/L has resulted in altered macroinvertebrate community structure through significant reductions in species diversity and abundance (Ward *et al.* 1993). In separate studies, individual taxa such as the freshwater shrimp (*Paratya australiensis*) (Olima *et al.* 1997) and (*Daphnia carinata*) (Zalizniak 2006) exhibit tolerance if previously exposed to chlorpyrifos but interestingly in the latter their progeny were more susceptible. Such chronic exposure can result in arying sensitivities across time and space. In such cases the range of known tolerances will be more helpful. Water fleas (*Daphnia carinata*) are extremely abundant in the aquatic environment and are considered an important source of food for juvenile fish. With negative reproductory effects occurring at prolonged exposure to 0.005 µg/L, even low concentrations cannot be considered safe for the aquatic environment (Zalizniak 2006).

It would appear that chlorpyrifos is not toxic to algae in the concentrations that would be encountered in the environment. This is supported by an Australian study of two species of micro-algae show (Zalizniak 2006) and by non-Australian studies for species such as *Anabaena* sp. (Lal *et al.* 1987) and marine algae (Walsh 1983).

Glyphosate

Glyphosate is a herbicide that blocks amino acid synthesis, starving growth in plants and is widely used for weed control. Overall, exposure data for Australian biota is scant with data generated by only a couple of research groups. This includes a good understanding of glyphosate action on frogs and a smaller effort on cladocera and microalgae. The lack of data is thought to reflect the attitude that animals are non-target organisms of the herbicide but it is now known that glyphosate can affect aquatic life at concentrations found in the environment.

It has a relatively low toxicity to terrestrial animals, while fish and aquatic invertebrates are considered more sensitive. It is the surfactant (polyoxyethylene amine) however, incorporated in commercial formulations the primary toxic agent (Zalizniak 2006). It will be important to distinguish the formulation of glyphosate in tolerance assumptions as Australian studies with frogs reveals a great variety in toxicities. For tadpoles, Roundup® Herbicide was found the most toxic of the glyphosate formulations at concentrations of between 2.9 and 11.6 mg/L glyphosate acid equivalent (Mann and Bidwell 1999). This was dependent on species tested. Other formulations such as Roundup® Biactive were found practically non-toxic with 48-hr LC50 values between 400 – 1,111 mg/L.

Its persistence in the aquatic environment for up to ten weeks has increased significance for short life cycle aquatic organisms such as microinvertebrates. This could have implications over several breeding seasons by gradually reducing populations over short periods of time.

Heavy Metals

Heavy metals are natural elements that occur in nature, although the levels of these metals in a given ecosystem can be increased by external inputs. Naturally metals are freed from the earths crust over time through both physical and chemical weathering. Externally, metals can enter waterways via industrial effluent, leaching, attached to sediment during a storm event or via a whole host of other routes (Anonymous. Metal Contaminants Webpage www.ozcoasts.org.au/indicators/metal_contaminants.js, accessed 19th Nov 2009). In trace amounts, many metals are essential to the normal function of plants and animals alike. However, in larger amounts, heavy metals can be toxic, and may accumulate in the body of an organism (Anonymous. Aquatic Ecosystem Health webpage /www.wsroc.com.au/wqm/ae_metals_in_water.html, accessed 19th Nov 2009). A brief description of the properties and effects of few of the heavy metals present within the Murray Darling are discussed hereafter with specific examples.

Mercury (Hg)

In the past, mercury compounds have been used in fungicides and antimicrobial mixtures, which are of course now prohibited due to the highly toxic nature of the metal. Mercury poisoning can occur through inhalation, ingestion, or through absorption via the skin (Sherwood 1987).

There have been no studies conducted within the Murray-Darling Basin that we have found that report on the mercury tolerance of frogs, fish, macroinvertebrates, zooplankton and microcrustacea, algae, cyanobacteria, macrophytes or trees.

Birds

The information available for birds concerns only the concentration of mercury found in the wings of sampled birds, not their tolerance per se. it was found that the levels of mercury (1984) in the wing tissue were not likely to cause any reproductive or behavioural changes (Bacher and Norman 1984).

Aluminium (Al)

Aluminium is the worlds most common metal (Sherwood 1987; Howells 1994). In its elemental form it is silvery white in colour (Howells 1994), and it is a component of most clays (Sherwood 1987). Despite its prevalence, it is generally present in low concentrations in freshwater. This is largely due to the fact that aluminium has very low solubility within the 'normal' pH range (5 to 8). Outside of this range aluminium's solubility increases markedly. Aluminium compounds are used in potable water treatment, and if a large amount of this treated water is flushed into a waterway biota are simultaneously exposed to high levels of aluminium and low pH, as release of these concentrated solutions decreases the pH of a water body (Howells 1994).

There have been no studies conducted within the Murray-Darling Basin that we have found that report on the aluminium tolerance of frogs, birds, fish, macroinvertebrates, zooplankton and microcrustacea, algae, cyanobacteria, macrophytes or trees.

Fish

Aluminium particles interfere with oxygen transfer across the gills in fish. This has an interactive effect with pH, as elevated acid conditions (lowered pH) results in elevated dissolved aluminium levels (Rees *et al.* 2004).

Cadmium (Cd)

Cadmium is a highly toxic metal, especially if one is exposed via inhalation. It is used extensively in electronics, and also cadmium plating. Most cadmium in the environment results from effluent from the latter, and has been known to poison biological sewage treatment plants (Sherwood 1987).

Macroinvertebrates

Khan and Nugegoda (2007) found that the 72 hour LC_{50} of the freshwater yabbie *Cherax destructor* was 673µg/L . The 96 hour LC_{50} of the freshwater hydra *Hydra viridissima* was 3µg/L with 0.8 µg/L found to be the lowest observed effect concentration (LOEC), and 0.4 µg/L produced no observable effect (NOEC). Another species of the same genera, *Hydra vulgaris,* had an 96 hour LC_{50} of 83 µg/L; 12.5 µg/L LOEC, and less than 12.5 µg/L was the NOEC (Holdway *et al.* 2001).

Over a much longer timeframe, 42 days, *Proisotoma minuta* of the family Isotomidae was exposed to 65µg/g (±24) and 125µg/g (±12) of cadmium before a change in reproductive behaviour was observed in 50% and 10% (EC_{50}, EC_{10}) of test subjects respectively (Nursita *et al.* 2005).

Copper (Cu)

Copper is essential in the synthesis of chlorophyll in plants. It is also found in a number of enzymes including the ubiquitous cytochrome c oxidase.

However, copper is toxic in large amounts. So much so that copper sulphate ($CuSO_4$) is used in Bordeaux mixture (fungicide) (Sherwood 1987).

Fish

Fish abundance was significantly reduced in a stream receiving a 'copper enriched' effluent (Pusey *et al.* 2004)

Macroinvertebrates

As with cadmium, the lethal and sublethal effects of copper on *Cherax destructor*, two hydra species, and *Proisotoma minuta* were investigated. The LC50 of test subjects of the species *Cherax destructor* was 509µg/L of copper over 72 hours (Khan and Nugegoda 2007). The LC50 of *Hydra viridissima* was 26 mg/L (±3.4) over 96 hours, while the LC50 of *Hydra vulgaris* was 8.5 mg/L (±0.3) over 96 hours (Pollino and Holdway (1999). Over 42 days, *Proisotoma minuta* was exposed to 209µg/g (±61) and 696µg/g (±154) of copper before a change in reproductive behaviour was observed in 50% and 10% (EC50, EC10) of test subjects respectively (Nursita *et al.* 2005).

Zooplankton and microcrustacea

The 48 hour LC50 of two daphnia species are available for the Murray Darling Basin. The LC50 values of of *Ceriodaphnia dubia* and *Daphnia carinata* were approximately 18 µg/L and 40µg/L respectively (Cooper *et al.* 2009)

Nickel (Ni)

Nickel is used in several industries including electroplating, as a component of alkaline batteries, and is used in steels and ceramics (Sherwood 1987; Howells 1994). Nickel is present in emissions from the combustion of fossil fuels, and can be found in runoff from roads. It is easily absorbed onto clay minerals, and thus could be prevalent in the sediment of an aquatic system.

Due to the sediment binding nature of the element, it is difficult to determine how much of the total nickel in a system is available for toxicological processes, which in turn makes it difficult to determine the true levels of nickel needed for a toxic effect (i.e. the toxicity of nickel). There has been little study on the effects of nickel on biota, but it has been found that nickel can interrupt protein metabolism and enzyme function (Howells 1994).

There have been no studies conducted within the Murray-Darling Basin that we have found that report on the nickel tolerance of frogs, birds, fish, zooplankton and microcrustacea, algae, cyanobacteria, macrophytes or trees.

Macroinvertebrates

Khan and Nugegoda (2007) found that 50% of test subjects of the crayfish species *Cherax destructor* were able to withstand a concentration of 71mg/L (61-84) of nickel over 72 hours (72h LC50).

Iron (Fe)

Iron is an important element in the functioning of aquatic ecosystems. Fe plays an important role in the cycling of P, N and S in aquatic ecosystems. It is also important in many biochemical processes, not the least as the oxygen carrier in haemoglobin. The only substantive study on iron toxicity to aquatic organisms in the MDB was on the yabby, *Cherax destructor*. The 72 hour LC_{50} for *C. destructor* was 468mg/L (Khan and Nugegoda, 2007).

Lead (Pb)

Lead is highly toxic, and lead compounds are readily absorbed by the body. Ten percent of ingested lead enters the blood stream, and 90 percent of this then builds up in the bones and tissues (Sherwood 1987). This is the main reason that lead is such a concern regarding bioaccumulation.

Zooplankton and microcrustacea

As with copper, the lead 48 hour LC50 of two daphnia species are available for the Murray Darling Basin. The LC50 values of *Ceriodaphnia dubia* and *Daphnia carinata* were about 200µg/L (160.1–272.2) and 450 µg/L respectively (Cooper *et al.* 2009). Over 92 hours, the LC50 of the ostracod *Diacypris compacta* was 3.1mg/L of lead (Brooks *et al.* 1995).

Zinc (Zn)

Zinc is the fourth most common metal in industrial use. In nature, it is an essential trace element. Zinc deficiency can cause leaf disease in trees, and it plays an important part in carbon dioxide (CO_2) metabolism in animal tissues (Sherwood 1987). Of all the heavy metals present in the Murray Darling Basin, there is by far the most toxicology information available for zinc.

Macroinvertebrates

The LC50 of *Hydra viridissima* was 935µg/L over 96 hours, 75µg/L was found to be the lowest observed effect concentration (LOEC), and 38µg/L produced no observable effect (NOEC). Another species of the same genera, *Hydra vulgaris,* had an LC50 of 2300 µg/L over 96 hours, 500µg/L LOEC, and 250µg/L was the NOEC (Holdway *et al.* 2001).

Over 42 days, *Proisotoma minuta* was exposed to 283µg/g (±70) and 61µg/g (±80) of zinc before a change in reproductive behaviour was observed in 50% and 10% (EC_{50}, EC_{10}) of test subjects respectively (Nursita *et al.* 2005). Perhaps not surprisingly given that zinc is an essential trace element, both the hydra species and *Proisotoma minuta* could withstand much higher levels of zinc than cadmium.

The zinc tolerance of the snail *Velesunio ambiguous* as determined by Millington and Walker (1983) was an LC_{50} of 66 ± 11 mg/L of zinc over 336 hours (14 days).

Zooplankton and microcrustacea

As with copper and lead, the zinc 48 hour LC50s of two daphnia species are available. The LC50 values of *Ceriodaphnia dubia* and *Daphnia carinata* were approximately 175 µg/L and 350 µg/L respectively (Cooper *et al.* 2009).

Over 92 hours, the LC50 of the ostracod *Diacypris compacta* was 2.1mg/L of zinc (Brooks *et al.* 1995).

Trees

Reichman *et al.* (2001) demonstrates that trees, using *Acacia holosericea* (Silver leaf wattle), *Eucalyptus camaldulensis* (River Red Gum), and *Melaleuca leucadendra* (Weeping Paperbark), have both an upper and a lower limit for zinc tolerance. Too little can cause cholorosis of the leaves and slight necrosis, and on the other hand, too much can trigger the development of collraoid roots, which is not ideal for the long term health of trees.

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