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## Mitigating and Managing Barriers to Fish Passage and Improving River Connectivity Final Report

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All fish need to move for breeding and reproduction, to find food resources, to access new territories and to maintain good genetic diversity. Barriers to fish movement have resulted in a substantial decline in freshwater fish populations Australia-wide. Reconnecting fragmented waterways is essential to restoring freshwater biodiversity. Image: Ed Meyer

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Cover image: Small concrete culverts, like this one in south each Queensland, are an ubiquitous part of waterway infractructure in Australia. Culverts can pose substantial barriers to fish movement by creating velocity barriers, disrupting water movements and altering light levels. Image: Ed Meyer

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Mitigating and Managing Barriers to Fish Passage and Improving River Connectivity - Final Report 3

### **Executive summary**

Man-made barriers to fish passage (e.g. dams, weirs and road crossings/culverts) have contributed to major declines in native fish numbers, with > 5,000 barriers to fish migration occurring in NSW alone. For this reason, barriers to fish movement can lead to population fragmentation and decline even in species not generally considered migratory. Given the pervasive impact of barriers on freshwater fish populations, considerable effort is being invested in developing remediation solutions, yet the efficacy of these designs for Australian small bodied fish remains largely untested. This project took an integrative, experimental approach to understanding and remediating a suite of barrier types for Australian native fish. Firstly, we undertook a comprehensive assessment of fish swimming performance to construct a database of performance characteristics to inform the revision of NSW DPI Fisheries Fish Passage Guidelines. Secondly, we explored the effects of a suite of culvert baffle designs to understand their potential negative impact on the upstream movement of small-bodied fish species. Thirdly we developed a novel longitudinal beam structure to improve fish movement through culverts. Fourth, we explored the effects of acute temperature reductions consistent with cold water pollution (CWP) events on the physiological performance of five species of Australian native fish and three species of invasive fish, and finally, we examined the effects of a non-physical anthropogenic (chemical) barrier, nitrate pollution, on fish performance.

Road crossings and culverts are arguably some of the most pervasive barriers to fish passage in Australian waterways. In order to address fish passage requirements for Australian fish species, we need a better understanding of the performance limitations that Australian fish operate under. We measured the swimming performance capacities of 22 species of Australian small bodied fish species and juveniles of larger bodied species using a standardised set of criteria. We found that the swimming performance of most Australian fish was weak relative to many high-performing north American fish species (i.e. salmonids). Across all species, body size was a robust predictor of swimming. The current NSW culvert water velocity guideline of 0.3 m s<sup>-1</sup> for fish passage was found to be appropriate for all but the two weakest species (purple spotted gudgeons and juvenile freshwater catfish) in our dataset. We also assessed how fish body size affected the relationship between water velocity and water depth on fish swimming performance and endurance capacity. We found that there was a strong non-linear relationship between water velocity, depth and transverse success, however the precise relationship between these factors was strongly species- and body size-specific. This data will assist civil engineers and fisheries managers in Australia to mitigate the impact of in-stream structures on local fish populations.

Efforts to remediate the negative effects of high water velocities in culverts for fish passage have led to the adoption of vertical baffles in serval Australian jurisdictions, however their efficacy for Australian small-bodied fish has not been well elucidated. We examined a suite of baffle designs on upstream fish movement in a 12 m, near full scale channel. We found that in all cases, small fish were more likely to successfully traverse the channel when no vertical baffle structures were present, suggesting that vertical baffles may be ineffective for facilitating passage in many small bodied Australian fishes. We also developed a novel culvert remediation design that will allow the 0.3 m s<sup>-1</sup> guideline to be met in culverts without substantially altering the discharge capacity of the culvert. The longitudinal beam design sits on the lateral wall of the culvert, just above the base and creates a small, low velocity zone in the space beneath the beam. This design was effective at improving the transverse capacity of 6 species of small bodied native fish species at bulk channel velocities higher than the 0.3 m s<sup>-1</sup> guideline. Longitudinal beams may allow infrastructure managers to provide fish passage without the engineering and economical constraints of meeting the 0.3m/s guideline across the entire culvert.

Nonphysical barriers to fish passage can be substantial, albeit be less obvious, consequences of large, instream structures. Cold water released from the base of thermally stratified dams can pollute downstream waters with unseasonably cold water that can compromise the physiological capacity of exposed fish. Our study found that realistic, acute 10°C water temperature reductions resulted in a substantial decrease in native fish performance, which for most species, did not change markedly over an ensuing 8-10 week acclimation period. The rate of temperature change also did not seem to influence thermal acclimation capacity (i.e. the ability to thermally compensate for the depressive effects of low temperatures). While the same drop in performance with cold water exposure was observed in two invasive fish species (carp and Gambusia), they had a greater ability to physiologically compensate for the low water temperatures than many of the native species. We are continuing to analyse our final datasets to determine whether there is a minimal acceptable downstream water temperature that asset owners can attempt to achieve to minimise severe impacts on native fish populations.

Anthropogenic nutrient pollution, including nitrate, is regarded as one of the most common and widespread forms of habitat degradation, and which can have significant impacts on fish physiological performance. Aquatically respiring animals (e.g. fish, amphibians, crustaceans) are particularly vulnerable to elevated nitrate concentrations. Our work showed that chronic sublethal exposure to nitrate levels at the current environmental guideline of 50 mg/L or above can impair energy homeostasis and aerobically supported traits (e.g. swimming, righting, growth) due to disruptions in blood-oxygen carrying capacity. This study indicates that although the current nitrate guidelines were not outright lethal to Australian native fish and crayfish, these exposures could have unintended sublethal consequences for native fauna and future ecotoxicological studies should consider the significant impacts of sublethal exposures on animals.

Artificial barriers to fish movement have contributed to worldwide freshwater fish population declines. This project demonstrated that conventional experimental physiology approaches provide simple, cost-effective suite of tools to assess the behavioural and physiological capacities of fish, which can inform 'fish-friendly' policies and support remediation practices.

## **General introduction**

Freshwater environments are widely regarded as some of the most highly threatened ecosystems on the planet. Anthropogenic competition for, and misuse of, aquatic resources through excessive waterway regulation, unsustainable water extraction practices, habitat destruction, and pollution has led to a decline in freshwater fish numbers and species. For this reason, barriers to fish movement such as instream structures, altered flow regimes, water extraction practices, and pollution have led to population fragmentation and decline even in non-migratory species (Paul and Meyer, 2001, Gibson et al., 2005, Harris et al., 2017). These changes to water flow or water quality become barriers to fish movement when they: (1) physically block fish movement up or down stream; (2) alter the hydrodynamic conditions within a structure such that they impede and exceed fish swimming capacities, or disorient and confuse fish; (3) reduce the behavioural motivation of fish to move into the modified environment; or (4) affect the capacity of fish to perform at an optimal level of swimming performance. In the Murray Darling Basin (MDB) there are an estimated 10,000 physical barriers to fish passage caused by dams, weirs, culverts, locks and barrages (Baumgartner et al., 2014). Unsurprisingly, current estimates suggest that native fish populations in the MDB are at 10% of pre-European numbers (Baumgartner, et al., 2014).

Experimental approaches can provide a controlled, iterative, integrative and cost-effective approach to assess the physical, physiological and behavioural limitations of fish that can be used to provide evidence-backed information to support or develop remediation practices. Unlike traditional impact assessments that utilise metrics with long lag-times such as recruitment success and population abundance, physiological responses to environmental change are often rapid, and can be easily incorporated as part of a holistic suite of approaches to provide critical insight into how and why specific environmental changes can be problematic for fish (i.e. establishing cause and effect, Seebacher and Franklin, 2012). Empirical experimental fish swimming performance studies, for example, can provide important baseline data that can be used by managers to guide the design of fish passage structures, or to provide evidence-backed advice on appropriate water use practices to limit impacts on fish populations (Cooke et al., 2017a).

In this project we took an experimental approach to improving connectivity for fishes in freshwater habitats. In particular, our research directly targeted two threatening processes for fish in the MDB: (i) Instream structures and other mechanisms that alter natural flow; and (ii) alteration to the natural temperature regimes of rivers and streams. Threatened species that are known to migrate over meso (100 km) and macro (100's km) scales and likely to benefit from the outputs of this project include Silver Perch (Critically Endangered - EPBC Act 1999), Trout Cod (Endangered - EPBC Act 1999), Murray Cod (Critically Endangered - IUCN; Vulnerable – EPBC Act 1999), Eel-tailed Catfish (MDB Population: Endangered- New South Wales Fisheries Management Act 1994, Threatened- Victorian Flora and Fauna Guarantee Act 1988, Protected - South Australian Fisheries Management Act 2007), Agassiz's Glassfish (endangered Population - New South Wales Fisheries Management Act 1994), and Macquarie Perch (Endangered - EPBC Act 1999). Additionally, the Lowland Darling River (including the Barwon, Border, Gwydir, Namoi, and Macquarie Rivers), Lachlan River, and Murray River (including the Murrumbidgee River) are listed as Endangered ecological communities under the New South Wales Fisheries Management Act 1994. The project's aims were to provide fundamental information on the movement physiology and behaviour of key freshwater species which can be used to assess the susceptibility of native fish in response to altered environmental conditions as well as influencing the invasion potential of introduced species. We aimed to develop reliable fish passage management tools which integrate fish swimming performance with bio-hydrodynamics to provide recommendations that will assist with the development of guidelines for managing fish connectivity within Australian waterways. These data will be used to better inform mitigation measures and management advice to improve fish passage and waterway connectivity.

## Swimming performance traits of twenty-two Australian fish species

#### Introduction

Road crossings and culverts are arguably some of the most pervasive barriers to fish passage in Australian waterways. Culverts can restrict fish movement by generating high water velocities in the structure, by creating behavioural barriers (low light, no vegetation or naturalistic structure) and by altering the natural hydrodynamic flow and turbulence regimes around the structure. Selecting a specific culvert design for a particular site is often complicated by the size of the barrier, site-specific constraints such as hydrology and gradient, and the ecology, behaviour and physiology of the fish that need to be passed (Palmer et al., 2007, Lapointe et al., 2013). Consequently, the implementation of a particular culvert design is a complex task, requiring the balancing of civil requirements with that of the local fish species while operating within budgetary constraints (Hyde, 2007).

Ultimately the, biology needs to drive the design of 'fish-friendly' structures. The key to providing effective fish passage through instream structures, is to ensure that the water velocities within them do not exceed the swimming capacities of the local fish species. Fish swimming performance studies can provide important baseline data that can be used by managers to guide the design of fish passage structures, or to provide advice on appropriate water use practices to limit impacts on fish populations (Cooke et al., 2017b). The performance of fish within fish passage structure is likely to be highly context-specific, and fish may use a variety of swimming gaits and behaviours to traverse a structure. The use of multiple performance tests that incorporate both volitional and non-volitional measures provides a more holistic assessment of the performance range of a species. Swimming performance data for native Australian fish is sparse and what does exist has been collected using different protocols and equipment, and so direct comparison across species is complicated. As a result, guidelines for the development of fish friendly culverts and road crossings are limited by a lack of understanding of the hydrological requirements of native fish species.

As an aid for fisheries managers in Australia and to inform fish passage guidelines, **we quantified the swimming performance of twenty-two native fish species (Watson et al., 2019).** Our focus was primarily on small-bodied species, and juveniles of larger growing fish species since these are the most vulnerable to the velocity challenges caused by man-made in-stream structures due to body size-dependent swimming performance capacity. Our study included 16 small-bodied species and juveniles of six species that are large bodied as adults

#### Methodology

We chose twenty-two native Australian fish species that span a range of body sizes, age classes, body and tail shapes, and behavioural and ecological traits (Table 1). The majority of fish were sourced from local commercial hatcheries, however, *R. semoni, M. duboulayi, P. signifier P. grandiceps, G. olidus* and *R. bikolanus* were wild-caught from south east Queensland waterbodies. We quantified the maximal sustainable swimming speed (Ucrit) and sprint speed (Usprint) Ucrit using a 185 L flow controlled recirculating flume (Loligo, Tjele, Denmar) (Figure 1A). We also measured fish endurance capacity and traverse capacity in a hydraulic channel (12 x 0.5 x 0.25 m) (Figure 1B,C). Water temperature was held consistent at  $25 \pm 1$  °C for all tests. Fish were swum individually, and all fish were introduced to the flume and left for five min to recuperate from handling stress prior to the start of each test. For *U*crit and *U*sprint tests, water velocities were increased by 0.05 m s<sup>-1</sup> every 5 min (*U*crit) or 10 sec (*U*sprint) until the fish fatigued. Endurance tests involved fish being swum at a fixed velocity for up to 60 min. Initial velocities were within 0.05ms<sup>-1</sup> of the species' mean *U*crit value, with the other two velocities chosen depending on how the fish performed at this initial velocity.



Figure 1. (A) Loligo<sup>M</sup> 185L recirculating swimming flume; (B) two 12 x 0.5 x 0.25m hydraulic channels used for endurance and traversability metrics; (C) stylised representation of hydraulic channels (from Shiau et al 2020).

Scientific name	Common name	Common adult size (mm)	Preferred flow environment	Migration classification	Movement distance <sup>†</sup>	Body shape	Tail shape
Ambassis agassizii	Olive perchlet	60	Slow	Potamodromous	Micro	Compressiform	Forked
Bidyanus bidyanus	Silver perch	300	No preference	Potamodromous	Macro	Compressiform	Emarginate
Craterocephalus stercusmuscarum	Fly-specked hardyhead	55	Slow	Potamodromous	Meso	Fusiform	Emarginate
Galaxias brevipinnis	Climbing galaxiid	100	Slow	Potamodromous	Meso	Sagitiform	Truncate
Galaxias olidus	Mountain galaxiid	100	Slow	Potamodromous	Micro	Sagitiform	Truncate
Hypseleotris compressa	Empire gudgeon	80	No preference	Amphidromous	Meso	Compressiform	Rounded
Hypseleotris galii	Firetail gudgeon	35	Slow	Potamodromous	Micro	Compressiform	Rounded
Leiopotherapon unicolor	Spangled perch	300	No preference	Potamodromous	Meso	Compressiform	Truncate
Maccullochella peelii	Murray cod	700	No preference	Potamodromous	Meso	Compressiform	Rounded
Macquaria ambigua	Golden perch	500	No preference	Potamodromous	Macro	Compressiform	Truncate
Melanotaenia duboulayi	Crimson-spotted rainbowfish	80	Slow	Potamodromous	Micro	Compressiform	Emarginate
Melanotaenia fluviatilis	Murray river rainbowfish	85	Slow	Potamodromous	Micro	Compressiform	Emarginate
Mogurnda adspersa	Purple spotted gudgeon	80	Slow	Potamodromous	Meso	Compressiform	Rounded
Nannoperca australis	Southern pygmy perch	60	Slow	Potamodromous	Micro	Compressiform	Rounded
Percalates novemaculeata	Australian bass	400	No preference	Catadromous	Macro	Compressiform	Emarginate
Philypnodon grandiceps	Flathead gudgeon	80	Slow	Amphidromous	Micro	Depressiform	Rounded
Pseudomugil signifer	Pacific blue-eye	40	No preference	Amphidromous	Micro	Fusiform	Emarginate
Redigobius bikolanus	Speckled goby	40	Slow	Amphidromous	Meso	Compressiform	Rounded
Retropinna semoni	Australian smelt	60	Slow	Amphidromous	Micro	Fusiform	Forked
Rhadinocentrus ornatus	Ornate rainbowfish	40	Slow	Potamodromous	Micro	Fusiform	Emarginate
Tandanus tandanus	Eel-tailed catfish	450	No preference	Potamodromous	Meso	Compressiform	Pointed
Trachystoma petardi	Freshwater mullet	400	Slow	Catadromous	Macro	Fusiform	Emarginate

 Table 1. Physical and behavioural characteristics of 22 Australian freshwater fish species. All fish were < 15cm TL.</th>

<sup>†</sup>Adapted from Mallen-Cooper and Zampatti (2012) where micro < 100 m, meso is 100s m to 10s km, and macro is 100s km.

#### Results

Of the 22 examined species, *Retropinna semoni* (pacific blue eyes) had the fastest body length-corrected mean *U*crit and *Usprint*, while *Morgunda adspersa* (southern purple spotted gudgeon) had the slowest mean *U*crit and *Tandanus tandanus* (freshwater catfish) had the slowest mean *Usprint* speed. There was a significant effect of body length and tail shape, and a significant effect of species for both *U*crit and *Usprint* models. In addition, species with emarginate tails had higher *U*crit speeds that those with rounded tails, while those species with emarginate tails had borderline significantly higher *Usprint* speeds than both pointed and rounded tail species (Figure 2). Migration classification, movement distance, preferred flow environment and body shape did not contribute significantly to the models. Unsurprisingly, larger individuals of all species had higher Ucrit and Usprint speeds than smaller individuals. Increasing the velocity decreased the average swimming endurance time for all species and reduced the propensity for fish to successfully transverse the 12m channel.



*Figure 2.* Maximum sustained (Ucrit; A) and maximum achievable (Usprint) swimming performance in 22 (21 for Usprint) Australian freshwater fish species grouped by tail shape. Tail shape and body size were significant predictors of swimming capacity, with smaller fish swimming slower than larger fish.

#### Discussion

Here we have collected and reported an important baseline dataset characterising the swimming performance of twenty-two native Australian fish species. The purpose of this dataset is to enable fisheries managers and engineers to design appropriate fish-friendly instream structures (e.g. culverts and fishways), and to enable the remediation of existing problem sites based on quantified data. For these purposes it is important for policy makers to have high-quality scientific data to support and enforce their decisions.

These data can now feed directly into guidelines for designing fish friendly road crossings in Australia. For example, current NSW guidelines recommend that baseline flows within culverts do not exceed 0.3 m s<sup>-1</sup> (Fairfull and Witheridge, 2003). The determination of this velocity was based on limited swimming performance data for Australian freshwater fish. Using the traversable distance models generated from the average Ucrit data generated in the current study, we found that the 0.3 m s<sup>-1</sup> recommendation was reasonably well supported, with all but two species unable to traverse 8 m, a distance equivalent to a culvert underneath a two-lane rural road crossing which predominate most waterway crossings (NSW DPI Fisheries unpublished data). This data indicates that there needs to be at least a contiguous region of the structure where water velocities do not exceed 0.3 m s<sup>-1</sup> to facilitate the successful upstream passage of most Australian small-bodied fish. However, the weakest species such as purple spotted gudgeon and juvenile freshwater catfish may still fail to traverse culverts even at the current water velocity guidelines. Understanding the native fish communities requiring passage at culvert structures should inform water velocity recommendations.

The utility of laboratory-defined fish swimming performance data for the design of fish passage structures has been in wide usage throughout much of the world (Peake et al., 1997, Scruton et al., 1998, Bice and Zampatti, 2005, Starrs et al., 2011, Link et al., 2017, Cai et al., 2018, TL et al., 2018, Katopodis et al., 2019). However, a similar resource did not exist for Australian freshwater fish species meaning that many fish passage structures were inappropriate for their passage requirements. We have collected the first comprehensive database of fish swimming performance capacities for Australian fish. From this data we have been able to identify key morphometric traits that can be used to improve estimates of swimming capacities in similar species for which performance data does not exist. Moreover, these data focus on the most vulnerable size class of fish in the environment, small individuals, less than 10 cm TL. These data represent a significant resource for fisheries managers and water infrastructure engineers in Australia to inform and develop more species-specific 'fish-friendly' fish passage in Australian waterways and to promote passage in a wider range of body shapes and sizes than before.

## Interactions between water depth, velocity and body size on fish swimming performance

#### Introduction

Understanding how fish traverse man-made barriers (e.g. road-crossings and culverts) ensures that engineering and design guidelines achieve positive outcomes for fish communities. Water velocity, depth and fish body size are interrelated factors that influence fish passage through culverts. Velocity barriers have been a major focus of culvert remediation efforts, however wide culvert designs that aim to limit high water velocities, often create shallow depths in the culvert barrel that can potentially impede fish passage. Despite best practises in the design and construction of new culverts or remediation practices, the desired velocity and depth can ultimately be limited by local precipitation and weather extremes. This can be exacerbated in existing culverts with historically poor designs where undesirable features, such as a shallow invert, reduced water depth during baseline and low flows combine (Harris, et al., 2017). Both high water velocities and shallow water depths can produce difficult hydraulic conditions for fish passage (Wolter and Arlinghaus, 2003, Harris, et al., 2017). Small-bodied fish have poorer swimming performance than larger fish and may struggle to negotiate higher water velocities (Nikora et al., 2003, Baker, 2014) while shallow depths disproportionately affect larger fish movements (Domenici, 2001, Bourne et al., 2011).

The MDB relies on flow events to stimulate fish to migrate and spawn. Under natural conditions, baseline flows can be highly restrictive to fish movement, particularly in the upper catchments where culverts are more numerous. This makes the **optimisation of culvert design and construction to fit both environmental and civil requirements difficult, particularly if minimum water depths and maximum velocities during baseline flows need to pass the entire fish community. Here, we quantified how water velocity, depth and fish body size interact to affect the swimming performance (endurance time) of three native Australian fish species and their ability to traverse a real-scale experimental channel.** Understanding the interactive effects of these abiotic and biotic components of fish passage will help inform fisheries management and the iterative improvement of culvert design guidelines. We hypothesized that: (1) a decrease in water depth, coupled with increasing water velocity, will reduce endurance swimming times; and (2) the probability of traverse success of fish through eight metres of the experimental channel will decrease with decreasing water depth and increasing fish size.

#### Methodology

We assessed the interactive effects of water velocity, depth and body size of swimming capacity and transverse success in three species of native freshwater fish: silver perch (*Bidyanus bidyanus*), Murray cod (*Maccullochella peelii*) and eel-tail catfish (*Tandanus tandanus*). These were chosen to represent a range of body shapes, swimming styles and capabilities. We swam each of the three species in nine treatments, which consisted of three water velocities (species specific, to reflect their different swimming capabilities) at three water depths (5, 10 and 15 cm, kept constant across the test species). We measured how long it took the fish to fatigue (capped at 60 minutes) and their traverse success rates over 8 m of flume length. We then determined how the three factors of water velocity, depth and fish body size interacted to affect the swimming performance of the three fish species. For *M. peelii* and *T. tandanus*, we quantified swimming *B. bidyanus*, we quantified swimming performance as the time taken for the fish to traverse up 8 m of the flume from the point of release, which all fish could do at all test velocities.

#### **Results**

Swimming endurance times decreased with decreasing water depth and increasing water velocities for Murray cod and eel-tailed catfish, but not silver perch. Larger Murray cod and eel-tailed catfish could not swim for longer than their smaller counterparts, particularly at shallow depths and high velocity waters. Silver perch were the strongest swimmers, with larger fish having shorter traverse times independent of both water depth and velocity (Figure 3). For all species, traverse success increased with increasing water depth: fish were less likely to fatigue and to show longer swimming endurance times in deeper water. Conversely, fatigue set in earlier at shallower depths: fish were significantly more likely to fatigue when swum at 5 cm depths compared with fish swum at 15 cm depths.

High water velocities generally predicted poor fish traverse success across all three species; however, the performance of big fish (>200 mm) was less severely affected by high water velocities than that of small fish. Nevertheless, when large fish were swum at high water velocities and shallow water depth, their performance was significantly poorer than when they were swum at the same velocity in deeper water.

Importantly, for Murray cod and eel-tailed catfish, the magnitude of the impact of water velocities and water depths was non-linear ('U' shaped) across the range of body sizes examined in this study. This means that water depth and water velocity had disproportionally larger negative effects on the smallest and the largest fish of the species (i.e., fish that were less than 100 mm or greater than 250 mm in length). Smaller-sized fish had an increased risk of fatigue and shorter endurance times in high water velocities compared to larger-sized fish; however, larger fish were only advantaged until shallow water depths limited their ability to swim.

Despite silver perch displaying stronger swimming capabilities and a higher probability of traverse success for all test velocities, the largest individuals were hindered by shallow water depths, with some observed resorting to swimming on their sides. This mode of swimming is likely more energetically costly, and may explain why silver perch larger than 250 mm were less likely to traverse the full length of the flume at the shallowest water depth than smaller members of the species.



*Figure 3.* A large and small Murray cod swimming in the same shallow water conditions in the experimental channel. The large fish struggles to make headway in the shallow water while the small fish has progressed much further along the channel.

#### Discussion

Our data shows that decreasing water depth and increasing water velocity can interact to reduce fish endurance swim times and decrease the likelihood of successfully traversing 8 m of an experimental channel (Shiau et al., 2020). As hypothesised, swimming endurance times decreased with decreasing water depth and increasing water velocities, with larger sized *M. peelii* and *T. tandanus* more heavily affected than smaller sized conspecifics. On the contrary, the traverse time for *B. bidyanus* was not influenced by water depth or velocity, only fish size. The probability of *B. bidyanus* successfully traversing the channel was greater than both *M. peelii* and *T. tandanus* irrespective of body size. Our results show that elevated water velocities remain a strong predictor of poor fish passage success for *M. peelii* and *T. tandanus*, but that bigger fish performed better than smaller fish at higher water velocities. However, the performance of large fish decreased with decreasing water depth, suggesting that shallow water depths can surpass velocity as the main challenge to their swimming performance and traverse success rates.

The effects of depth and velocity on *M. peelii, T. tandanus* and *B. bidyanus* can be related back to their different physical characteristics and ecology. Increases in velocity reduced the distance fish could traverse, and suggested that the interspecific differences may be dependent on physical and behavioural traits (Haro, 2004). Although *M. peelii* may undertake seasonal upstream migrations of up to 130 km, they and *T. tandanus* generally inhabit slower moving waters and move < 10 km from their home range (Reynolds, 1983, Lintermans, 2007, Koehn et al., 2009). In contrast, *B. bidyanus* are excellent swimmers that prefer fast-flowing waters and migrate at the macro scale (> 500 km upstream) (Reynolds, 1983, Lintermans, 2007). Despite adult *B. bidyanus* having exceptional swimming capabilities, their large physical size (body height) restricted their ability to swim in shallow water depths, with some of the largest individuals observed resorting to swimming on their sides.

This would have markedly increased drag and dramatically reduced their ability to produce thrust, resulting in much higher energetic costs to swim (Webb et al., 1991, Hughes, 2004). While our sample size range for *M. peelii* and *T. tandanus* did not include their maximal adult size ranges, it can be reasonably assumed that individuals larger than those examined would also be impaired by very shallow water. This could have significant implications for migrating adult animals. This was reflected in our results showing *B. bidyanus* that were longer than 250 mm were on average less likely to traverse the full length of the flume at the shallowest water depth than smaller conspecifics.

The complexity is amplified by the effects of climate change on flow regimes will continue to be a major challenge for fish passage management in the MDB. With reduced precipitation and prolonged drought periods extending the dry season (Balcombe et al. 2011), the negative effects of shallow water in culverts and causeways (< 10 cm) will likely contribute to reduced migratory spawning movements of large-bodied *B. bidyanus* and *M. peelii*.



Water depth is a significant issue that affects the utility of culverts for fish passage. Our study revealed that the impacts of water depth on fish passage is likely highly species- and life stage-specific, and complicated by water velocity. Image: John Keep

## Low light intensities increase avoidance behaviour of diurnal fish species

#### Introduction

Inadequately designed culverts may act as behavioural barriers to fish passage if they affect the willingness of fish species to enter or pass through the structure due to altered ambient light conditions. For most fish, vision is an important aspect of their sensory repertoire and their behaviour is linked with diel light cycles. It is increasingly apparent that anthropogenic disturbances to natural lighting regimes can have detrimental impacts on affected fish populations (Becker et al., 2013). Structures that limit natural light penetration can alter the behaviour of diurnally active fish (Jones et al., 2017, Jones and Hale, 2020).

Avoidance of darkened environments within covered fishways contributes to the reduced movement of several small-bodied Australian freshwater fish species (Jones, et al., 2017). Abrupt changes in light intensity such as at the entrance/exits of fishways or culverts can also cause avoidance behaviour (Moser and Mesa, 2009, Ono and SimenstadSchool, 2014). However, other studies have demonstrated that upstream migrating salmon, trout, eels, and common galaxias in Australia, are apparently largely unaffected by reduced light intensity in civil structures (Rogers and Cane, 1979, Gowans et al., 2003, Fjeldstad et al., 2018). These conflicting accounts of the effect of light on fish movement suggest a range of species-specific behavioural responses to different lighting conditions (Rogers and Cane, 1979, Gowans, et al., 2003, Fjeldstad, et al., 2018), with such variability also indicating **that our understanding of the effects of altered lighting regimes on fish movement is poor, despite this issue being raised in several fish passage guidelines** (Fairfull and Witheridge, 2003, Franklin et al., 2018).

Accordingly, research is required to better understand the potential for low light intensity within culverts to impact fish movement and behaviour to inform the regulation of new culvert structures and to guide the remediation of existing structures. **The aim of this study was to quantify the effect of reduced light intensity on the movement behaviour of four species of small-bodied or juvenile Australian native fish.** Three of the species, Fly-specked Hardyhead, Australian Smelt and Silver Perch, are more active during the daytime (Mallen-Cooper, 1999, Stuart and Mallen-Cooper, 1999, Clunie and Koehn, 2001, Baumgartner et al., 2008), while Australian Bass are generally crepuscular but can be active at other times of the day and night (Harris, 1985, Smith et al., 2011). We hypothesised that Fly-specked Hardyhead, Australian Smelt and Silver Perch would prefer an illuminated environment, and that Australian Bass would prefer a darker environment. We then aimed to establish the minimum lighting thresholds for the species that displayed a preference for an illuminated environment. Finally, we surveyed light intensities within a subset of common culvert designs and sizes in Brisbane, Queensland, Australia to understand whether low light intensity has the potential to affect use of culverts by fish based on our experimental observations.

#### Methodology

To understand how reduced light intensity might affect fish behaviour in culverts, we performed a behavioural choice experiment quantifying the amount of time individual fish spent in dark and illuminated areas of a controlled experimental channel. Juvenile Australian bass and juvenile silver perch were sourced from commercial hatcheries; adult fly-specked hardyheads and adult Australian smelt were wild-caught. Behavioural trials were conducted in a 12m hydraulic channel (Figure 1.B,C). The light around and within the entire channel was controlled using blackout plastic sheeting to create an environment with zero ambient light (0 lux). Half of the channel was illuminated using 4000 K correlated colour temperature LED lighting, suspended 1 m above the water surface and the other half left unilluminated. The light intensity above the illuminated half was set to 2535 lux (measured at the water surface), the same as in the housing aquaria. Four treatments were required to control for the direction of water flow in the channel that could not be changed and the illuminated and the upstream darkened. Fish were placed into the channel and their movements between the darkened and illuminated sections of the channel were directly observed for 30 min through a small observation point at the transition zone between the illuminated and darkened areas. The channel bulk velocity was set to 0.3 m s–1 and the depth was set to 0.15 m.

To understand the minimum illumination intensity that would encourage species that showed an avoidance of darkened environments to enter a darkened channel, we sequentially increased the light intensities in the darkened section of the flume until fish moved into it; the light levels in the illuminated section remained the same. To put these light levels into context, we measured illumination patterns in 15 culverts in Brisbane.

#### Results

To understand how reduced light intensity might affect fish behaviour in culverts, we performed a behavioural choice experiment quantifying the amount of time individual fish spent in dark and illuminated areas of a controlled experimental channel. We found that behavioural responses were largely reflective of the species' diel activity patterns; the diurnal species *Craterocephalus stercusmuscarum* and *Retropinna semoni* preferred illuminated regions, spending 97.2% and 86.3%, respectively, of their total trial time in the illuminated zone (Figure 4). The nocturnal/crepuscular *Macquaria novemaculeata* preferred the darkened region of the channel, spending on average 91.3% of their time there. *Bidyanus bidyanus* were strongly rheotactic, and their behaviour was influenced more by water flow direction than ambient light level. Neither the release point nor the illumination condition at the release point was found to affect the time spent in either the light or dark zones.

Given that both fly-specked hardyhead and Australian smelt displayed strong avoidance of the darkened environment in the channel, we gradually increased the illumination in the darkened (treatment) zone to determine the light threshold that would encourage these species to enter. Overall, the number of individuals entering the treatment region of the channel increased with increasing illumination. Fly-specked hardyheads began entering the darkened region of the flume at 5 lx, with 50% of fish moving into the darkened region at ~ 200 lx. Australian smelt started to enter the darkened region at 2.5 lx with 50% of individuals moving at just 5 lx. Further increasing the light intensity to 25 lx resulted in more than 75% of individuals entering the treatment zone.

We quantified the illumination intensities in 15 culverts in south-east Queensland to determine how many reached the minimum lighting thresholds required to encourage 70% of Australian smelt and fly-specked hardyhead to successfully move into a darkened environment. The modelled threshold values corresponded to 100 and 200 lx for Australian smelt and fly-specked hardyhead, respectively. We found that light intensities at the culvert entrance/exit averaged 71 lx (range: 5.6-123.1). In all culverts, light intensities dropped to less than 3 lx in the centre (range 0-2.3 lx) – less than the minimum movement threshold for flyspecked hardyheads and Australian smelt.

#### Discussion

This study shows that the levels of available light significantly affected the behaviour of three of the four Australian fish species examined, and that the results were mostly consistent with the study's hypothesis that species behavioural responses to light intensities would relate to their daily activity patterns (i.e., diurnal vs. nocturnal) (Keep et al., 2020). The largely diurnal Australian smelt and fly-specked hardyhead showed near absolute avoidance of the completely darkened environment, whereas the nocturnal Australian bass strongly avoided the illuminated section. For the two species that avoided the darkened environment, we found that the threshold light intensities needed to encourage individual fish to enter the darker half of the test channel were quite low. Moreover, providing even very low levels of light with artificial lighting could remove the behavioural barrier that low light intensities in culverts may pose to diurnally active fish species.

Road-crossing pipe and box culverts located in the Brisbane region had maximum light intensities that were less than the minimum movement thresholds for fly-specked hardyhead and Australian smelt (<3 lx), therefore potentially posing a behavioural barrier to these species. Although the authors only measured light intensities on just 1 day at each culvert, we measured at the brightest time of day (morning) and year (Austral summer), so if prohibitively low light intensities were detected under these conditions, they are likely to be light barriers at other times of year. Culvert lighting requirements need to be considered in the context of other culvert design features to ensure that efforts to mitigate hydraulic barriers to fish passage (e.g., significant slope or excessive water velocities) do not inadvertently create behavioural obstacles to fish movement.

# Remediating culverts to improve fish passage in urban drainages

#### Introduction

Culverts are a major cause of habitat fragmentation in freshwater ecosystems, are a barrier to fish movement, and can be regarded as a significant contributor in the decline of freshwater fish populations globally. To address this significant conservation issue, various culvert remediation strategies have been designed, including the installation of vertical baffles and the provision of naturalistic (rock) substrates both of which aim to slow water flow and create a degree of turbulence that fish may be able to exploit to make upstream passage. Although these strategies do reduce the velocity of water flowing through the structure, there is often resistance to their application in some jurisdictions because the resultant reduction in culvert discharge can increase upstream flood risks and they can contribute to debris clogging and increased culvert maintenance costs. In addition, some baffle designs markedly increase water turbulence to such an extent that they may be detrimental to passage by some small-bodied fish species or larval or juvenile size classes. Here we tested a range of some novel remediation designs that exploit the reduced water velocity in boundary layers along the culvert wall to enhance fish passage without significantly compromising discharge capacity.

#### Methodology

We tested several novel lateral 'rib' designs that could run down the length of culverts to increase the size of the reduced velocity zone (RVZ) that forms at the wall-base intersection (Figure 5 A-D). We hypothesised that the longitudinal structures would increase the boundary layer effect to increase the size of RVZ and provide a small, low velocity movement path for fish. We then optimised the most successful design configuration by extending the lateral protrusion of the beam (to 100mm) and by incorporating roughening to the wall of the sub-beam space (Figure 5 E-G) which has been shown to increase the size of the boundary layer and improve the swimming performance of small-bodied fish (Liao et al., 2003, Rodgers and Franklin, 2017, Goodrich et al., 2018). We hypothesised that increasing the horizontal dimension would increase the size and magnitude of the RVZ under the beam, which in turn would improve fish swimming performance and rates of traverse success up the experimental channel. We also tested the efficacy of several vertical baffle designs for traverse success (Figure 5 H-K). The vertical baffles included two rightangled triangle designs (Wang et al., 2018) and two rectangular panels; both designs were attached perpendicular to the lateral wall of the channel and projected into the centre of the channel. We measured a suite of swimming performance metrics (endurance capacity, transverse success), behaviour (use of the sub-beam space) and water flow hydrodynamics. We chose a range of small bodied or juvenile (< 10 cm total length) native Australian fish species to quantify the effect of our culvert remediation designs: Juveniles of golden perch (Macquaria ambigua), Murray cod (Maccullochella peelii), eel-tailed catfish (Tandanus tandanus), and silver Perch (Bidyanus bidyanus) and adults of empire gudgeons (Hypseleotris compressa), glassfish (Ambassis agassizii), pacific blue eyes (Pseudomugil signifier), carp Gudgeon (Hypseleotris klunzingeri), fly-specked Hardyhead (Craterocephalus stercusmuscarum), and crimsonspotted rainbowfish (Melanotaenia duboulayi).



**Figure 5.** Schematic representation of flume setup and cross section profiles of the longitudinal rib and baffle designs tested. We tested the (A) control channel against a (B) double square (50mm) beam, (C) circular beam, (D) ledge, (E) single 50 mm square beam, (F) single 100 mm beam, (G) single 100mm beam + sub-beam roughening, (H) triangular baffle, (I) vented triangular baffle, (J) 50 mm vertical baffle, and (K) 150mm longitudinal baffle. B–G ran longitudinally through the channel; positioned 50 mm above the base of the channel. H and I were spaced 0.66 m apart and covered 9m of the channel; J baffles were spaced 10-20 cm apart, K baffles were spaced 30-60 cm apart.

#### Results

Velocity contour plots of channel cross-sections show that the square, circle and ledge designs (Figure 5 B-D) all expanded the RVZ in the sub-beam space adjacent to the wall and channel bed, when compared to the same area in the control channel (Figure 6). The turbulence profile of the square beam rib was most similar to the control channel and this design created the largest RVZ in the sub-beam space. The square, semicircle and ledge beams all significantly improved fish traverse success rates when compared to the smooth control channel (Figure 7), however, the square longitudinal beam was the only modification to also significantly increase the swimming endurance performance of all species when compared to the control channel.

We then conducted a follow-up study to see whether we could improve the design of the square longitudinal beam. The first experimental stage aimed to quantify how expanding the width of the beam from 50 mm to 100 mm into the centre of the channel would affect the RVZ below the beam, and the second stage was to assess whether the addition of surface roughening to the lateral wall of the sub-beam channel further reduced the water velocity in the space. The naturalistic surface roughening consisted of  $45 \pm 5$  mm road base aggregate glued to the wall of the channel. Both modifications, the 100 mm beam and the 100 mm beam with roughening, reduced the average point velocity in the sub-beam space by between 25 and 70% relative to the control channel, which undoubtedly contributed to the improved upstream passage in the tested fish species. There was no difference in utilisation of the sub-beam space in the 50 and 100 mm beam treatments with approximately 49 and 53% of the total swimming time spent underneath the 50 and 100 mm beams, respectively. There was a considerable improvement in utilisation rates under the 100 mm beam + naturalistic roughening, compared to the 50 mm beam, with fish spending on average 72% of their total swimming time under the 100mm beam + roughening (Figure 8). While both modifications increased fish performance in the channel, the beam with roughening provided the most benefit to the greatest number of species (4 of 7). In terms of water flow around the modified designs, the 100 mm beam and 100 mm beam + roughening did resulted in larger changes to the mean channel flow velocity compared to the 50 mm beam. Both designs increased channel afflux by approximately 15%.

We undertook testing to examine the efficacy of a suite of vertical baffle designs for upstream fish movement in a controlled culvert-like channel. These ranged from standard baffle deigns in current fish passage guidelines to a relatively new triangular baffle (Wang, et al., 2018). We examined use of the inter-baffle spacing, upstream passage and endurance capacity. Overwhelmingly, we found that vertical baffles, despite providing low-velocity zones behind the baffle that fish could rest in, negatively affected fish performance and reduced channel traversability (Figure 9). Baffles created very high velocities around the structures, and the 150 mm vertical baffle caused considerable afflux. Although fish would generally sit behind baffles, they were often unable or unwilling to move around the baffle to make upstream passage.

Videos of fish interacting with baffles and beams:

- 1. Gudgeon small 50mm vertical baffle discharge 0.029m<sup>3</sup>s<sup>-1</sup> https://youtu.be/JB8Mbxhl0H8
- 2. Silver perch small 50mm vertical baffle discharge 0.029m<sup>3</sup>s<sup>-1</sup> https://youtu.be/6HUepvVVvxg
- 3. Glassfish triangular baffle discharge 0.011 m<sup>3</sup>s<sup>-1</sup> https://youtu.be/Th\_4ONeZ2U0
- 4. Silver perch triangular baffle discharge 0.038 m<sup>3</sup>s<sup>-1</sup> https://youtu.be/QLz9\_pT2QZc and https://youtu.be/ fUQ86nUgujM
- 5. Juvenile Murray cod with 100mm beam + sub-beam roughening https://youtu.be/dJdo0iZZNss
- 6. Juvenile Murray Cod under 100mm beam no roughening https://youtu.be/0bJ3GJXjXsM
- 7. Small fish under 50mm baffle 0.5ms<sup>-1</sup> https://youtu.be/HVD7LnskDnA



*Figure 6.* The reduction in water velocity at the wall-bed intersection in a control channel and in the same location under the square beam (blue square). The average velocity in the sub-beam space was almost 25% lower than in the control channel.



*Figure 7. (A)* Transverse success and *(B)* endurance times of six species of native freshwater fish in the presence of the 3 longitudinal beam designs (in addition to the control channel).



**Figure 8.** Kaplan-Meier survival curves showing the probability fish would not fatigue by the end of the 600s (10 min) high velocity swimming trial in the presence of the four experimental beam treatments. In the 'all species combined' panel, it was clear that the presence of the 100mm beam with sub-beam roughening resulted in an ~75% chance of fish not fatiguing during the trial compared with < 10% for fish in the control channel. The 7 panels to the right show the breakdown in responses by species. In the presence of at least one of the beam treatments, all species performed better than in the control channel, though which beam configuration was the 'best' differed between species.



**Figure 9.** Vertical baffles generally did not promote, or even reduced, traverse success in several species of Australian small-bodied freshwater fish. 'Small' and 'large' pertain to the two vertical rectangular baffles of 50 and 150mm width (projection distance into the main channel). Control is the unmodified 12 m channel. The bar colours reflect different discharge rates.

#### Discussion

The novel beam designs all provided a significant improvement in the endurance and traversability of a wide range of small-bodied and juvenile fish species when compared to the vertical and triangular baffle designs or the control channel (Watson et al., 2018). Further, we showed that baffles can have a negative impact on small fish, largely due to the generation of excessive turbulence at high discharge rates relative to their swimming capacity. By contrast, all longitudinal beam designs produced comparably little turbulence and had little impact on channel discharge capacity. The low velocity zone between the beam and the base of the channel reduced the velocity of water by between 20 and 75% of the bulk channel flow. This means that they may be suitable in culverts where there might be a risk of upstream flooding due to reduced culvert discharge capacity. The longitudinal beams allow high bulk discharge rates whilst providing a small velocity refuge that small fish can swim again. Widening the longitudinal beams and well as the addition of naturalistic roughening increased the size of the reduced velocity zone and the magnitude of velocity reduction in the sub-beam channel. All seven of the Australian fish species tested under these conditions displayed increased swimming performance capacity or increased traversability in at least one of the designs tested, regardless of their body morphology, size or swimming capabilities. However, the three designs did differ in their impact on overall channel discharge capacity which needs to be considered if these are to be retrofitted to existing culverts.

Our analysis of common vertical baffle designs suggest that these may be problematic for small-bodied Australian fish species. Of the four species examined in our study, all derived little benefit in terms of upstream passage relative to the control channel. We would caution the use of vertical baffles for remediation of culverts to promote fish passage.

## Using fish thermal physiology to improve the management of cold-water pollution from large dams.

#### Introduction

In addition to physically restricting fish movements, regulation of water flows in the MDB, in combination with rapidly changing seasonal water flow patterns, drought and agricultural runoff can result in substantial changes to the physiochemical properties of the water that can impede fish movements (Koehn, et al., 2009). Increasingly, cold-water pollution (CWP) is a considered to be significant threat to aquatic communities downstream of large, bottom-release dams. Cold water releases typically occur during summer when storage dams release unseasonably cold and anoxic hypolimnetic waters, which can decrease the temperature of downstream. The effects of this cold water thermal pollution can extend for up to 400 km downstream and may reduce downstream water temperatures by up to 16°C (Preece, 2004, Boys et al., 2009, Lugg and Copeland, 2014). Depending on the release duration, these hypothermic conditions can persist for many months. Importantly, these release periods often coincide with migration and spawning of key native fish species, and in some areas of the MDB, water temperatures now seldom approach levels required for spawning of key native fish species (Lugg and Copeland, 2014). Fish and other thermally sensitive organisms can be significantly impacted by thermal pollution, and different life stages can be differentially sensitive to exposure to unseasonably cold water. On top of this, the introduction of invasive fish species, many of which have a wider thermal tolerance range than our native species, means that thermal pollution may give invasive species an advantage in thermally polluted environments, contributing to the loss of ecological community structure in affected areas. Presently, no guidelines exist at the Commonwealth or State level on the ecologically safe temperature range for water releases.

The capacity of ectothermic species to tolerate or rapidly adjust to acute temperature changes may determine the nature and magnitude of the impact of CWP on affected species. The aim of this project was to determine the physiological consequences of CWP-like acute reductions in water temperature on native and invasive fish species, and to understand whether physiological plasticity might allow fish to 'adjust' to these water temperature to offset the negative impacts on low temperature on performance. We also tested several potential remediation actions that involve slowing the rate of temperature decline to allow fish to adjust gradually to the low temperatures, as well as testing the minimum tolerable temperatures that did not result in a substantial loss of performance.

#### Methodology

The thermal sensitivity and plasticity of four MDB fish species (juveniles of silver perch, golden perch, and spangled perch, and adult Murray rainbow fish) and three invasive fish species (Gambusia holbrooki, juvenile Carp and juvenile Tilapia) were experimentally assessed following an acute simulated CWP event (a 10°C reduction in water temperature from 24°C to 14°C over 12 h). We measured swimming performance (burst [Usprint] and sustained [Ucrit]) and energy expenditure (routine and maximal metabolic rates) at intervals over the following 10-12 weeks to track acclimation responses. A 185 liter recirculating swimming flume (Figure 1A) was used to measure fish swimming performance and intermittent flow respirometry to measure oxygen consumption. All fish were less than 10 cm in total length. For juveniles of the larger growing species (perch), growth rates were measured over the 10 week experimental period. In a separate experiment, we examined whether the rate of change in water temperature affected the ability of Murray cod to physiologically adjust performance to cope with low water temperatures. In this study, water temperatures were again lowered by 10 degrees but over 1, 5 or 10 days. Again we measured swimming performance (burst [Usprint] and sustained [Ucrit]) and energy expenditure (routine and maximal metabolic rates) at intervals over the following 10-12 weeks to track acclimation responses. In a final experiment, we examined whether smaller water temperature reductions were better tolerated by embryonic and juvenile Murray cod. We collected freshly laid eggs from a commercial hatchery and incubated eggs at different water temperatures: 14, 17, 20, 24°C. We recorded hatching success, time to hatch and size at hatching. We then took a separate cohort of fish reared at 20°C and tested their swimming performance after being acutely exposed to one of the 4 test temperatures.

#### **Results**

We measured swimming performance (burst [*U*sprint] and sustained [*U*crit]) and energy expenditure (routine and maximal metabolic rates) in 5 native and 3 invasive fish species to track performance responses to the acute temperature reduction over 10-12 weeks. For juveniles, growth rates were also measured over the 10 week experimental period. For all species, the acute reduction in water temperature had a significant effect on swimming and metabolic performance with all traits decreasing by 30 - 50%. Importantly, performance capacity remained largely unaffected by prolonged exposure to the low temperature in both the native and invasive fish species (Figure 10). There was some evidence of partial compensation for the low temperatures in swimming and/or metabolic performance in all species, but only 1 species (Murray cod) was capable of compete thermal compensation in all traits after 10 weeks of low temperature exposure. Growth rates were suppressed in all juvenile fish that were exposed to low water temperatures.

This work demonstrated acute and chronic depressive effects of a rapid 10°C decrease in water temperature on growth, swimming performance and metabolic rates of fish, however it was unclear if a slower rate of temperature decrease could mitigate the negative effects of rapid cold water releases on fish. To test this, juvenile Murray cod were exposed to one of three different thermal reduction regimes: gradual (over 10 days), intermediate (over 5 days) or rapid (over 1 day) change from 24 to 14°C. Regardless of the rate of initial temperature decrease, cold exposure had a significant acute and lasting depressive effect on several physiological metrics. Murray cod showed evidence of compensation to low temperature, with partial compensation of *U*crit and complete compensation of *U*sprint and metabolic rate after 8 weeks of cold-acclimation. The results of this study indicate that regardless of the rate of temperature decrease, the short-term effects of CWP-like reductions in temperature are more significant for this species than the long-term effects.

In the third part of this project we investigated the effect of low temperature exposure regimes during early lifehistory stages on the survival, physiological performance and developmental plasticity of fish. We hypothesised that the exposure of eggs and larvae to low temperatures consistent with CWP would decrease survival, development, growth and swimming performance in subsequent life history stages (i.e. in larvae and juveniles). We found that exposure of Murray cod eggs to 14°C water caused significant embryonic mortality, delayed hatching time and reduced larval size at hatching. Exposure to 17°C water resulted in greater hatching success and greater body size, but delayed hatching time. There was no significant effect on water at either 20 or 24°C on any embryonic metric. Our results suggest that exposure of Murray cod eggs to waters of below 17°C are like to have significant impacts on recruitment success in this species. We continue to analyse data on the physical performance of larval fish and juveniles.



**Figure 10.** The effects of acute cold water exposure on the swimming performance capacity of 5 native freshwater fish species and 3 invasive species. NB. Tilapia suffered acute mortality after transfer to cold treatment so no further testing was possible. \* = treatments are significantly different at this timepoint. Absence of significant differences in performance at later time points indicates some thermal compensation was occurring.

#### Discussion

Acute reductions in water temperature associated with unseasonably cold water releases from thermally stratified dams could have significant effects on the physiological function of aquatic ectotherms in the downstream reaches of the waterway (Astles et al., 2003; Michie et al., 2020b; Parisi et al., 2020). We examined this issue in juvenile Murray cod, silver perch, golden perch, spangled perch and Murray river rainbow fish (Parisi et al., 2020). In all species, acute exposure to 14°C water resulted in decreased swimming performance and metabolic rate measures, however juvenile Murray cod did demonstrate a good capacity to compensate for the depressive effects of low temperature, however it took considerable time. The other species showed differing degrees of thermal compensation capacity, and also their relative compensation capacities differed across physiological metrics. We also hypothesised that managing the speed of the thermal change by allowing physiological compensation. However, in juvenile Murray cod, initial cold water exposure rates did not influence the degree or rate of thermal compensation. This study has implications for the management of dam releases and indicates that slowing the rate of temperature decline to rates tested here may not be a successful mitigation strategy for CWP effects on Murray cod. More broadly, this form of thermal pollution does not appear to promote rapid thermal compensation in native fish and so can act as a barrier to fish movement, causing reduced recruitment, ecological community shifts and disruptions to timing and success of reproduction.

Generally, invasive species possess physiological traits that enable them to tolerate a wider range of environmental conditions relative to native species (Zerebecki and Sorte, 2011, Kelley, 2014). Maintaining performance across a wide range of environmental temperatures likely plays a critical role in the ability of invasive species to establish in disturbed environments (Schnell and Seebacher, 2008, Bae et al., 2018). We assessed whether three invasive fish species were better able to tolerate the effects of CWP-like changes in water temperature which might provide them with a competitive advantage over native Australian species. Like the native fish, acute exposure to low temperature water decreased locomotor performance and metabolic function in all invasive species. However, for Tilapia, cold-shock lead to a significant and abrupt loss of physical condition. Although varying degrees of thermal compensation were seen across performance traits in Gambusia and Carp, these were not evident until late in the exposure period. Our data suggest that management actions designed to limit the negative impacts of CWP on native fish species are likely to also improve the performance of invasive species, which has important implications for the management of CWP in affected waterways.



Hume dam Image: Rebecca Cramp

## Anthropogenic nitrate as a non-physical barrier to fish in the MDB

#### Introduction

Nutrient pollution (i.e. nitrogen and phosphorous) can reduce water quality and contribute to the loss of connectivity in freshwater environments. Nitrogen pollution is regarded as one of the most common and widespread forms of freshwater habitat degradation. Nitrate pollution is particularly prominent in areas of high fertiliser use where agricultural land practices (crop and livestock) have increased nitrate loadings in surrounding waters, causing eutrophication, algal blooms, anoxic dead zones, altered food webs and nitrate toxicity to residing species (Camargo et al., 2005, Camargo and Alonso, 2006, Gilbert, 2017). For example, after heavy rainfall, the use of a nitrate-based fertiliser (ammonium nitrate) caused the concentration of nitrate in surface-water to spike from 2 mg NO<sup>3</sup>-L<sup>-1</sup> to 100 mg NO<sup>3</sup>-L<sup>-1</sup> in sites adjacent to agriculture plantations (Jaynes et al., 2001). The impacts of nitrate, however, can be mediated by the presence of other environmental challenges in the environment in ways that are currently unrecognised (e.g. induced susceptibility or induced resistance; Ortiz-Santaliestra and Marco, 2015; Smallbone et al., 2016). Understanding how nitrate interacts with other stressors may be particularly important for the management and conservation of animals (or populations) inhabiting anthropologically degraded environments, but also important for other sectors such as aquaculture where nitrate concentrations can be especially high (often exceeding 500 mg NO<sup>3</sup>-L<sup>-1</sup>) and are often coupled with other physiochemical challenges (e.g. elevated temperatures, hypoxia; (Reid et al., 2019)).

Physiological and behavioural responses to hypoxia may be compromised under high levels of nitrate pollution. When water-nitrate levels are elevated, nitrate enters the body of aquatically respiring organisms through the gills which may compromise oxygen-dependent processes (Jensen, 1996). Nitrate may also interact with other environmental stressors to magnify their effects on animals (Gomez Isaza et al., 2018, Gomez Isaza et al., 2020b, Gomez Isaza et al., 2020a, Gomez Isaza et al., 2021b, Gomez Isaza et al., 2021a)

#### Methodology

In the first part of this work, we conducted a systematic review to quantify the impacts of elevated nitrate exposure on key fitness related traits in aquatic fauna using meta-analytical tools and sought to examine whether organismal survival is affected by nitrate and its interaction with other stressors. Subsequently, we examined the interactive effects of nitrate plus pH, temperature and hypoxia on fish. Environmental pH is one factor that may modify the toxicity of nitrate by exacerbating its uptake and disrupting key physiological performance traits. In the first experiment, spangled perch (Leiopotherapon unicolor) were exposed to one of two pH levels (pH 5.0 and 7.0) and three nitrate concentrations (0, 50 and 100 mg  $NO^3$ - L<sup>-1</sup>). Aerobic scope (maximal minus standard oxygen uptake rates) was measured at six time points during exposure, and fish blood-oxygen carrying capacity were assessed after 28 days of exposure. In the second experiment, nitrate-induced reductions to oxygen transport were expected to lower species' tolerance of, and impede their capacity to compensate for prolonged exposure to, elevated temperatures. To test this prediction, fish (silver perch, Bidyanus bidyanus) were exposed to 28 or 32°C and simultaneously exposed to one of three nitrate concentrations. Indicators of fish performance, aerobic scope and upper thermal tolerance were assessed after 6-weeks. Finally, the impact of elevated nitrate on the efficacy of behavioural and physiological responses of fish to acute hypoxia were investigated. Fish were exposed to one of three nitrate treatments for three weeks, then behavioural avoidance, haematological changes, opercular beat frequency (OBF), swimming performance and aquatic surface respiration (ASR) responses to progressive hypoxia were quantified.

#### Results

The first aim of this project was to quantify the impacts of elevated nitrate exposure on key fitness related traits using meta-analytical tools and to examine whether organismal survival is affected by nitrate and its interaction with other stressors. Across studies, exposure to elevated nitrate decreased the activity, growth, and survival of aquatic taxa. Further, antagonistic interactions between nitrate and other stressors were commonplace.

In a second study, we tested the hypothesis that anthropogenic environmental acidification may increase the toxicity of nitrate by exacerbating its uptake. To test this spangled perch (*Leiopotherapon unicolor*) were exposed to one of two pH levels (pH 5.0 and 7.0) and three nitrate concentrations (0, 50 and 100 mg NO<sup>3</sup>– L<sup>-1</sup>). In fish, aerobic scope was compromised by the interaction between low pH and nitrate and resulted in increased energy expenditure. We also observed a reduction in swimming performance, which likely was the consequence of nitrate impacts on oxygen carrying capacity.

We also looked at the interactive effects of elevated water temperatures on physiological performance in silver perch. We found that nitrate exposure in animals acclimated to a high, but not stressfully high temperature of 28°C reduced their tolerance of higher temperatures. However, in fish acclimated to a stressfully high temperature of 32°C, nitrate exposure actually increased their thermal tolerance. These results are suggestive of a cross-tolerance interaction, whereby the mechanisms employed to respond to one stressor increase their tolerance of the other stressor.

Lastly, the impact of elevated nitrate on behavioural and physiological responses of silver perch to acute hypoxia were investigated. We found that fish exposed to elevated nitrate had a lower tolerance of aquatic hypoxia and had to surface at a higher dissolved oxygen level during progressive hypoxia to access oxygen in the air-water interface. Nitrate exposure reduced blood oxygen carrying capacity increased gill ventilation rates. These physiological disturbances during nitrate exposure had pronounced negative effects on the swimming performance and hypoxia tolerance of fish and indicate that nitrate pollution is likely to increase the susceptibility of fish to aquatic hypoxia.

#### Discussion

Although nitrate is generally regarded as non-toxic, our studies found guantitative evidence that exposure to nitrate poses consistent, negative effects on aquatically respiring organisms (amphipods, amphibians and fish) living in freshwater. Nitrate toxicity increases with increasing nitrate concentration and exposure durations. However, most importantly, the risk from nitrate pollution can be modulated by the presence of other environmental stressors within the environment. Our studies showed that 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 significant consequences. Overall, the presence of nitrate and additional stressors impaired energy homeostasis, such that aerobic scope is reduced and compromised the functioning of aerobically supported traits (e.g. swimming, growth), due to disruptions of the blood-oxygen carrying capacity and the additional energy required to detoxify and eliminate absorbed nitrate. Although these stressors may induce only moderate levels of stress and long term persistence may be possible, trade-offs in energy allocation (e.g. energy for growth and reproduction) may confer lasting fitness consequences with ramifications for populations (Gomez Isaza, 2020). In some cases, however, physiological compensation (acclimation/acclimatization) can and does offset the effects of nitrate, possibly explaining some antagonistic interactions observed in our study. Overall, our findings show that looking at nitrate in isolation greatly oversimplifies and underestimates its effect on aquatic animals. This project highlights the unpredictability of stressor interactions and underscores the importance of experimental assessments in addressing the eco-physiological constraints of species in a rapidly changing world.

## **Conclusions and application of research**

This project used a suite of physiological tools to investigate a variety of barriers to fish movement in freshwater systems and demonstrates how physiological approaches can contribute to evidence-based conservation and management solutions. A basic understanding of the ecology of fish, including their capacity to adjust to anthropogenic disturbances in both the short and longer terms, is important to effectively manage fish passage requirements in an increasingly fragmented landscape. The utility of experimental physiology means that the environmental boundaries within which fish operate can be determined from the level of a single stressor (e.g. water velocity) to more ecological realistic multi-stressor studies (e.g. water movement through a particular type of fish pass structure). Although fish passage management in general has been relatively good at utilising data from scientific research to inform on-ground actions, much remains to be done to improve the usefulness, accessibility and incorporation of primary fish physiology research into management actions.

One historical hurdle for the design of effective fish passage guidelines for Australian species is that globally, management of fish passage has been dominated by salmonid-focused research. Salmon are among the highest performing freshwater fish and so are not a suitable proxy species for generalising freshwater fish movement capacity, particularly for Australian species. Despite this, many Australian fish passage structures and 'fish-friendly' culvert designs have been based on adult salmon swimming capabilities (Mallen-Cooper and Brand 2007, Baumgartner et al. 2014). Likewise, remediation efforts such as vertical baffles that are designed to promote adult salmon movement (Mallen-Cooper and Brand 2007, Baumgartner et al. 2014) do not perform well for small-bodied species, or juveniles of larger growing species. Our project provided the first significant assessment of fish swimming capacities for some of the weakest-performing species and life stages of Australian freshwater fish. Given that smaller fish are the most likely to be affected by poorly designed culverts and fish pass structures, design criteria that considers the limitations on these individuals are likely to benefit a broader section of the fish population. These data can now be used to directly inform the design of fish passage structures targeted to the requirements and capacities of Australian species. Moreover, they provide empirical justification for fish passage guidelines that establish key velocity, depth and lighting recommendations that will ensure the greatest chance of successful fish passage.

The need to remediate existing structures so that they better function to support the movement requirements of aquatic fauna is widely recognised (Bernhardt et al., 2005, Bernhardt and Palmer, 2007). While billions of dollars every year are spent globally in an effort to restore connectivity to waterways and enhance ecosystem services (Bernhardt, et al., 2005, Lapointe, et al., 2013), many fail in part because of a lack of understanding of the behaviour and locomotory capacities of the fish using the structures (Palmer, et al., 2007, Lapointe, et al., 2013). Lab-based experimental fish studies uniquely positioned to provide proof of cause-and-effect relationships between hydrodynamic variables (Cooke, et al., 2017a, Cramp et al., 2020) and responses to remediation strategies can be modelled and tested in an iterative, controlled, and cost-effective manner. Our project revealed that many vertical baffle designs currently employed in Australian culverts could be negatively impacting upstream movements of small-bodied species and juvenile fish. A lack of empirical testing of these designs for Australian fish means that in some cases, they may be doing more harm than good.

The provision of effective fish passage in culverts requires a thorough understanding of the ecological communities likely to use the structure, their movement requirements and performance capacities. Yet this data does not exist for most species, and a general lack of post-installation monitoring often means that the efficacy of these structure for providing passage is never assessed. Our data suggest that the value of installing vertical baffles for fish passage must be carefully considered particularly for small fish. Our analysis of fish behaviour in culvert-like channels revealed that naturally low velocity boundary layers are critically important for proving passage for small fish, and remediation of existing box culverts for small fish could be improved by installing longitudinal beams that expand these low velocity zones. While our laboratory studies suggest that longitudinal beams are highly efficacious for small fish, like vertical baffles, their efficacy in the field needs to be fully understood before they can be recommended as a remediation tool. Other approaches that preserve natural water movement by maintaining the natural structure of the waterway with vegetation, rocks, waterway bends etc., are likely to be the most

effective way of maintaining connectivity in freshwater systems for small fish.

Not all barriers to fish movement are physical; physical structures can also alter hydrological, hydrodynamic, thermal, oxygen, heavy metal and nutrient conditions up-and downstream. There are 103 'large' dams and hundreds of smaller dams or weirs in the MDB with the potential to cause thermal pollution in downstream water reaches (Boys, et al., 2009, Lugg and Copeland, 2014). Release of unseasonably cold water to supply agricultural requirements often occurs at spawning time for the critically endangered (EPBC Act 1999) Murray Cod, Silver perch, and Trout cod. However, little is known about the thermal physiology of Australian fish from the MDB and whether they have the capacity to adjust physiological performance following acute reductions in water temperature that occur with large water releases. Our studies showed that acute water temperature drops of 10°C are likely too extreme for many native fish to contend with, however some handled the temperature change better than others. Remediation approaches that reduce the thermal stratification of deep dam impoundments or provide additional higher water offtake sites could reduce thermal pollution impacts on native fish populations. However, they are also likely to improve outcomes for invasive fish species simultaneously. Currently, Tilapia do not extend into the majority of the MDB, which in part may be due to their intolerance of low water temperatures. The restoration of natural MDB thermal regimes could promote conditions for Tilapia incursion, and so careful monitoring and management of remediation actions is required. Our work also showed that chronic sublethal exposure to nitrate pollution at the current environmental guideline of 50 mg/L or above can impair energy homeostasis and aerobically supported traits (e.g. swimming, growth etc.) due to disruptions in blood-oxygen carrying capacity. Our studies indicate that although the current nitrate guidelines were not outright lethal to Australian native fish, these exposures could have unintended sublethal consequences for native fauna and future ecotoxicological studies should consider the significant impacts of sublethal exposures on animals.

Although the challenges of taking data from largely laboratory-based studies to inform field policies and practice are acknowledged, traditional experimental approaches can provide important baseline information on fish performance and behaviour, particularly where other data collection approaches (e.g. long-term population monitoring, biologging in small bodied animals) may be limited. Lab-based, 'proof-of-principle' approaches allow conservation managers to explore how combinations of factors might influence performance in fish passage structures at small scale, and in an iterative and cost-effective manner. An understanding of the biological limitations that fish operate within provides a framework from which remediation efforts and the design of novel fish passage structures can be based (Cramp, et al., 2020).

## Impact of the research

This research now underpins the New South Wales Department of Primary Industries Fisheries' Road Crossing Design Guidelines, and will help reduce the impact of man-made barriers that limit native fish from accessing key habitat.

The project has produced the largest quantified dataset on native Australian fish swimming ability and a practical and cost-effective new design to promote fish passage through culverts. It is expected that the data and design will be of substantial benefit to waterway managers and engineers in the design of retrofitted and new waterway culverts to improve native fish passage.

Outputs from this project are feeding directly into NSW DPI Fisheries Policy and Guidelines and the NSW Interagency Group on Cold Water Pollution, while also addressing key threats identified within individual threatened species recovery plans.

## **Future research priorities**

Swimming performance data for small bodied Australian species and juveniles is still severely underrepresented in the literature. Despite this, small bodied species and juvenile fish make up a large proportion of the fish undertaking both local and larger-scale migrations. Future attention needs to be given to describing the variation in swimming performance capacities in small bodied species and across life history stages. The traditional experimental swimming performance approaches use in this study are particularly useful for small bodied and juvenile animals that may be too small to monitor with telemetry.

The potential for novel culvert remediation designs (longitudinal beams) to add to the fish passage design and remediation toolkit is well supported by our results presented here. However, while all designs performed well under laboratory conditions, they require extensive field testing before being recommended as an appropriate remediation strategy. There may be differences in how fish respond to the longitudinal beams in a natural setting with the benefit of natural motivation for movement and migration being present. The inherent complexity of field trials means the design, execution and interpretation of results must be carefully undertaken.

Multi-stressor studies increase the ecological realism and can reveal important ecological interactions that can be of considerable interest to fish passage managers and infrastructure designers (Cooke et al., 2017c). While some of our projects did incorporate multi-stressor studies, future work should prioritise multi-stressors experimental designs to better reveal complex interactions that may shape our understanding of fish physiological responses and more effectively guide management interventions. A strategy that combines a range of physiological assessment approaches and more ecological complexity will provide a more holistic picture of the physiological limits to performance and the consequences of performance at a fish's physiological limit. 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. It is critical that water temperatures remain within optimal thermal ranges for both fish performance and reproduction in the breeding season, to promote the success and recovery of native fish species in fragmented and thermally polluted waterways. To achieve this, thermal ranges need to be identified for target species at various life stages and from those data, temperature targets within thermal ranges can be set for dam releases. Where possible, release windows should occur during non-critical periods (i.e. outside of breeding season) in cases where releases are going to rapidly reduce downstream temperatures. Another strategy is to raise the temperature (and/or reduce thermal stratification) of water released from thermally stratified dams, however achieving this is an ongoing challenge.

## Data sets

- 1. Exposure to nitrate increases susceptibility to hypoxia in fish https://doi.org/10.14264/c24338f
- 2. Simultaneous exposure to nitrate and low pH reduces the blood oxygen-carrying capacity and functional performance of a freshwater fish https://doi.org/10.14264/80b3353
- 3. Assessment of the effects of microPIT tags on the swimming performance of small-bodied and juvenile fish https://doi.org/10.14264/3f9d440
- 4. Interactions between water depth, velocity and body size on fish swimming performance: Implications for culvert hydrodynamics https://doi.org/10.14264/f065d83
- 5. Thermal acclimation offsets the negative effects of nitrate on aerobic scope and performance https://doi.org/10.14264/fe5e038
- 6. Thermal plasticity of the cardiorespiratory system provides cross-tolerance protection to fish exposed to elevated nitrate https://doi.org/10.14264/c5155b2
- 7. Utilising the boundary layer to help restore the connectivity of fish habitats and populations https://doi.org/10.14264/7115884
- 8. Low light intensities increase avoidance behaviour of diurnal fish species: implications for use of road culverts by fish https://doi.org/10.14264/ee01f97
- 9. Can the impacts of cold-water pollution on fish be mitigated by thermal plasticity? https://doi.org/10.14264/762a2da
- 10. Swimming performance of 22 Australian fish species (to follow)

## Recommendations

- Small fish are comparably weaker swimmers than larger fish. Body size is a reasonable predictor of fish swimming performance and can be used to estimate the performance capacities of rare or data-deficient fish species;
- Current fish passage guidelines that limit maximum water velocities in culverts to < 0.3ms<sup>-1</sup> will effectively pass the majority of small bodied Australian fish species and juveniles of larger growing species. However, only a small contiguous portion of the culvert needs meet the 0.3ms<sup>-1</sup> guideline to provide passage to small bodied fish;
- Water depth in culverts can limit fish passage by affecting size- and water velocity-dependent performance in fish. Consideration of the specific relationships between these variables is important for understanding and remediating culverts as barriers to fish passage.
- Attention to lighting levels in civil structures is important for fish passage. Low light levels in culverts can reduce passage success particularly in diurnally active fish species. Most common culvert designs do not permit sufficient light to penetrate to promote fish movement during the day. Small amounts of natural light via sky-lights or artificial LED lighting could ameliorate this issue.
- Vertical baffles cause considerable turbulence which can limit upstream movements of small bodied species. We do not recommend the use of vertical baffles in culverts for promoting passage by small bodied species or juveniles.
- Longitudinal beams offer a novel alternative to vertical baffles for remediating culvert fish passage. Longitudinal beams provide a small, low velocity refuge that fish can utilise to move upstream. Laboratory testing has shown them to be highly effective, but field testing is required.
- Cold water pollution (CWP) significantly reduces fish physiological performance which in part explains the
  persistent downstream negative effects on native fish populations. Thermal acclimatisation does ameliorate
  some of the acute negative effects on fish performance, but these adjustment may take many months to develop.
  Where possible, release windows should occur during non-critical periods (i.e. outside of breeding season) in cases
  where releases are going to rapidly reduce downstream temperatures.
- Acute CWP-like changes in temperature negatively affects invasive carp, Tilapia and Gambusia performance, but they have a high capacity to positively respond to the low temperatures. Management actions designed to improve downstream water temperatures for native species are also like to benefit invasive species.
- We recommend that concentrations of nitrate in fresh water be kept below the current recommendation of 50 mg/l which resulted in impairments to fish and crayfish physiology and behaviour 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.
- Short-term testing of toxicity is unlikely to show the full impact of exposure to nitrate. 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.
- Waters high in nitrate 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.

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## **Ethics statement**

All studies were conducted with the approval of The University of Queensland Animal Ethics Committee, the QLD Department of the Environment and Science and the Queensland Department of Fisheries.

## **Resulting publications**

- Gomez Isaza DF, Cramp RL, Franklin CE (2021) Exposure to nitrate increases susceptibility to hypoxia in fish. *Physiol Biochem Zool* 94: 124-142
- Gomez Isaza DF, Cramp RL, Franklin CE (2021) Thermal plasticity of the cardiorespiratory system provides cross-tolerance protection to fish exposed to elevated nitrate. *Comp Biochem Physiol* C 240: 108920
- Isaza DFG, Cramp RL, Franklin CE (2020) Living in polluted waters: A meta-analysis of the effects of nitrate and interactions with other environmental stressors on freshwater taxa. *Environmental Pollution* 261: 114091
- Isaza DFG, Cramp RL, Franklin CE (2020) Simultaneous exposure to nitrate and low pH reduces the blood oxygencarrying capacity and functional performance of a freshwater fish. *Conservation Physiology* 8: coz092
- Isaza DFG, Cramp RL, Franklin CE (2020) Thermal acclimation offsets the negative effects of nitrate on aerobic scope and performance. *Journal of Experimental Biology* 223: jeb224444
- Keep JK, Watson JR, Cramp RL, Jones MJ, Gordos MA, Ward PJ, Franklin CE (2020) Low light intensities increase avoidance behaviour of diurnal fish species: implications for use of road culverts by fish. *Journal of Fish Biology*: jfb.14604
- Parisi MA, Cramp RL, Gordos MA, Franklin CE (2020) Can the impacts of cold-water pollution on fish be mitigated by thermal plasticity? *Conservation Physiology* 8: coaa005
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