# Science for Saving Species

Research findings factsheet Project 3.3.7



# The impact of nitrate pollution in waterways on freshwater species

# In brief

Nitrate pollution in freshwater environments is caused by runoff from fertilisers, urban waste and debris. Despite evidence of the negative effects of nitrate on aquatic species, it is considered to be relatively non-toxic. Yet after heavy rain, it can found in concentrations up to 100 times the current safe guideline of 50 mg/l.

We investigated how nitrate interacts with other environmental stressors (acidity, high temperatures and low oxygen) to impact freshwater fish and crustaceans through a series of tank and flume experiments on three common native species.

We found that exposure to nitrate makes freshwater species more susceptible to the other stressors, and likewise that these other stressors make animals more susceptible to nitrate toxicity.

We found that fish exposed to high levels of nitrate grew less and swam slower than fish not exposed to nitrate. We also found that nitrateexposed crayfish had reduced claw strength. Of even greater concern is that exposure to nitrate reduces the capacity of fish to tolerate high temperatures, acidic conditions and low oxygen levels.

We recommend that the guideline for nitrate concentration in freshwater be reduced to less than 50 mg/l. Nitrate pollution should also be limited during summer months when heatwaves and heavy rains causing runoff are more frequent, and in waterways that suffer from low oxygen conditions.



The Fitzroy River, Queensland, some parts of which suffer from increased nitrate concentrations due to anthropogenic causes. Image: Craig Franklin

#### Background

Human activity has significantly altered freshwater environments in Australia and worldwide. This exposes aquatic species such as freshwater fish, amphibians and small crustaceans like shrimps and yabbies to pollution, invasive species and climate change. These stressors often create negative effects for freshwater species that are more severe in combination than in isolation.

The runoff from fertilisers, urban waste and debris causes nitrate pollution in waterways. Currently, nitrate guidelines are set at 50 mg/l. This level was established based on the level at which half a population of a species will die. However, despite its documented impacts on aquatic fauna, nitrate is considered to be relatively nontoxic. Yet, following heavy rain nitrate concentrations can be up to 100 times higher than the guideline level. Freshwater species like fish, amphibians and crustaceans can take up these high concentrations of nitrate, with impacts to their health and behaviour even if they are not killed. Furthermore, exposure to high levels of nitrate can make freshwater species more susceptible to other human-induced threats, such high temperatures, low oxygen levels and acidity.





BELOW: Juvenile silver perch being measured after being exposed to high nitrate plus current-day summer temperatures (28°C, left) and nitrate plus elevated temperatures (32°C, right). Fish exposed to 32°C were significantly smaller than fish maintained at 28°C. Image: Daniel Gomez Isaza

#### Background (continued)

Fish and crayfish are known to use physiological compensation strategies in response to elevated levels of nitrate and other stressors and these strategies potentially impact fitness and survival.

Understanding how nitrate interacts with other stressors is important for managing and conserving species that live in environments degraded by human activity. It is also important for aquaculture, where nitrate concentrations can be particularly high, often exceeding 500 mg/l.

#### **Research** aims

We investigated how nitrate interacts with the other environmental stressors of acidity (low pH), high temperatures and low oxygen to impact the physiology and behaviour of freshwater species of fish and a species of crustacean, and the implications for fitness and survival.





#### What we did

We selected three native freshwater species of the Murray–Darling Basin including two fish and a crayfish: spangled perch (*Leiopotherapon unicolor*), silver perch (*Bidyanus bidyanus*) and the common yabby (*Cherax destructor*).

We measured the species' aerobic capacity at different levels of nitrate concentration and in combination with different water temperature, oxygen and pH levels. We kept the species in tanks exposed to different levels of nitrate for at least four weeks to simulate long-term exposure. We then tested aerobic capacity by swimming fish in flume until fatigue, and testing the claw strength of yabbies by measuring the force produced by the closing of the claws under combinations of the following conditions:

- Nitrate concentrations at 0, 50, and 100 mg/l. Concentrations above 50 mg/l were considered high.
- Temperatures from 28°C to 38°C. Temperatures above 28°C were considered high.
- Oxygen concentrations between 2 mg/l and 8 mg/l.

Concentrations below 5.6 mg/l were considered low.

• Acidities from pH 4.0 to pH 7.0. Below pH 5.0 was considered acidic.

Aerobic capacity is the amount of energy available for activities that require oxygen – like growth, swimming performance (for fish) and claw strength (for crustaceans).

Swimming performance is a good measure of a fish's aerobic capacity, as it involves the workings of many organs of the body. It has also been shown in prior research to be reduced by elevated levels of nitrate, and to be sensitive to other stressors and combinations of stressors.

When fish are faced with low oxygen conditions, they swim to the surface of the water to try to take up oxygen from the atmosphere. We tested how exposure to nitrate affects the surfacing behaviour of fish when exposed to low oxygen conditions. Surfacing behaviour was assessed by placing fish in a tank and then progressively lowering the oxygen level of water.

## Key findings

The findings of this study advance our understanding of how stressors interact, specifically, how nitrate toxicity is influenced by other stressors in freshwater environments to harm aquatic species.

Our first important finding was that fish exposed to high concentrations of nitrate showed lower rates of growth and could not swim as quickly as fish that had not been exposed to high nitrate levels. Aquatic animals living under high levels of nitrate face effects that may be deadly and, even when they do not kill immediately, will reduce growth, lower activity levels and reduce survival. The threats apply from life stages from larvae to adult, and affect fish and crustaceans to a similar degree.

An even more important finding was that when fish are exposed to high levels of nitrate it reduces their ability to tolerate high temperatures, acidic (low pH) conditions and low oxygen levels.

Nitrate and high temperatures: Fish exposed to both elevated temperatures and elevated nitrate levels at the same time showed reduced aerobic capacity and poorer swimming performance. Nitrate exposure also lowered the capacity of fish to tolerate heat wave conditions.

Nitrate and low pH: Exposure to both nitrate and low pH caused steep declines in growth, swimming performance and claw strength.

Nitrate and low oxygen levels: Fish exposed to high levels of nitrate are more susceptible to the ill effects of periods of low oxygen. Exposure to nitrate also forced fish to move to the surface of the water sooner when exposed to low oxygen conditions, and this behavioural strategy to avoid hypoxia may reduce survival.

Our findings show that looking at nitrate in isolation greatly oversimplifies and underestimates its effect on aquatic animals. The combined effects of two stressors tends to be greater than the effect of one stressor in isolation. Nitrate interacts with numerous other stressors such as pH levels, UV-B radiation, oxygen levels, herbicides and pesticides that can amplify the toxic effects of nitrate with potentially unimagined consequences.

We found that nitrate plus low pH and high temperatures tended to reduce the aerobic capacity of species, with the effects varying depending on the levels or intensity of the stressors. This is because animals suffering from nitrate toxicity will divert energy away from aerobic activities like growth and fast swimming towards cellular repair and regulating their bodily functions. Under moderate levels of nitrate stress, long-term persistence is possible, but trade-offs in how an individual spends energy (or conserves it) can reduce its fitness and survival.

In response to high levels of nitrate in combination with other stressors we observed fish and crayfish make compensatory changes to their behaviour and physiology, which could help them cope with poor water conditions. Behaviourally, we found that nitrate-exposed fish avoided low oxygen conditions sooner by coming up to the surface of the water, but this behavioural strategy can make fish more prone to predation. When exposed to high temperatures, fish underwent physiological changes that improved their aerobic capacity, by growing larger hearts and larger gills, but these changes were not enough to overcome the negative effects of high temperatures and nitrate exposure on growth and perform fast swimming. Overall, we found that species living in degraded environments can sometimes respond to the presence of multiple stressors via various kinds of compensations - behavioural, physiological and adaptive changes. Although these compensation strategies can be beneficial in the short-term, in the long-term, fish and crayfish exposed to multiple stressors are likely to have reduced fitness and survival due to increased risk of predation and reduced fertility.



# Cited material

Gomez Isaza, D. (2020). Anthropogenic disturbances to freshwater taxa: Interactions between nitrate and additional stressors on various physiological traits. PhD thesis, The University of Queensland. DOI: 10.14264/ea9709d.

## Implications and recommendations

The results provide key information for improving the management of threatened freshwater species of the Murray-Darling Basin.

In the Murray–Darling Basin climate change is predicted to increase temperatures and the frequency of heat waves. Our study found that fish exposed to elevated temperatures and elevated nitrate levels at the same time showed poorer swimming performance.

In parts of the Murray–Darling Basin there are extreme fluctuations in water pH due to the presence of acid sulphate soils. Our study found exposure to both nitrate and low pH caused steep declines in growth, swimming performance and claw strength.

The Murray–Darling Basin faces periods of low oxygen in areas of high agricultural pollution. Fish exposed to high levels of nitrate are more susceptible to the ill effects of periods of low oxygen, and show behavioural strategies to avoid hypoxia that may reduce survival.

Our findings show that looking at nitrate in isolation greatly oversimplifies and underestimates its effect on aquatic animals. The combined effects of two stressors tends to be greater than the effect of one stressor in isolation. Nitrate interacts with numerous other stressors such as pH levels, UV-B radiation, oxygen levels, herbicides and pesticides that can amplify its toxic effects with potentially severe consequences. We recommend that concentrations of nitrate in fresh water be kept low, that is, below the current recommendation of 50 mg/l. Although there was not high mortality of individuals at this nitrate level, we nevertheless measured impairments to physiology and behaviour that are likely to reduce fitness and survival. We recommend that the nitrate guidelines therefore be changed to address levels that result in nitrate poisoning as demonstrated in this study rather than simply lethal levels.

Also, we recommend that when assessing levels of nitrate toxicity, both the short- and long-term effects of nitrates on aquatic animals need to be taken into account. Shortterm testing of toxicity is unlikely to show the full impact of exposure to nitrate. Indeed, nitrate toxicity increases with longer exposure, so that short-term exposure may appear harmless. A priority should be placed on managing environments that experience sustained periods of nitrate pollution.

To prevent large-scale mortality of aquatic species, it is especially important for nitrate pollution to be limited during the summer months when temperatures and heat waves peak and heavy rains are more frequent. This applies most particularly to waterways that show low levels of oxygen. Waters high in nitrate also should be supplemented with additional aeration devices during peak summer months or following large runoffs of debris which deplete water of oxygen as they break down.

Nitrate concentration guidelines need to be more stringent in areas of the Murray–Darling Basin that are affected by acid sulphate soils and waterways receiving acidic effluent.

Finally, this and other studies so far have examined how aquatic animals respond to exposure to constant levels of stressors at the same time. However, this does not reflect the reality of most environments, where stressors vary dynamically. Future work could explore how freshwater species respond to variations in the timing (daily, seasonal) and the magnitude of environmental stressors. Also, future work could look at how species respond to removal of a stressor. It is currently unknown, for example, whether removing nitrate would allow aquatic animals to better cope with other stressors like low pH levels. A shift in focus of future research towards more realistic experiments on wild or semi-wild animals, such as sampling natural fish populations along a nitrate pollution gradient or among similar streams could also go some way to closing current gaps in our knowledge.

# **Further Information**

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